Prevalence of macrolide and fluoroquinolone resistance-associated mutations in *Mycoplasma genitalium* in metropolitan and overseas France

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ABSTRACT

Objective Limited macrolide and fluoroquinolone resistance data are available in France for *Mycoplasma genitalium*. We performed a multicentre cross-sectional study to investigate the prevalence of macrolide and fluoroquinolone resistance-associated mutations in *M. genitalium*-positive patients in metropolitan France between 2018 and 2020 and in overseas France in 2018 and 2019.

Methods Each year, a 1-month prospective collection of *M. genitalium*-positive specimens was proposed to metropolitan French microbiology diagnostic laboratories, and a similar 3-month collection was proposed to overseas French laboratories. Resistance-associated mutations were detected using commercial kits and sequencing.

Results A total of 1630 *M. genitalium*-positive specimens were analysed. In metropolitan France, the prevalence of macrolide resistance-associated mutations ranged between 34.7% (95% CI 29.4% to 40.4%) and 42.9% (95% CI 37.1% to 49.0%) between 2018 and 2020 and was significantly higher in men (95% CI 52.4% to 60.2%) than in women (95% CI 15.9% to 22.2%) (p<0.001). These prevalences were significantly higher than those of 6.1% (95% CI 3.7% to 10.3%) and 14.7% (95% CI 10.9% to 19.6%) observed in overseas France in 2018 and 2019 (p<0.001), where no difference between genders was noted. The prevalence of fluoroquinolone resistance-associated mutations was also significantly higher in metropolitan France (14.9% (95% CI 11.2% to 19.5%) to 16.1% (95% CI 12.1% to 21.2%)) than in overseas France (1.3% (95% CI 0.4% to 3.7%) and 2.6% (95% CI 1.3% to 5.3%) in 2018 and 2019, respectively) (p<0.001), with no difference between men and women regardless of the location.

Conclusion This study reports the high prevalence of macrolide and fluoroquinolone resistance-associated mutations in *M. genitalium* in metropolitan France and highlights the contrast with low prevalence in overseas France. In metropolitan France, macrolide resistance-associated mutation prevalence was three times higher in men than in women, which was likely to be driven by the proportion of men who have sex with men. This suggests that gender and sexual practice should also be taken into account for the management of *M. genitalium* infections.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Antimicrobial resistance has been increasing worldwide in Mycoplasma genitalium, but limited data are available in France.

WHAT THIS STUDY ADDS

⇒ This study reports high prevalence of both macrolide and fluoroquinolone resistance-associated mutations in metropolitan France, which reached 42% and 16% in M. genitalium, respectively.

⇒ First French overseas resistance prevalence data showed significant lower resistance-associated mutation prevalence than in metropolitan France, especially fluoroquinolone resistance-associated mutation prevalence, which was below 3%.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Careful monitoring of the macrolide resistance-associated mutation prevalence is necessary in metropolitan France, as well as in overseas France because a significant increase may have occurred between 2018 and 2019 (6.1% and 14.7%, respectively).

INTRODUCTION

Mycoplasma genitalium is a recognised STI bacterium, for which the macrolide azithromycin and the fluoroquinolone moxifloxacin are the first-line and second-line treatments in most available guidelines, as part of sequential treatments or not.[1–3] The prevalence of macrolide and fluoroquinolone resistance-associated mutations has been increasing in Europe and worldwide for the last 10 years,[4 5] but limited resistance data are available in France, where there is no national antimicrobial resistance monitoring system for *M. genitalium*. A previous study conducted in Bordeaux, southwestern France, on 344 *M. genitalium*-positive patients reported a prevalence of macrolide and fluoroquinolone resistance-associated mutations of only 17% and 6% in 2013–2014, respectively.[6] Additionally, no resistance data are available in overseas France, where *M. genitalium* has also been reported.[7] To fill this gap, we performed a multicentre cross-sectional study to investigate the prevalence of macrolide and fluoroquinolone resistance-associated mutations in *M. genitalium*-positive men and women in metropolitan France between 2018 and 2020 and in overseas France in 2018 and 2019.

MATERIALS AND METHODS

Patients, specimens and data collection

A 1-month systematic prospective collection of remnants of *M. genitalium*-positive specimens was proposed between 15 September and 15 October 2018, 2019 and 2020 to public and private microbiology diagnostic laboratories performing *M. genitalium* detection throughout the metropolitan French territory. A similar 3-month collection between 1 August and 31 October 2018 and 2019 was proposed to overseas French microbiology diagnostic laboratories located on La Réunion Island and in Mayotte in the Indian Ocean region, in French Guyana on the northeastern coast of South America, and in New Caledonia and French Polynesia in the South Pacific region. Remnants of *M. genitalium*-positive patient specimens from metropolitan and overseas France were sent to the French National Reference Centre for Bacterial STI in Bordeaux at +4°C and at −20°C, respectively. Patient age, gender, type of samples and sample collection sites were anonymously collated by the microbiologist of each laboratory at the time the specimen was detected as *M. genitalium*-positive.

Specimen processing

DNA extraction was performed using the MagNA Pure 96 DNA and viral NA small-volume kit and the MagNA Pure 96 instrument (Roche Diagnostics). Macrolide resistance-associated mutations[4] were detected using the ResistancePlus MG assay (SpeeDx, Australia). Specimens harbouring mutated *M. genitalium* were submitted to 23S rRNA sequencing to determine the type of mutation.[8] Mutations in the quinolone resistance-determining region (QRDR) of the *parC* gene were searched as previously reported.[6]

Statistical analysis

Categorical data are presented as numbers and frequencies (percentages), and 95% CIs of antimicrobial resistance-associated mutation prevalence were calculated using the exact binomial distribution. The frequencies were compared by the χ^2 test or Fisher's exact test, as appropriate. P values below 0.05 were considered significant. Statistical analyses were performed using the biostaTGV website (https://biostatgv.sentiweb.fr/).

RESULTS

Specimens and patients included

A total of 339, 391 and 315 *M. genitalium*-positive specimens were collected in metropolitan France in 2018, 2019 and 2020 from 20, 19 and 21 laboratories, respectively, and 287 and 344 specimens were collected in overseas France in 2018 and 2019 from 5 and 3 overseas territories, respectively (tables 1 and 2 and online supplemental figure S1). Specimens were mainly cervicovaginal swabs in women and first-void urines in men (table 1). The percentage of rectal swabs ranged between 29.2% and 39.9% in metropolitan France and between 4.1% and 5.1% in overseas France.

A total of 1630 *M. genitalium*-positive patients were included in the study. The proportion of men and women included in metropolitan France was similar, whereas the proportion of women was significantly higher than the proportion of men in overseas France (p<0.001) (table 2). The mean age of the patients ranged between 27.7 years and 31.6 years (table 2). The percentage of patients for whom an amplification of 23S rRNA and *parC* gene was achieved was 78.6% (1281/1630) and 77.1% (1256/1630), respectively.

Prevalence of macrolide resistance-associated mutations in metropolitan and overseas France

The prevalence of macrolide resistance-associated mutations in *M. genitalium* ranged between 34.7% (95% CI 29.4% to 40.4%) and 42.9% (95% CI 37.1% to 49.0%) over 2018–2020 in metropolitan France (table 3). The resistance was stable throughout the 3 years because the percentages of macrolide resistance-associated mutations calculated among the 13 centres that participated in the 3 years of the study were 33.1% (56/169) in 2018, 34.8% (81/233) in 2019 and 36.4% (60/165) in 2020 (p=0.82). A significant prevalence difference was noted between men and women, ranging between 52.4% and 60.2% in men vs

Table 1 Characteristics of the studied specimens

	Metropolitan France 15 September–15 October			Overseas France 1 August–31 October	
	2018	2019	2020	2018	2019
Total number of specimens	339	391	315*	287†	344‡
Specimens from women	141	189	145	188	218
Cervicovaginal swabs	128 (90.8)	166 (87.8)	127 (87.6)	133 (70.7)	167 (76.6)
First-void urines	12 (8.5)	16 (8.5)	17 (11.7)	29 (15.4)	49 (22.5)
Rectal swabs	1 (0.7)	4 (2.1)	1 (0.7)	3 (1.6)	2 (0.9)
Throat swabs	0	1 (0.5)	0	2 (1.1)	0
Unknown origin		2 (1.1)	0	21 (11.1)	0
Specimens from men	198	202	160	99	122
First-void urines	98 (49.5)	119 (59.1)	91 (56.9)	56 (56.6)	108 (88.5)
Rectal swabs	79 (39.9)	59 (29.2)	57 (35.6)	5 (5.1)	5 (4.1)
Throat swabs	14 (7.1)	7 (3.5)	5 (3.1)	2 (2.0)	0
Urethral swabs	7 (3.5)	17 (8.4)	7 (4.4)	4 (4.0)	4 (3.3)
Semen	0	0	0	3 (3.0)	5 (3.3)
Unknown origin	0	0	0	29 (29.3)	0

Data are presented as numbers with percentages in parentheses.
*Nine patients of unknown gender and one transgender patient.
†A total of 207 specimens from La Réunion, 50 from Mayotte, 14 from French Guyana, 15 from French Polynesia and 1 from New Caledonia.
‡Four patients with unknown gender. A total of 259 specimens from La Réunion, 73 from French Guyana, and 12 from French Polynesia.

Table 2 Characteristics of the studied population

	Metropolitan France Sept 15–Oct 15			Overseas France Aug 1–Oct 31	
Year	2018	2019	2020	2018	2019
Number of patients	329	379	302	278	342
Number of participant centres or overseas territories	20	19	21	5*	3†
Gender					
Male	189 (57.4)	194 (51.2)	150 (49.7)	96 (34.5)	122 (33.5)
Female	140 (42.6)	185 (48.8)	142 (47.0)	182 (65.5)	216 (63.2)
Transgender	0	0	1 (0.3)	0	0
Unknown	0	0	9 (3.0)	0	4 (1.1)
Age					
Mean age (years)	31.3	30.1	31.6	27.7	29.5
Median age (years)	29	27.5	29.2	26.0	27
Range (years)	15–68	16–76	15–67	13–65	14–63
Sample collection sites					
Infectious disease department	58 (17.6)	36 (9.5)	49 (16.2)	–	–
Gynaecology department	57 (17.3)	67 (17.7)	54 (17.9)	–	–
STI clinic	43 (13.1)	132 (34.8)	80 (26.5)	–	–
General practitioner	50 (15.2)	41 (10.8)	9 (3.0)	–	–
French Red Cross	0	0	0	–	–
Emergency department	3 (0.9)	18 (4.7)	14 (4.6)	–	–
Penitentiary centre	12 (3.7)	10 (2.7)	13 (4.3)	–	–
Obstetrics department	4 (1.2)	5 (1.3)	3 (1.0)	–	–
Abortion centre	17 (5.2)	13 (3.4)	4 (1.3)	–	–
Family planning centre	10 (3.0)	1 (0.3)	18 (6.0)	–	–
Others‡	17 (5.2)	6 (1.6)	21 (6.9)	–	–
Unknown	58 (17.6)	50 (13.2)	37 (12.3)	–	–

Data are presented as numbers with percentages in parentheses.
In overseas France, in 2018 and 2019, nine and two patients had infection at two different sites, respectively.
– denotes information not available.
*La Réunion, Mayotte, French Guyana, New Caledonia and French Polynesia.
†La Réunion, French Guyana and French Polynesia.
‡Other includes dermatology, reproductive medicine, forensic medicine, gastroenterology, rheumatology, and urology departments.
§In metropolitan France, in 2018 and 2019, two and three patients had specimens at two different dates and eight and nine patients had *Mycoplasma genitalium* infection in two different anatomical sites, respectively. In 2020, in metropolitan France, nine patients had infection at two different sites and two patients had infection at three different sites.

15.9% and 22.2% in women (p<0.001). When the prevalence of macrolide resistance-associated mutations was examined in rectal swabs from men, the percentages of macrolide resistance-associated mutations were 70.6% (48/68) in 2018, 65.8% (25/38) in 2019 and 75.8% (25/33) in 2020, whereas the prevalence of macrolide resistance-associated mutations in other male sample types was lower, 51.1% (48/92, p<0.05) in 2018, 49.6% (56/113, p=0.08) in 2019, and 54.1% (46/85, p<0.05) in 2020 (data not shown).

In overseas France, the prevalence of macrolide resistance-associated mutations was significantly lower than that in metropolitan France, with a prevalence of only 6.1% (95% CI 3.7% to 10.3%) in 2018 (p<0.001) and 14.7% (95% CI 10.9% to 19.6%) in 2019 (p<0.001) (table 3). In contrast to metropolitan France, there was no difference in the percentage of macrolide resistance-associated mutations between men and women (table 3). A significant increase in the proportion of macrolide resistance-associated mutations was noted between 2018 and 2019, 6.1% vs 14.7% (p=0.002), respectively, and the difference was observed in both men and women.

Among the 279 mutated *M. genitalium* strains of the study for which 23S rRNA sequencing was successful, the most frequent mutation was A2059G (53.0%, 148/279) (*Escherichia coli* numbering), followed by A2058G (25.8%, 72/279), A2058T (16.8%, 47/279), a mix of A2058G and A2059G (2.5%, 7/279), A2058C (0.7%, 2/279), A2062T (0.7%) and A2059T (0.4%, 1/279), with no significant difference between years and geographical locations.

Prevalence of fluoroquinolone resistance-associated mutations in metropolitan and overseas France

The prevalence of amino acid changes in the QRDR of the ParC protein is presented in table 3. The Ser83Ile, Ser83Arg, Asp87Tyr, and Asp87Asn changes were considered likely of clinical significance because increased Minimum Inhibitory concentrations (MICs) have been observed in mutated isolates[9 10] or because moxifloxacin treatment failures were reported in the presence of these mutations.[11–14] The Gly81Cys mutation was also considered significant because increased moxifloxacin MIC were reported.[15] In contrast, the ParC Ser83Asn alteration, which did not significantly increase fluoroquinolone MICs,[10] was not included in the ParC alterations likely to have clinical significance.

ParC amino acid changes likely of clinical significance ranged between 14.9% (95% CI 11.2% to 19.5%) and 16.1% (95% CI 12.1% to 21.2%) in metropolitan France over 2018–2020, with no significant difference between years (table 3). In overseas

Table 3 Prevalence of macrolide and fluoroquinolone resistance-associated mutations in metropolitan and overseas France. Data are presented as the percentage of patients harbouring mutated isolates with 95% CI in brackets

	Metropolitan France				Overseas France			
	Total	Men	Women	P value	Total	Men	Women	P value
Macrolide resistance-associated mutations								
2018	42.9* (37.1 to 49.0)	59.6 (51.7 to 66.9)	18.7 (12.4 to 27.1)	<0.001	6.1† (3.7 to 10.3)	6.5 (2.8 to 14.3)	6.0 (3.2 to 10.9)	1.0
2019	34.7* (29.4 to 40.4)	52.4 (44.3 to 60.3)	15.9 (10.7 to 22.9)	<0.001	14.7† (10.9 to 19.6)	13.5 (8.1 to 21.8)	15.6 (10.8 to 22.1)	0.7
2020	42.1* (36.1 to 48.4)	60.2 (51.2 to 68.6)	22.2 (15.6 to 30.5)	<0.001	ND	ND	ND	ND
ParC amino acid changes in QRDR								
2018	18.1 (13.9 to 23.3)	19.8 (14.2 to 27.1)	16.0 (10.3 to 24.2)	0.5	3.9 (2.1 to 7.2)	5.8 (2.5 to 12.9)	2.7 (1.1 to 6.8)	0.3
2019	17.6 (13.7 to 22.6)	19.8 (14.2 to 27.1)	15.4 (10.7 to 24.2)	0.3	6.3 (4.0 to 9.9)	3.1 (1.1 to 8.8)	8.3 (3.1 to 8.6)	0.09
2020	19.2 (14.4 to 25.2)	20.4 (13.6 to 29.4)	19.2 (12.6 to 28.0)	0.8	ND	ND	ND	ND
xParC amino acid changes likely of clinical significance‡								
2018	16.1§ (12.1 to 21.2)	18.2 (12.8 to 25.2)	13.2 (8.0 to 20.9)	0.2	1.3 (0.4 to 3.7)	2.3 (0.6 to 8.1)	0.7 (0.1 to 3.7)	0.5
2019	14.9§ (11.2 to 19.5)	17.1 (11.9 to 24.1)	12.5 (7.9 to 19.1)	0.3	2.6 (1.3 to 5.3)	3.1 (1.1 to 8.8)	2.4 (0.9 to 5.9)	0.7
2020	15.8§ (11.4 to 21.4)	17.3 (11.1 to 26.0)	15.2 (11.4 to 21.4)	0.7	ND	ND	ND	ND
Dual macrolide resistance-associated mutations and ParC amino acid changes likely of clinical significance								
2018	10.7¶ (7.3 to 15.6)	15.0 (9.8 to 22.2)	4.7 (1.8 to 11.6)	<0.05	0	0	0	ND
2019	9.2¶ (6.2 to 13.5)	12.7 (7.9 to 19.6)	5.3 (2.5 to 11.1)	<0.05	0.9 (0.2 to 3.3)	1.3 (0.2 to 7.0)	0.7 (0.1 to 4.0)	1
2020	12.8¶ (8.7 to 18.5)	17.6 (11.1 to 27.2)	8.8 (4.6 to 16.6)	0.08	ND	ND	ND	ND

P values compare prevalence between men and women.
*The percentages of macrolide resistance-associated mutations calculated among the 13 centres that participated in the 3 years of the study were 33.1% (56/169) in 2018, 34.8% (81/233) in 2019 and 36.4% (60/165) in 2020 (p=0.82).
†Significant increase in the prevalence of macrolide resistance-associated mutations between 2018 and 2019. The percentages of macrolide resistance-associated mutations calculated among the three overseas territories that participated both years of the study were 7.3% (14/191) in 2018 and 14.7% (38/258) in 2019 (p=0.01).
‡Only parC Asp87Tyr, Asp87Asn, Ser83Ile, Ser83Arg and Gly81Cys alterations, which are likely to have clinical significance, were counted.[9–15 23]
§The percentages of ParC amino acid changes calculated among the 13 centres that participated in the 3 years of the study were 15.4% (27/175) in 2018, 15.3% (40/262) in 2019 and 17.9% (24/134) in 2020 (p=0.77).
¶The percentage of dual macrolide resistance-associated mutations and ParC amino acid changes likely of clinical significance calculated among the 13 centres that participated in the 3 years of the study were 9.7% (14/145) in 2018, 10.6% (20/189) in 2019 and 13.1% (16/122) in 2020 (p=0.65).
QRDR, quinolone resistance-determining region; ND, not determined.

France, the prevalence of fluoroquinolone resistance-associated mutations was significantly lower than that in metropolitan France, with prevalence of only 1.3% (95% CI 0.4% to 3.7%) in 2018 (p<0.001) and 2.6% (95% CI 1.3% to 5.3%) in 2019 (p<0.001) (table 3). There was no significant difference in the prevalence of fluoroquinolone resistance-associated mutations in overseas France in 2018 and 2019 (p=0.35). In addition, no significant difference in the prevalence of fluoroquinolone resistance-associated mutations was observed between men and women either in metropolitan France or in overseas territories.

ParC alterations are described in table 4. The Ser83Ile mutation was the most frequent change in metropolitan France, representing approximately 70% of ParC mutations, with a stable proportion between 2018 and 2020. In contrast, Ser83Ile was less frequent in overseas French territories, representing only 33.3% and 11.8% of ParC alterations in 2018 and 2019, respectively. Overall, mutations likely of clinical significance in ParC were detected in 82.0% (32/39) to 89.1% (41/46) of patients harbouring ParC alterations in metropolitan France but in only 33.3% (3/9) to 41.2% (7/17) of patients from overseas France (table 4).

Prevalence of dual resistance-associated mutations in metropolitan and overseas France

The prevalence of dual resistance-associated mutations ranged between 9.2% (95% CI 6.2% to 13.5%) and 12.8% (95% CI 8.7% to 18.5%) between 2018 and 2020 in metropolitan France, with no significant difference between years (table 3). Dual resistance-associated mutation prevalence was significantly more frequent in men than in women in 2018 and 2019 (p<0.05). In overseas France, no dual resistance-associated mutations were noted in 2018, and prevalence was only 0.9% (95% CI 0.2% to 3.3%) in 2019, with no significant difference between men and women.

DISCUSSION

It is important to monitor the prevalence of macrolide and fluoroquinolone resistance-associated mutations in countries

Table 4 Number and proportion of patients with alterations in the QRDR of parC in metropolitan and overseas France. Data are presented as the number of patients harbouring an isolate mutated in ParC with the percentage of total in parentheses.

	Metropolitan France			Overseas France	
	2018	2019	2020	2018	2019
Alterations in the QRDR of ParC likely of clinical significance*					
Ser83(80)Ile	34 (73.9)	34 (68.0)	28 (71.8)	3 (33.3)	2 (11.8)
Ser83(80)Arg	0	3 (6.0)	0	0	0
Asp87(84)Asn	4 (8.7)	2 (4.0)	2 (5.1)	0	2 (11.8)
Asp87(84)Tyr	2 (4.3)	3 (6.0)	2 (5.1)	0	2 (11.8)
Gly81(78)Cys	1 (2.2)	0	0	0	1 (5.8)
Total	41 (89.1)	42 (84.0)	32 (82.0)	3 (33.3)	7 (41.2)
Alterations in the QRDR of ParC unlikely of clinical significance*					
His80(77)Arg	0	0	1 (2.6)	0	0
His80(77)Asp	0	0	1 (2.6)	0	0
Ser83(80)Asn	3 (6.5)	2 (4.0)	3 (7.7)	0	2 (11.8)
Asp87(84)Gly	1 (2.2)	0	0	0	0
Arg91(88)Lys	0	0	0	0	5 (29.5)
Arg91(88)Trp	0	0	0	0	1 (5.8)
Ser95(92)Asn	0	1 (2.0)	1 (2.6)	2 (22.2)	0
Ser95(92)Cys	0	1 (2.0)	0	0	0
Ser104(101)Phe	0	0	0	0	1 (5.8)
Ile105(102)Phe	0	0	0	0	1 (5.8)
His106(103)Tyr	1 (2.2)	0	0	2 (22.2)	0
His106(103)Gln	0	1 (2.0)	0	0	0
His106(103)Arg	0	0	0	1 (11.1)	0
Gly107(104)Asp	0	0	1 (2.6)	0	0
Ser111(108)Leu	0	1 (2.0)	0	0	0
Asn116(114)Asp	0	2 (4.0)	0	0	0
Ala119(117)Ser	0	0	0	1 (11.1)	0
Total	5 (10.9)	8 (16.0)	7 (18.0)	6 (66.7)	10 (58.8)
Total	46 (100)	50 (100)	39 (100)	9 (100)	17 (100)

**Mycoplasma genitalium* numbering with *Echerichia coli* numbering in parentheses. Only ParC Asp87Tyr, Asp87Asn, Ser83Ile, Ser83Arg and Gly81Cys alterations, which are likely to have clinical significance, were counted.[9–15 23]
QRDR, quinolone resistance-determining region.

where *M. genitalium* infections are widespread. In France, we reported the prevalence of macrolide and fluoroquinolone resistance-associated mutations in a similar sampling frame restricted to southwestern France in 2013–2014, with percentages of 17% and 6%, respectively.[6] As a comparison, the present study including approximately 20 French microbiology laboratories throughout the territory each year suggests a significant increase in the prevalence of both macrolide and fluoroquinolone resistance-associated mutations, with prevalence up to 42.9% and 16.1% in 2018, respectively. The macrolide resistance-associated mutation prevalence appears higher than that recently reviewed in several European non-Nordic countries,[4 5] but the studies included in both reviews were based on data collected until 2017. Indeed, the prevalence of macrolide resistance-associated mutations above 40% is now in accordance with the French macrolide consumption level of 1600 defined daily doses/1000 persons/year, as analysed by Kenyon and Manoharan-Basil in the association drawn between prevalence of macrolide resistance-associated mutations and macrolide consumption.[16] However, compared with a similar study performed in 2017–2018 by the UK National Reference Laboratory, the French prevalence of macrolide resistance-associated mutations was significantly lower than the 70.7% UK prevalence ($p<0.001$).[17] On the other hand, the French prevalence of moxifloxacin resistance-associated mutations was significantly higher than the prevalence of 8.0% reported by the UK National Reference Laboratory ($p<0.01$)[17] and by other previous European studies[4 5] but was in the range of 10.5%–28.7% reported in the Western Pacific regions.[4] Of note, the French prevalence of dual resistance-associated mutations was similar to the prevalence of fluoroquinolone resistance-associated mutations. Indeed, clinically relevant fluoroquinolone resistance-associated mutations were often associated with macrolide resistance-associated mutations, likely as a consequence of macrolide treatment failure or pre-existing macrolide resistance-associated mutation prior to the use of moxifloxacin, as recommended in the European *M. genitalium* infection guidelines.[3]

One noticeable point of this study is the significant difference observed in macrolide but not in the fluoroquinolone resistance-associated mutation prevalence between men and women in metropolitan France. This finding was previously reported in Australia[18] and was also retrieved in a recent meta-analysis.[4] In the present study, patient sexual orientation could not be collated. Because rectal swabs are almost always collected from men who have sex with men (MSM) and very rarely collected from heterosexual men in France, rectal swabs may serve as a proxy of samples from MSM. The percentage of macrolide resistance-associated mutations up to 75.8% found in rectal swabs from men is consistent with previous resistance studies focusing on MSM.[4 19–21] Thus, the difference in macrolide

resistance-associated mutation prevalence between genders in metropolitan France is likely to be driven by MSM status, with higher levels of screening for STIs, higher levels of macrolide consumption in the subgroup of MSM likely to carry and exchange macrolide-resistant strains in sexual networks.[4 19–22] This finding has some implications for clinical care. If macrolide treatment may be conceivable without previous detection of resistance-associated mutations in women, resistance-guided therapy is highly required in men, especially in MSM. Regarding fluoroquinolones, with resistance-associated mutation prevalence ranging between 14.9% and 16.1% with no difference between genders, detection of fluoroquinolone resistance-associated mutations prior to moxifloxacin treatment may be considered but is debatable. To date, the ParC Ser83Ile mutation has been shown to be more common among patients with moxifloxacin treatment failure[12] and is associated with increased MICs.[9] Some moxifloxacin treatment failures were also reported in patients infected by *M. genitalium* harbouring Ser83Arg,[11] Asp87Asn and Asp87Tyr,[13] along with increased MICs of the isolates harbouring these mutations.[10 23] However, a few patients infected by *M. genitalium* strains harbouring ParC mutations likely of clinical significance were reported to clear the infection with moxifloxacin treatment,[24 25] even if this fact might also be attributed to spontaneous clearance of the bacterium.[26 27] In addition, from a technical point of view, amplification and sequencing of fluoroquinolone targets are time-consuming and hardly usable in a routine strategy. Very few commercial kits have been developed to detect mutations in the *parC* gene.[28 29] Kits usually detect groups of mutations at positions Ser83 and Asp87, with no distinction between the ParC alterations likely to be associated with treatment failure, which reduces the clinical interest of their results.

This study reports the first resistance data in overseas France, where the prevalence of *M. genitalium* infection was recently reported to be up to 4.88% in an STI clinic on La Réunion Island.[7] In overseas France, prevalence of macrolide and fluoroquinolone resistance-associated mutations was dramatically lower than that in metropolitan France, with no difference between men and women. The difference in prevalence between metropolitan and overseas France may be attributable to differences in the enrolled population. Whereas the population mean age was similar, women represented 64.2% of enrolled patients in overseas France vs 46.2% ($p<0.001$) of patients enrolled in metropolitan France (table 2). Because in 2019, the prevalence of macrolide resistance-associated mutations was identical in women from metropolitan and overseas France (15.9% vs 15.6%, respectively, table 3), the difference in prevalence was more likely attributable to overseas men who appeared less affected by resistant strains than metropolitan men (52.4% vs 13.5% in 2019, $p<0.001$). A difference in the proportion of MSM may be a parameter as only a few rectal swabs were collected in overseas territories (table 1), reflecting a small number of MSM. Although the guideline for the management of *M. genitalium* infections are the same in metropolitan and overseas France (2016 guideline, updated in 2021), the reduced prevalence of resistance-associated mutations in overseas territories may also be associated with a lower antibiotic selection pressure because of lower accessibility to STI treatments of populations living in remote areas. Interestingly, ParC mutation types were different in metropolitan France and in overseas France. In metropolitan France, 82.0%–89.1% of the ParC mutations were likely of clinical significance, whereas in overseas France, this proportion was only 33.3%–41.2% (table 4). Although these results need to be confirmed in a higher number of patients, this suggests that ParC mutations may be associated with a higher antibiotic selection pressure in metropolitan France, whereas in overseas territories, ParC alterations may more accurately reflect the diversity of single-nucleotide polymorphisms in the *parC* gene. Overall, because of low resistance-associated mutation prevalence and in contrast to the situation in metropolitan France, non-resistance-guided treatment of *M. genitalium* infections may still be used at low risk in overseas France where resistance tests are hardly available. However, it should be noted that a significant increase in the prevalence of macrolide resistance-associated mutations may have occurred between 2018 and 2019 (6.1% vs 14.7%, $p=0.002$). This increasing trend will need to be confirmed and carefully monitored in the future because the potential increased use of azithromycin in STI treatments as well as imported resistance cases may impact prevalence.

The strength of this study is the analysis of 1676 *M. genitalium*-positive samples collected in multiple centres in France and in overseas territories where no national antimicrobial resistance monitoring system exists for *M. genitalium*. The main limitation is the lack of information regarding the sexual orientation of patients. Additionally, all centres did not equally contribute to the number of specimens (online supplemental figure S1) and, in overseas France, La Réunion Island contributed four times as many samples as French Guyana. Mutations in the *gyrA* gene were not searched in this study, but they have been reported at low frequency, have almost never been detected in the absence of ParC mutations, and have not been correlated alone with *M. genitalium* MIC increase or treatment failure.[11 12 18 30]

In conclusion, this study reports the high prevalence of macrolide and fluoroquinolone resistance-associated mutations in *M. genitalium* in metropolitan France and highlights that the prevalence of macrolide resistance-associated mutations differs between genders in link with the proportion of MSM in the male population. The prevalence of resistance-associated mutations is low in overseas France, especially fluoroquinolone resistance-associated mutation prevalence, which is below 3%, but careful monitoring of this mutation prevalence is necessary. Whereas it remains conceivable to treat *M. genitalium* infections with macrolides without prior detection of macrolide resistance-associated mutations in overseas France, resistance-guided treatment is required in metropolitan France, especially in men.

Handling editor Nigel Field

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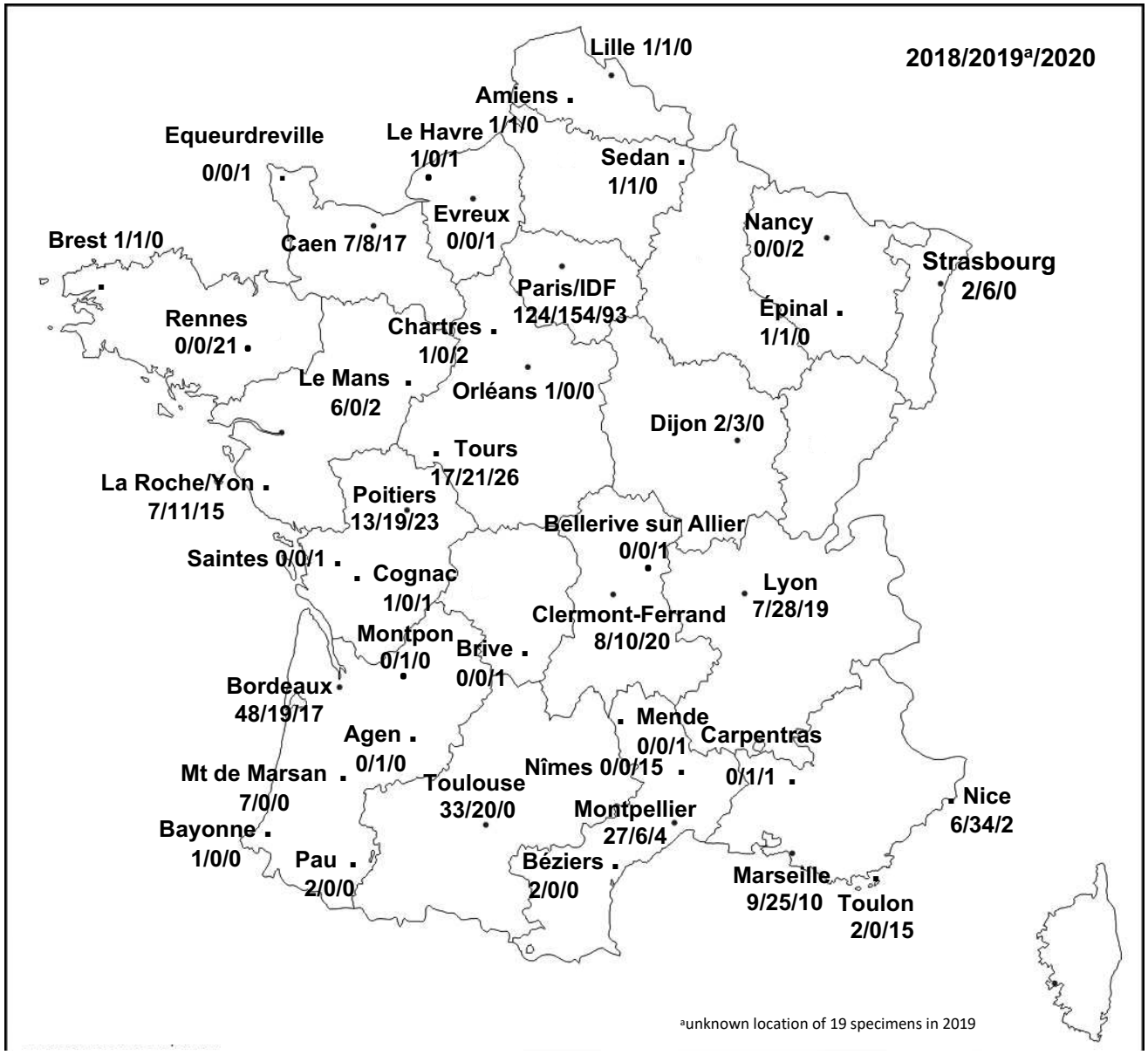
2018/2019[a]/2020
Lille 1/1/0
Amiens 1/1/0
Equeurdreville 0/0/1
Le Havre 1/0/1
Sedan 1/1/0
Evreux 0/0/1
Brest 1/1/0
Caen 7/8/17
Nancy 0/0/2
Strasbourg 2/6/0
Paris/IDF 124/154/93
Épinal 1/1/0
Rennes 0/0/21
Chartres 1/0/2
Le Mans 6/0/2
Orléans 1/0/0
Dijon 2/3/0
Tours 17/21/26
La Roche/Yon 7/11/15
Poitiers 13/19/23
Bellerive sur Allier 0/0/1
Saintes 0/0/1
Cognac 1/0/1
Lyon 7/28/19
Clermont-Ferrand 8/10/20
Montpon 0/1/0
Brive 0/0/1
Bordeaux 48/19/17
Mende 0/0/1
Carpentras 0/1/1
Agen 0/1/0
Nîmes 0/0/15
Mt de Marsan 7/0/0
Toulouse 33/20/0
Nice 6/34/2
Montpellier 27/6/4
Bayonne 1/0/0
Pau 2/0/0
Béziers 2/0/0
Marseille 9/25/10
Toulon 2/0/15
[a]unknown location of 19 specimens in 2019

Abstract

Objectifs

Peu de données sur la résistance aux macrolides et aux fluoroquinolones sont disponibles pour *Mycoplasma genitalium* en France. Nous avons réalisé une étude transversale multicentrique pour déterminer la prévalence des mutations associées à la résistance aux macrolides et aux fluoroquinolones chez des patients positifs à *M. genitalium* en France métropolitaine entre 2018 et 2020 et en Outre-mer entre 2018 et 2019.

Méthodes

Pour chaque année, une collecte prospective d'un mois des échantillons positifs à *M. genitalium* a été proposée aux laboratoires d'analyses en France métropolitaine. Une collecte similaire de 3 mois a été proposée aux laboratoires d'Outre-Mer. Les mutations associées à la résistance ont été détectées avec des trousses commerciales et par séquençage.

Résultats

Un total de 1630 échantillons positifs à *M. genitalium* a été analysé. En France métropolitaine, la prévalence des mutations associées à la résistance aux macrolides était comprise entre 34,7% (intervalle de confiance 95% (IC 95%), 29,4-40,4) et 42,9% (IC 95%, 37,1-49,0) entre 2018 et 2020 et était significativement plus élevée chez les hommes (52,4% to 60,2%) que chez les femmes (15,9% to 22,2%), (p<0,001). Ces prévalences étaient significativement plus élevées que celles observées en Outre-mer en 2018 (6,1%, IC 95%, 3,7-10,3) et 2019 (14,7%, IC 95%, 10,9-19,6) (p<0,001), où aucune différence entre les sexes n'était observée. La prévalence des mutations associées à la résistance aux fluoroquinolones était aussi significativement plus élevée en France métropolitaine (14,9% (IC 95%, 11,2-19,5) à 16,1% (IC 95%, 12,1-21,2)) qu'en Outre-mer (1,3% (IC 95%, 0,4-3,7) et 2,6% (IC 95%, 1,3-5,3) en 2018 et 2019, respectivement) (p<0,001), sans différence entre homme et femme quelle que soit la localisation.

Conclusion

Cette étude rapporte une prévalence élevée des mutations associées à la résistance aux macrolides et aux fluoroquinolones chez *M. genitalium* en France métropolitaine et souligne le contraste avec la faible prévalence de ces mutations observée en Outre-mer. En France métropolitaine, la prévalence des mutations associées à la résistance aux macrolides est trois plus élevée chez hommes que chez les femmes, en rapport avec la proportion d'hommes ayant des relations sexuelles avec les hommes. Ces résultats suggèrent que le genre et les pratiques sexuelles doivent être prises en compte pour la prise en charge des infections à *M. genitalium*.