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Original article

Clinical, epidemiological and therapeutic characteristics of *Mycoplasma genitalium* infection in a French STI center☆

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ABSTRACT

Objectives: We report the characteristics of *Mycoplasma genitalium* (MG) infection in patients from a STI center in Paris. We evaluated outcomes after treatment.
Methods: We included all patients tested for MG, *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) infection in our center from January 2017 to December 2018, using multiplex PCR on urine specimen, vaginal or rectal swabs. We collected data regarding sex, age, HIV status, PrEP use, sexual behavior, NG and CT co-infection, symptoms and treatment.
Results: MG infection prevalence was 7% (397/5586) (95% CI 6.4–7.8). It ranged from 4.6% in patients consulting for routine STI testing (3.9% in women, 5% in men), to 16% in HIV-positive patients and 25% in PrEP users. Among the 397 MG infected patients, 351 (88%) were asymptomatic and 87 (22%) were co-infected with NG or CT. Among the 270 (68%) treated patients, 249 (92%) received azithromycin. Failure rate was 74% in the 103 patients tested post-treatment. Treatment failure tended to be higher with azithromycin single dose than with 5-day azithromycin (88% vs. 70%; $P=0.07$). Azithromycin and moxifloxacin were used as second-line treatment in 24 and 23 patients, respectively. Post-treatment PCR remained positive in 55% of the 44 tested patients with a better eradication rate with moxifloxacin than with azithromycin (70% vs. 33%; $P=0.04$).
Conclusion: MG infection is highly prevalent in PrEP users and HIV-positive patients and is mostly asymptomatic. Management of MG infection should be tailored and adapted to the risk of antibiotic resistance and reinfection.

1. Background

Mycoplasma genitalium (MG) infection was first described as a sexually transmitted infection (STI) in 1981, when it was isolated from two men with non-gonococcal urethritis [1]. MG has emerged over the last few decades as a sexually transmitted pathogen causing acute and chronic inflammation in the urogenital tract by adhesion to epithelial cells.

While MG is the third cause of urethritis after *Neisseria gonorrhoeae* (NG) and *Chlamydia trachomatis* (CT), with prevalence of 10–35% according to the studies [2], its pathogenicity in women remains questioned. However, since recent meta-analyses have shown a significant association between MG infection and cervicitis, pelvic inflammatory disease, preterm birth and spontaneous abortion [3,4], some authors suggest systematic MG detection in women of childbearing age [5].

Most infected people are asymptomatic and spontaneously clear the MG [6]. In a meta-analysis including nine studies performed in the general population, MG was detected in 1% to 3% of the tested subjects [7]. High sexual activity, co-infection with other sexually transmitted pathogens, HIV infection and use of HIV Pre-Exposure Prophylaxis (PrEP) have been described as risk factors of MG acquisition [8–12].

Widened use of MG nucleic acid amplification testing (NAAT) has increased MG diagnoses and treatment, resulting in widespread emergence of antimicrobial resistance [13]. In 2018 in France, 43%

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of the strains collected at the National Reference Center were resistant to macrolides and 18% to fluoroquinolones [14].

For two years, we systematically performed MG NAAT in cases of STI testing in our center. We assessed MG infection prevalence, clinical presentation, and risk factors in our population, as well as the therapeutic issues in infected patients.

2. Methods

2.1. Study population, procedures and data collection

We conducted this monocentric retrospective study at Pitié-Salpêtrière hospital in Paris France, in two different departments (Infectious Disease department and STI center) from January 2017 to December 2018. We included all the consecutive patients tested for MG, CT and NG infection, using multiplex PCR on urine sample, vaginal and/or anal swab. The multiplex PCR was the Hologic Aptima® (Hologic, Inc. San Diego, CA 92121 USA) nucleic acid amplification test, performed according to the manufacturer's protocol. Reasons for the patients' visits were routine STI screening, possible STI symptoms or follow-up for PrEP or HIV infection.

Specimens from patients with macrolide or fluroquinolone failure were sent to the French National Reference Center to search for resistance. Resistance to azithromycin and fluoroquinolone was investigated at the National Reference Center for Bacterial STIs, Bordeaux University Hospital, in Bordeaux, France. We checked for 23S rRNA mutations associated with azithromycin resistance by using the ResistancePlus™ MG test (SpeeDx, Australia) directly on specimens and amplifying and sequencing the 23S rRNA. We screened for moxifloxacin-associated mutations by amplifying and sequencing the quinolone resistance-determining region (QRDR) of the *parC* gene [15].

We collected data regarding sex, age, HIV status and use of PrEP for all patients, and also data about sexual behavior, NG and CT co-infection, symptoms and therapeutic management in cases of MG infection.

We considered patients lost to follow-up if they did not come back for their test results, and they were not included in the therapeutic analysis. Patients were considered cured if test of cure using PCR performed 4 to 12 weeks after completing the antibiotic therapy was negative; otherwise, treatment failure was assumed.

2.2. Statistical analysis

We expressed qualitative variables in absolute numbers (number of cases) with percentages, and quantitative variables as medians with interquartile ranges (IQR). We performed descriptive and univariate analysis to identify significant differences between MG positive and negative patients, using Student t or Chi^2 tests ($P < 0.05$). We used a logistic regression model (multivariate analysis) to assess factors associated with MG infection. Investigated factors were sex, age, sexual orientation, HIV status, PrEP use, having symptoms, NG and CT co-infection. Odds ratios were presented with 95% confidence interval (95% CI). We performed all analyses with XLSTAT.

2.3. Ethics

Patients at Pitié-Salpêtrière Hospital are routinely monitored using the *Nadis* database [16]. Data from *Nadis* are collected prospectively, for which all patients provided signed consent (registration number with the "*Commission nationale de l'informatique et des libertés*, CNIL": 770134). All data were anonymized before analysis. No additional biological samples or questionnaires were used for this study.

3. Results

Among the 5586 patients included, 3694 (66%) were men with average age of 29 years (IQR 23.4–39.0) and 1884 (34%) women with average age of 23.5 years (IQR 20.9–27.9), 810 (14.5%) were HIV positive (HIV+) and 207 (3.7%) were on PrEP. Among the 397 (7%) patients whose MG PCR was positive (MG+ patients), 319 (80%) were men and 78 (20%) were women, 135 (34%) were HIV+ and 52 (13%) were HIV negative (HIV−) men on PrEP (Fig. 1). MG infection prevalence varied according to the reason for consultation. Among the patients visiting the center for routine STI screening (HIV− and not on PrEP), prevalence was 4.6% with no significant difference between women and men (3.9% vs. 5%, respectively). The MG infection rate was 16.6% in HIV+ patients and tended to be higher in HIV+ men than in HIV+ women (17.6% vs. 9.3%, respectively, OR 1.9, 95% CI 0.9–3.8, $P = 0.08$). HIV− men on PrEP had the highest MG infection rate at 25%. In univariate analysis, MG+ patients were more often HIV+ (OR 3.4, 95% CI 2.7–4.3) and more often on PrEP (OR 4.9, 95% CI 3.5–6.8) than MG− patients (Table 1).

Among the 78 MG+ women, 20 (26%) were co-infected with CT but none with NG, 73 (93%) were asymptomatic and 5 (7%) reported genito-urinary symptoms (3 cervicitis, 2 dysuria). Among the 319 MG+ men, 41 (13%) were co-infected with CT, 27 (8.5%) with NG, 9 (3%) with both, 278 (87%) were asymptomatic, 26 (8%) reported urethral symptoms (itch, discomfort, discharge or dysuria) and 13 (4%) reported anorectal symptoms (itch, discomfort, pain or bleeding) (Table 2).

Among the 397 MG+ patients, 270 (68%) received an antibiotic treatment (Fig. 1). Treatment abstention was more common in asymptomatic men than in asymptomatic women (17.8% and 3.9%, respectively, OR 5.3, 95% CI 1.2–22, $P = 0.02$).

Among the treated patients, 249 (92%) received azithromycin as first-line treatment, 192 (77%) for 5 days (500 mg followed by 250 mg daily for 4 days) and 57 (23%) 1 g single dose. Other treatments are detailed in Fig. 1. Test of cure was carried out in 103 (38%) patients within 8 weeks (IQR 4-12). Test of cure was performed in 66 (34%) patients treated with 5 days azithromycin showing a 70% treatment failure rate, and in 33 (57%) treated with a single dose showing an 88% treatment failure rate (OR 3.0, 0.9–9.7), $P = 0.07$). Among the patients initially co-infected, 14 underwent a test of cure and all remained positive for MG.

Fourteen samples were tested for macrolide and fluoroquinolone resistance. Mutations associated with macrolide resistance were detected in 9 samples, 3 of them also being associated with fluoroquinolone resistance. No strain showed single resistance to fluoroquinolone.

As second-line treatment, 24 (41%) patients received azithromycin for 5 days, 23 (39%) moxifloxacin and 8 (13%) doxycycline. No patient received single dose azithromycin as second-line treatment. Test of cure was carried out in 43 (73%) patients within 10 weeks (IQR 6–12), with a 44% eradication rate. Test of cure was performed in 18 (75%) patients treated with azithromycin showing a 67% treatment failure rate, and in 17 (74%) treated with moxifloxacin showing a 30% treatment failure rate (OR 4.8, 1.1–20, $P = 0.04$).

Third-line treatment is reported in Fig. 1.

4. Discussion

This study highlights some characteristics of MG infection in men and women. Prevalence in our population approximated 7%. Nearly 90% of the patients were asymptomatic and failure rates after first- and second-line treatments exceeded 50%.

MG infection prevalence varied according to type of patient and to reasons for consultation. In patients consulting for a routine STI

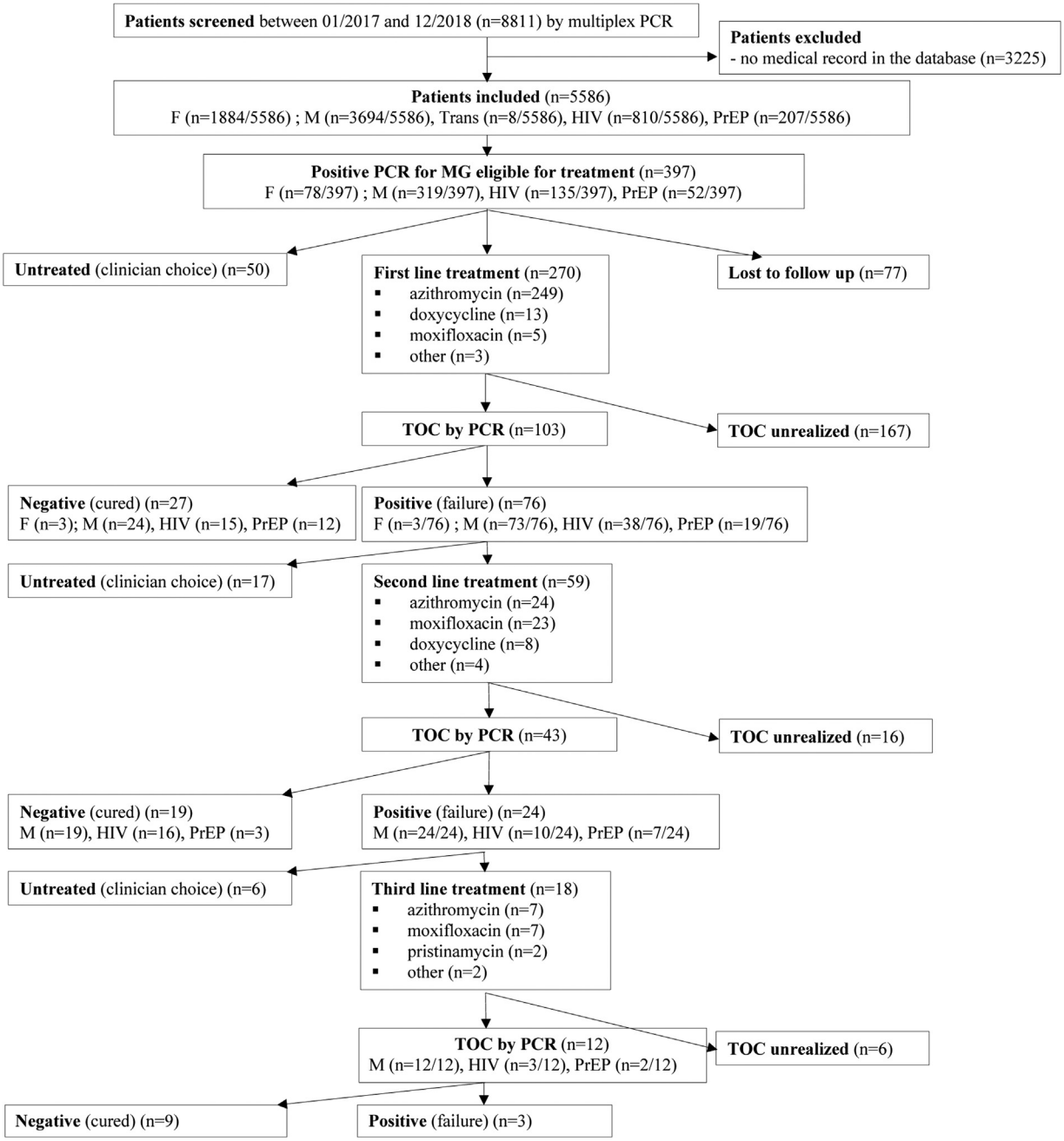

Fig. 1. Flow chart. TOC: test of cure; PrEP: HIV pre-exposure prophylaxis; MG: *Mycoplasma genitalium*; F: female; M: male; Trans: transexual.

Table 1
MG infection rate according to the reason for medical consultation.

	Screened patients (n = 5586)	MG+ patients (n = 397)	MG infection rate	OR (95% CI)	P-value
HIV-negative patient, not on PrEP (routine screening)	4569	210	4,6%	Reference	–
HIV follow-up consultation	810	135	16.6%	3.4 (2.7–4.3)	P < 0.0001
PrEP follow-upconsultation	207	52	25%	4.9 (3.5–6.8)	P < 0.0001

MG: *Mycoplasma genitalium*; PrEP: HIV pre-exposure prophylaxis.

screening, it was 3.9% in women and 5% in men. Such rates are similar to those reported in other studies conducted in STI clinics where they range from 3% to 7.5% in women, and from 5.3% to 11.4% in men [16–19]. In HIV+ patients and in men on PrEP, it was drastically higher (16% and 25%, respectively). These rates are similar to those found in MSM in Germany, i.e., 18.4% in HIV+ patients and 19.4% in patients on PrEP.

Some German authors found that being HIV+ or using PrEP were independent risk factors for being tested MG+ (OR 1.7 and 2.0, respectively) [20]. It has been demonstrated that thigh MG

Table 2
Epidemiological and clinical characteristics in 319 men and 78 women found to have MG infection.

	Women $n = 78$	Men $n = 319$	P-value
Age (median, IQR)	25 (21.9-27.6)	32 (26.9-39.4)	–
Sexual health data *n* (%)			
Homosexual relationship	1 (1%)	118 (37%)	$P < 0.001$
Heterosexual relationship	64 (82%)	106 (33%)	$P < 0.001$
Unknown	13 (17%)	95 (30%)	$P = 0.015$
HIV-positive *n* (%)	9 (11%)	126 (39%)	$P < 0.0001$
Sampling site			
Urine	NA	139 (44%)	NA
Anus	4 (5%)	180 (56%)	NA
Vaginal	77 (99%)	NA	NA
Co-infections			
CT	20 (26%)	41 (13%)	$P = 0.006$
NG	0	27 (8,5%)	$P = 0.008$
Clinical			
Asymptomatic	73 (93%)	278 (87%)	$P = 0.12$
Symptomatic			
Genitourinary symptoms	5 (7%)	26 (8%)	$P = 0.6$
Ano-rectal symptoms	0	13 (4%)	NA
Other	0	2 (<1%)	$P = 0.9$
Co infection CT/NG	2 (40%)	16 (39%)	$P = 1$

CT: chlamydia trachomatis; NG: neisseria gonorrhoeae.

infection prevalence is associated with a large number of sex partners and unprotected sexual intercourse [8,13,20]. However, HIV infection could independently exacerbate the diagnostic sensitivity of MG infection due to immune cell dysfunction [21]. Frequency of STI screening may also influence MG prevalence in men on PrEP, systematic two-site screening (anus and urine sample) being performed every 3 months, as part of treatment monitoring.

In our study, MG + patients were frequently co-infected with CT or NG, 22% of all patients and up to 40% in case of symptoms, and the failure rate was 100% in the co-infected patients having undergone a test of cure after first-line treatment. Having another STI was previously characterized as an independent risk factor for MG infection [10]. Compared to another study [26], there was no NG co-infection in women. A recent study reported a strong association between MG and CT infection but not NG, suggesting that it could result not only from shared risk factors but also from a causal association between CT and MG or an epidemiological association [27]. They also showed that MG macrolide and fluoroquinolone resistance were more likely to be found in patients with a co-infection [25,27].

Our results show a poor outcome with a 74% failure rate after first-line and 55% after second-line treatment for patients having undergone a test of cure. During the study period (2017–2018), there were no clear guidelines for the screening and management of asymptomatic MG infection, a factor possibly explaining the discrepancies between clinicians' practices. Systematic detection of MG coupled with detection of NG and CT (multiplex PCR) increased the diagnosis of MG infections, leading to several therapeutic difficulties. For example, the use of 1 g single dose azithromycin as empiric treatment, which concerned 1/5 of the first-line treatments in our study, is now known to contribute to the emergence of macrolide resistance [22]. A more recent study underscored the enhanced efficacy of a sequential treatment with doxycycline (7 d) followed by azithromycin (5 d) or moxifloxacin (7 d) depending on the results of macrolide resistance testing, and a test of cure was advised 5 weeks after cessation of antibiotic intake [24].

In our HIV+ patients and men on PrEP, the MG treatment was particularly challenging. This is probably linked to several factors, including a greater risk of reinfection (given the MG prevalence in this population), a high number of sexual partners, absence of safe sex, high frequency of sexual intercourse and a higher risk of antibiotic resistance due to repeated antibiotic therapies [22,23]. Given the logistical difficulty of outsourcing samples, very few of them were sent the National Reference Center to be tested for resistance. Therefore, the resistance rate (i.e., 9/14, 64%) was probably overestimated due to the fact that samples were taken only from patients with treatment failure. However, our result is similar to those reported in other studies where resistance rates range from 74.1% to 80% in HIV+ patients [12,13] and from 58% to 78.8% in men on PrEP [13,23]. New combinations of antibiotics have shown positive results in the management of patients with macrolide-resistant MG for whom no classic treatment options are available. For patients in whom moxifloxacin has failed or is contraindicated, the association of doxycycline and pristinamycin (10 d) was effective in 75% of 73 participants [28] and the combination of doxycycline and sitafloxacin (7 d) cured 11/12 patients after failure of sequential therapy by doxycycline and moxifloxacin and second-line treatment by pristinamycin [29]. In the future, acquisition of PCR directly coupled with the search for resistance will allow a targeted treatment from the outset.

Given the 87% rate of asymptomatic patients found in our study and the absence of studies showing a risk of sequelae in cases of unnoticed MG infection in men, we agree with the current recommendations limiting screening to symptomatic patients, the objective being to prevent the emergence of untreatable strains [23]. Given possible pathogenicity in women, the above practices should perhaps be limited to men having sex exclusively with men.

In women, the risk of long-term sequelae in case of untreated MG infection remains uncertain. In a meta-analysis of 1080 studies published between 1980 and 2014, Lis and al found that MG infection was significantly associated with increased risk of cervicitis (OR, 1.66), pelvic inflammatory disease (OR, 2.14), preterm birth (OR, 1.89) and spontaneous abortion (OR, 1.82) [4]. In our study, the management of women infected with MG did not pose any difficulties and the infection rate was lower. For these women, given the possible long-term sequelae of an unnoticed MG infection, systematic MG screening should consequently be discussed.

This study has several limitations, including retrospective data collection, which entailed missing data for a substantial number of patients. While the absence of a standardized protocol for testing and therapeutic management limited analysis, this observational work nevertheless highlighted the difficulties of MG infection management in real-life situations. Lastly, we did not systematically test

for mutations associated with macrolide and fluoroquinolone resistance, which nonetheless represents a key element for discussion.

5. Conclusion

This study highlights pronounced variability in the management of MG infection, depending even in the same center on type of patient and reason for consultation. MG infection was significantly associated with HIV infection and PrEP intake. While most patients are asymptomatic, they are often at high risk of reinfection, and represent a major challenge for clinicians.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the 1964 Helsinki declaration and its later amendments.

Contribution of authors

C. Br, R.P and G.M drafted the manuscript. N.G, J.R, R.A provided information and participated in writing the manuscript. E.C, C.Be and A.S reviewed and revised the manuscripts. All authors read and approved the final manuscript.

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Disclosure of interest

The authors declare that they have no competing interest.

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