Original research

An alarming high prevalence of resistance-associated mutations to macrolides and fluoroquinolones in *Mycoplasma genitalium* in Belgium: results from samples collected between 2015 and 2018

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ABSTRACT

**Objectives** The number of reported cases of multiresistant *Mycoplasma genitalium* (MG) is increasing globally. The aim of this study was to estimate the prevalence of macrolide and possible fluoroquinolone resistance-associated mutations (RAMs) of MG in Belgium.

**Methods** The study was performed retrospectively on two sets of MG-positive samples collected in Belgium between 2015 and 2018. The first set of samples originated from routine surveillance activities and the second set came from a cohort of men who have sex with men (MSM) using pre-exposure prophylaxis to prevent HIV transmission. Detection of RAMs to macrolides and fluoroquinolones was performed on all samples using DNA sequencing of the 23S ribosomal RNA gene, the gyrA gene and the parC gene.

**Results** Seventy-one per cent of the MG samples contained a mutation conferring resistance to macrolides or fluoroquinolones (ParC position 83/87). RAMs were more frequently found among men compared with women for fluoroquinolones (23.9% vs 9.1%) and macrolides (78.4% vs 27.3%). Almost 90% of the MG infections among MSM possessed a RAM to macrolides (88.4%). In addition, 18.0% of the samples harboured both macrolides and fluoroquinolone RAMs; 3.0% in men and 24.2% in MSM. Being MSM was associated with macrolide and fluoroquinolone RAMs (OR 15.3), fluoroquinolone RAMs (OR 3.8) and having a possible multiresistant MG infection (OR 7.2).

**Conclusion** The study shows an alarmingly high prevalence of MG with RAMs to macrolides and fluoroquinolones in Belgium. These results highlight the need to improve antimicrobial stewardship in Belgium in order to avoid the emergence of untreatable MG.

INTRODUCTION

*Mycoplasma genitalium* (MG) is an emerging STI and its global burden of disease is not very well known so far.1

Prevalence varies among different risk populations—and is generally higher in men who have sex with men (MSM) and female sex workers compared with other heterosexual individuals. A systematic review and meta-analysis up to 2017 found the prevalence of MG to be 0.9% in pregnant women, 3.2% in MSM and 15.9% in female sex workers.2 The prevalence of MG in MSM, is, however, now reaching 10% according to the most recent publications.3–6

MG is a cause of non-gonococcal urethritis in men and cervicitis in women, but MG is also frequently found in asymptomatic individuals.3 The natural history and clinical consequences of asymptomatic MG infections are still poorly understood and including MG in screening programmes among high-risk groups is, therefore, not recommended. Furthermore, along with *Neisseria gonorrhoeae* (NG), MG is one of the STIs that can acquire resistance to different classes of antibiotics at an alarmingly rapid rate.7 In fact, MG cases with macrolide-resistant-associated mutations (RAMs) have been reported globally, and prevalence of these mutations is exceeding 80% in MSM using pre-exposure prophylaxis (PrEP) to prevent HIV.3–4

As a result, guidelines mainly suggest testing and treating for MG in cases of non-gonococcal, non-chlamydia urethritis or cervicitis. Moreover, MG-positive samples should be further analysed for the presence of macrolide RAMs to tailor patient treatment and management.7

European guidelines recommend the use of moxifloxacin, a fourth-generation fluoroquinolone, in cases where macrolide treatment failure is detected.7 The prevalence of resistance to fluoroquinolones has been noted to be increasing in a number of regions. A recent systematic review from Europe estimated that the prevalence of fluoroquinolone resistance was 5% in this region, but there were considerable gaps in the data, such as from Belgium.8

With this study, we aimed to estimate the prevalence of RAMs to macrolides and fluoroquinolones in MG samples received by the Belgian National Reference Centre (NRC) from 2015 to 2018. In addition, we explored risk factors associated with the presence of RAMs in order to inform clinical and testing practice.

METHODS

**Samples**

Since 2015, genital, anorectal or urine samples from patients with clinical suspicion of MG could be sent to the NRC, located at the Institute of
Tropical Medicine (ITM), Antwerp for MG identification (hereafter referred to as surveillance samples). Sociodemographic and clinical data, such as age, postal code, gender, country of birth, sexual orientation, HIV status, presence of symptoms and presence of other STIs is requested for each case.

Samples from a PrEP demonstration study that was ongoing at the ITM from 2015 to 2018 were also included. The Be-PrEP-ared study was a single site, open-label PrEP demonstration study which included 200 HIV-negative MSM.9,10 A total of 179 HIV-negative individuals were followed up for a period of 18 months and STI screening, including MG detection, was performed quarterly at the three anatomical sites (ie, urethra, anorectum and pharynx).10

For this retrospective study, both surveillance and study samples from 01 January 2015 to 31 December 2018 were included.

**Laboratory procedures**

MG detection was routinely performed at the NRC with an accredited in-house RT-PCR that targets the *pbhD* gene until 30 September 2018.12 After this, MG detection was performed using the S-DiaMGTV multiplex kit, according to manufacturer’s instructions (Diagene Diagnostics, Seraing, Belgium) on the m2000rt platform (Abbott Molecular Des Plaines, Illinois, USA). DNA extraction of all samples was performed using the Abbott *m2000sp* instrument and CT/NG extraction kit. Sample remnants and DNA extracts were stored below −20°C.

Sanger sequencing of the 23S rRNA, *parC* and *gyrA* genes was performed on the ABI 3670xl instrument of Applied Biosystems (USA) as described previously to detect the presence of RAMs to macrolides and fluoroquinolones.13 14 Mutations found in region V of the 23S rRNA gene, *parC* (nucleotides 164–483) and *gyrA* gene (nucleotides 172–402) are provided according to MG numbering. Samples with a silent mutation (without an alteration in amino acid) are not reported as mutations.

**Statistical analysis**

All data analyses were performed using STATA V.15.1. MG was defined as resistant to macrolides if a RAM in the 23S rRNA region V was detected. MG possessing an alteration in ParC at position 83 or 87 was defined as resistant to fluoroquinolones. MG was defined as multiresistant if mutations in both genes (23S rRNA and ParC position 83/87) were detected.

Prevalence of antibiotic resistance is estimated on samples of the MG cases with ParC mutations, and although this alteration is considered a true resistance mutation, it is not shown in the tables. Moreover, MG is considered susceptible to macrolides if a RAM was not detected in the macrolide resistance region of the 23S rRNA, and susceptible to fluoroquinolones.

Presence of possible resistance-associated mutations

Sequencing for both macrolide and fluoroquinolone RAMs was successful for 214 samples. Identified mutations and their resulting amino acid changes are shown in [table 2](#) for the 23S rRNA, *gyrA* and *parC* gene.

More than 80% of all MG infections (n=177/214; 82.7%; 95% CI 77.0 to 87.5) presented with a possible RAM to macrolides or fluoroquinolones. This applied to almost 90% of the samples collected from men (87.3%; 95% CI 81.5 to 91.8). Women were more likely to have no RAMs (42.4%) compared with men (12.7%) (OR 5.06; 95% CI 2.03 to 12.3; p<0.0001).

**Macrolide RAMs**

Mutations conferring macrolide resistance at nucleotide position 2071 or 2072 (corresponding to position 2058 or 2059 based on *Escherichia coli* numbering) of the 23S rRNA gene were detected in almost 75% of the samples (74.3%; n=159/214).

**All possible fluoroquinolone RAMs**

A total of 85 samples (39.7%) presented with possible RAMs in the *gyrA* gene or the *parC* gene ([table 2](#)). Only seven samples showed a mutation in the *gyrA* gene, and five of these samples possessed an additional mutation in ParC (amino acid position 83). In total, almost 40% of the samples had a mutation in the *parC* gene (38.8%; n=83/214), and of those, 66.3% (n=55/83) had a mutation at ParC location 83 or 87. Beside these two positions, mutation P62S (C184T) was found in 31.3% (n=26/83) of the MG cases with ParC mutations, and although this alteration was found in both genders, it was more present in women (27.3% vs 9.4%).

**Presence of RAMs to both antibiotics**

Almost one-third of the samples (31.3%; n=67/214; 95% CI 25.2 to 38.0) harboured possible RAMs to both macrolides and fluoroquinolones. A2071G/S83I was the most frequent combination (31.3%; n=21/67), followed by A2072G/S83I (19.4%; n=13/67) and A2071G/P62S (16.4%; n=11/67).

**Multiple MG episodes in individuals**

Online supplementary annex 1 documents the presence of RAMs in 48 individuals that experienced multiple MG episodes.
over the 4 years including antimicrobial treatment and RAMs if available. They contributed to 104 additional MG episodes and 23 of them were categorised as a new MG episode.

Six participants (included in the Be-PrEP-ared study) remained positive at every 3 monthly visit for 18 months and therefore none of these MG infections was considered to be new MG episodes. Nevertheless, in three of those participants, infection with different MG genotypes was found.

Microbiological failure (remaining positive to MG within 4 months and harbouring a mutation against the provided therapy) was documented in nine cases in eight individuals: six A2071G; one A2072G and two S83I mutations. We could not detect any de novo mutations.

Prevalence of antimicrobial resistance and associations between antimicrobial resistance and sociodemographic determinants

Due to the unknown relevance of the mutations found in GyrA and ParC, the exploration of risk factors for having fluoroquinolone resistance or multiresistance was conducted on samples which harboured a mutation in ParC at position 83 and 87.

In addition, we only included samples of a new MG episode. Of the 235 MG samples that were categorised as a new MG infection, sequencing for both 23S rRNA and parC gene was successful for 167 samples.

The prevalence of the different MG genotypes and the sociodemographic characteristics are presented in Table 3.

Table 1  Sociodemographic characteristics of the study population

|                  | All participants | Women | Men |
|------------------|------------------|-------|-----|
|                  | No (n=212)       | No (n=64) | No (n=148) |
| Median age (IQR) | 32               | 26–40 | 26  | 23–32.5 | 35 | 28–42 |
| Source           |                  |       |     |         |    |      |
| Surveillance     | 142              | 67.0  | 64  | 100     | 78 | 52.7 |
| Be-PrEP-ared     | 70               | 33.0  | 0   | 0       | 70 | 47.3 |
| Place of residence |                |       |     |         |    |      |
| Brussels         | 47               | 22.2  | 2   | 3.1     | 45 | 30.4 |
| Flanders         | 128              | 60.4  | 36  | 56.3    | 92 | 62.2 |
| Wallonia         | 37               | 17.5  | 26  | 40.6    | 11 | 7.4  |
| MSM              |                  |       |     |         |    |      |
| No               | 80               | 37.7  | 64  | 100     | 16 | 10.8 |
| Yes              | 97               | 45.8  | 0   | 0       | 97 | 65.5 |
| Unknown          | 35               | 16.5  | 0   | 0       | 35 | 23.7 |
| HIV positive     |                  |       |     |         |    |      |
| No               | 117              | 55.2  | 15  | 23.4    | 102| 68.9 |
| Yes              | 15               | 7.1   | 1   | 1.6     | 14 | 9.5  |
| Unknown          | 80               | 37.7  | 48  | 75.0    | 32 | 21.6 |
| Positive for *Mycoplasma genitalium* at more than one visit | | | | | |
| No               | 164              | 77.4  | 63  | 98.4    | 101| 68.2 |
| Yes              | 48               | 22.6  | 1   | 1.6     | 47 | 31.8 |
| Number of visits |                  |       |     |         |    |      |
| 2                | 27               | 12.7  | 1   | 100     | 26 | 55.3 |
| 3                | 19               | 3.3   | 3   | 6.5     | 7  | 14.9 |
| 4                | 6                | 2.8   | 6   | 12.8    |    |      |
| 5                | 1                | 0.5   | 1   | 2.1     |    |      |
| 6                | 1                | 0.5   | 1   | 2.1     |    |      |
| 7                | 6                | 2.8   | 0   | 0.5     |    |      |
| MSM              |                  |       |     |         |    |      |

MSM, men who have sex with men.

The number of macrolide-resistant MG cases were significantly higher in men (78.4%; 95% CI 70.4 to 85.0) than in women (27.3%; 95% CI 13.3 to 45.5; p<0.0001) (Table 3).

This difference in gender was also seen in fluoroquinolone-resistant MG cases (23.9% vs 9.1%, p=0.092), however, to a lesser extent and not statistically significant. The prevalence of multiresistant MG cases was remarkably higher in men (21.6%; 95% CI 15.0 to 29.6) than in women (3.0%; 95% CI 0.8 to 15.8; p=0.010). Furthermore, being MSM increased the prevalence of all antibiotic-resistant MG genotypes. Almost one quarter (24.2%; 95% CI 16.0 to 34.1, p=0.002) of the MG samples from MSM were multiresistant, and almost 9 out of 10 infections among MSM had a mutation in the 23S rRNA gene (88.4%; 95% CI 80.2 to 94.1, p<0.0001). Being of male gender and not MSM markedly decreased the presence of macrolide (53.9%; n=21/39) and fluoroquinolone resistance (18.0%; n=7/39). The number of wild-type MG samples increased to 43.6% (n=17/39), whereby the number of multiresistant MG cases dropped to 15.4% (n=6/39) (data not in table).

Being MSM was the only factor associated with quinolone resistance (OR 3.84; 95% CI 1.20 to 16.09; p=0.013) or having a multiresistant MG infection (OR 7.19; 95% CI 1.63 to 65.16; p=0.0033). Having an MG infection with macrolide resistance was besides being associated with being male and MSM, also associated with an anorectal site of infection.

Multivariate analyses however showed that being MSM was the only factor associated with a higher probability of having
### Table 2  Presence of *Mycoplasma genitalium* macrolide and possible fluoroquinolone resistance-associated mutations (RAMs) in Belgium based on *M. genitalium* numbering

| RAMs detected | All samples | Female samples | Male samples |
|---------------|-------------|----------------|--------------|
| Polymorphism (mutation) | No. (n=214) | % (95% CI) | No. (n=33) | % (95% CI) | No. (n=181) | % (95% CI) |
| **Macrolide RAMs (Escherichia coli numbering in parentheses)** | | | | | | |
| Wild type | 55 | 25.7 (20.0 to 32.1) | 24 | 72.7 (54.5 to 86.7) | 31 | 17.1 (11.9 to 23.4) |
| Mutations detected | 159 | 74.3 (67.9 to 80.0) | 9 | 27.3 (13.3 to 45.5) | 150 | 82.9 (76.6 to 88.1) |
| 23S rRNA gene region V | | | | | | |
| A0271G (A2058G) | 65 | 30.4 | 4 | 12.1 | 61 | 33.7 |
| A0271T (A2058T) | 8 | 3.7 | 1 | 3.0 | 7 | 3.9 |
| A0272G (A2059G) | 86 | 40.2 | 4 | 12.1 | 82 | 45.3 |
| **Possible fluoroquinolone RAMs** | | | | | | |
| Wild type | 129 | 60.3 (53.5 to 66.7) | 20 | 60.6 (43.3 to 75.6) | 109 | 60.2 (52.9 to 67.1) |
| Mutations detected | 85 | 39.7 (33.3 to 46.5) | 13 | 39.4 (24.4 to 56.7) | 72 | 39.8 (32.9 to 47.1) |
| **gyrA gene** | | | | | | |
| Wild type | 131* | 61.2 (54.3 to 67.8) | 20 | 60.6 (42.1 to 71.7) | 111 | 61.3 (53.8 to 68.4) |
| Missense mutations detected | 83 | 38.8 (32.2 to 45.7) | 13 | 39.4 (22.9 to 57.9) | 70 | 38.7 (31.5 to 46.2) |
| Mutations at 83 or 87 | 55 | 25.7 (20.0 to 32.1) | 3 | 9.1 (1.9 to 24.3) | 52 | 28.7 (22.3 to 35.9) |
| S83I (G248T) | 38§ | 17.8 | 1 | 3.0 | 37 | 20.4 |
| S83N (G248A) | 4 | 1.9 | 1 | 3.0 | 3 | 1.7 |
| S83R (A247C) | 1¶ | 0.5 | 0 | 0 | 1 | 0.6 |
| D87N (G259A) | 4 | 1.9 | 1 | 3.0 | 3 | 1.7 |
| D87Y (G259T) | 8 | 3.8 | 0 | 0 | 8 | 4.4 |
| Missense mutations at other locations in parC | | | | | | |
| D82N (G244A) | 1¶ | 0 | 0 | 1 | 0.6 |
| P62S (C184T) | 26** | 9 | 27.3 | 17 | 9.4 |
| A118E (C353A) | 1 | 1 | 3.0 | 0 | 0 |
| Silent mutations | 3 | 1 | 3.0 | 2 |
| G75S (G225A) | 1 | 1 | 0 | 0 |
| H78N (G234T) | 1 | 0 |
| V112V (G336A) | 1 | 0 |

*Ten samples were PCR negative and could not be sequenced for the gyrA gene.
†Samples presented an additional mutation in ParC S83 in three of the samples and S83N in two samples.
‡Three silent mutations were not counted as RAM and are categorized as wild type MG.
§Twenty-one samples had an additional silent mutation (H78N (G234T)).
¶One sample had an additional mutation P62S (C184T).
**Two samples presented an additional silent mutation (T16 H78N (G234T) and T199N (C297T)).
### Table 3: Sociodemographics and presence of the different *Mycoplasma genitalium* genotypes

| Total | Wild type | Macrolide resistant (23S rRNA) | Fluoroquinolone resistant (ParC pos. 83/87) | Multiresistant (23S rRNA+ParC pos. 83/87) |
|-------|-----------|-------------------------------|---------------------------------|---------------------------------|
|       | No. | %       | (95% CI) | No. | %       | (95% CI) | No. | %       | (95% CI) | No. | %       | (95% CI) |
| Year   |      |          |          |      |          |          |      |          |          |      |          |          |
| 2015–2016 | 51 | 30.5    |          | 45 | 88.2  | (76.1 to 95.6) | 12 | 23.5  | (12.8 to 37.5) | 5 | 9.4  | (5.1 to 20.7) |
| 2017   | 63 | 37.7    |          | 43 | 68.3  | (59.3 to 79.4) | 16 | 25.4  | (15.3 to 37.9) | 5 | 9.4  | (5.1 to 20.7) |
| 2018   | 53 | 31.7    |          | 47.2 | (33.3 to 61.4) | <0.0001 | 26 | 49.1  | (35.7 to 63.2) | 7 | 13.2  | (5.5 to 23.9) |
| Source of sampling |     |          |          |     |          |          |     |          |          |     |          |          |
| Surveillance | 94 | 56.3    |          | 50 | 53.2  | (46.6 to 63.6) | 16 | 17.0  | (10.1 to 26.2) | 5 | 9.4  | (5.1 to 20.7) |
| Be-PreP-aned study | 73 | 43.7    |          | 64 | 87.7  | (77.9 to 94.2) | 19 | 26.0  | (16.5 to 37.6) | 18 | 24.7  | (15.3 to 36.1) |
| Gender |     |          |          |     |          |          |     |          |          |     |          |          |
| Women  | 33 | 19.8    |          | 9  | 27.3  | (13.3 to 45.5) | 3  | 9.1   | (19.2 to 24.3) | 1  | 3.0   | (0.8 to 15.8) |
| Men    | 134 | 80.2   |          | 26 | 19.4  | (13.1 to 27.1) | 105 | 78.4  | (70.4 to 85.0) | 29 | 21.6  | (5.0 to 24.9) |
| Sexual orientation |     |          |          |     |          |          |     |          |          |     |          |          |
| Hetero | 47 | 28.1    |          | 15 | 31.9  | (19.1 to 47.1) | 4  | 8.5   | (2.4 to 26.4) | 2  | 4.3   | (0.5 to 14.5) |
| MSM    | 95 | 56.9    |          | 84 | 88.4  | (80.2 to 94.1) | 25 | 26.3  | (17.8 to 36.4) | 23 | 24.2  | (5.0 to 34.1) |
| Unknown | 25 | 15.0   |          | 15 | 60.0  | (38.7 to 78.9) | 6  | 24.0  | (9.4 to 45.1) | 5  | 20.0  | (6.8 to 40.7) |
| HIV status |     |          |          |     |          |          |     |          |          |     |          |          |
| Negative | 109 | 64.7  |          | 84 | 73.8  | (68.8 to 85.2) | 22 | 20.4  | (12.2 to 32.2) | 21 | 19.4  | (9.5 to 29.2) |
| Positive | 13 | 7.8    |          | 0  | 0.0  | (0.0 to 0.0) | 12 | 92.6  | (90.0 to 95.2) | 4  | 30.8  | (16.5 to 53.8) |
| Unknown | 46 | 27.5    |          | 18 | 39.1  | (25.1 to 54.6) | 9  | 19.6  | (9.4 to 33.9) | 6  | 13.0  | (4.9 to 26.3) |
| Type of visit |     |          |          |     |          |          |     |          |          |     |          |          |
| Initial visit | 151 | 71.3  |          | 102 | 67.6  | (59.5 to 74.9) | 30 | 19.9  | (13.8 to 27.1) | 27 | 17.3  | (12.1 to 24.9) |
| Return visit* | 16 | 28.7  |          | 12 | 12.5  | (6.6 to 21.9) | 5  | 31.3  | (16.0 to 51.5) | 2  | 12.5  | (5.0 to 24.9) |
| Site of infection |     |          |          |     |          |          |     |          |          |     |          |          |
| Genital | 118 | 70.7  |          | 73 | 61.9  | (52.5 to 70.6) | 24 | 20.3  | (13.5 to 28.7) | 19 | 16.1  | (10.0 to 24.0) |
| Anorectal | 45 | 27.0  |          | 38 | 84.4  | (75.5 to 93.5) | 10 | 22.2  | (11.2 to 37.1) | 10 | 22.2  | (11.2 to 37.1) |
| Pharyngeal | 3  | 1.8    |          | 3  | 1.00  | (0.0 to 0.0) | 1  | 33.3  | (0.8 to 90.6) | 1  | 33.3  | (0.8 to 90.6) |
| Unknown | 1  | 0.6    |          | 0  | 0.0  | (0.0 to 0.0) | 0  | 0.0  | (0.0 to 0.0) | 0  | 0.0  | (0.0 to 0.0) |
| STI-associated symptoms |     |          |          |     |          |          |     |          |          |     |          |          |
| No    | 65 | 38.9    |          | 53 | 81.5  | (74.0 to 89.0) | 14 | 21.5  | (12.3 to 32.5) | 14 | 21.5  | (12.3 to 32.5) |
| Yes   | 53 | 31.7    |          | 36 | 67.6  | (59.3 to 76.1) | 132 | 22.6  | (12.3 to 36.2) | 10 | 18.9  | (4.0 to 32.4) |
| Unknown | 49 | 29.3   |          | 25 | 51.0  | (36.3 to 65.6) | 9  | 18.4  | (8.8 to 32.0) | 6  | 12.6  | (4.6 to 24.0) |
| STI coinfections |     |          |          |     |          |          |     |          |          |     |          |          |
| No STI coinfection | 92 | 55.1  |          | 73 | 79.4  | (69.6 to 87.1) | 21 | 22.8  | (14.7 to 32.8) | 19 | 20.7  | (12.9 to 30.4) |
| Any STI coinfection | 25 | 15.0  |          | 20 | 80.0  | (59.3 to 92.3) | 1.00 | 24.0  | (9.4 to 45.1) | 4  | 16.0  | (4.5 to 36.1) |
| Unknown | 50 | 29.9   |          | 21 | 42.0  | (32.8 to 56.8) | 8  | 16.0  | (7.2 to 29.1) | 7  | 14.0  | (5.8 to 26.7) |

* Associations were calculated using Fisher’s exact test.
* P-values in bold are <0.05 so statistically significant.
* All samples from return visits were from MSM.
* Concurrent STI infections were found in 25 participants.
* MSM, men who have sex with men.
an MG infection with macrolide resistance (adjusted OR 15.33; 95% CI 3.52 to 66.77; p<0.0001).

DISCUSSION
A recent systematic review of macrolide and fluoroquinolone resistance in MG found that global macrolide resistance increased to 51.4% by 2017, while fluoroquinolone resistance remained stable at around 8%. In Europe, the fluoroquinolone resistance prevalence is now estimated to be 5%. We found considerably higher rates of resistance in Belgium. In fact, <30% of the MG strains analysed were wild type. Almost 20% (18.0%) had RAMs to both macrolides and fluoroquinolones, and this was particularly prevalent in MSM—24.2%. Previous reports from Belgium reported a lower prevalence of macrolide RAMs among female sex workers (6.9%) and MSM (44%). It should be noted that in both studies another technique than the gold standard Sanger sequencing was used.

While MG resistance has been described as occurring in MSM, the prevalence of RAMs in low-risk populations has not often been studied. The present study shows that RAMs to macrolides or fluoroquinolones are also prevalent in lower risk populations, such as women (33.3%) and heterosexual men (56.4%), but to a lesser extent than in MSM (90.5%). This may be explained by the relatively high consumption of both macrolides and fluoroquinolones in the general population in Belgium.

In MSM, almost 9 out of 10 MG infections were resistant to macrolides. An important driver of macrolide use in high STI-prevalence populations, such as PrEP cohorts, are chlamydia and gonorrhoea infections, where single dose azithromycin (alone or in combination) is a recommended therapy. Individuals with chlamydia or gonorrhoea are frequently co-infected with MG (as was the case for 20% in our study), which increases the probability of bystander antimicrobial selection pressure and which, in part, may explain the high prevalence of macrolide RAMs found in MSM. We could however not detect any de novo mutations, probably due to the already high prevalence of antimicrobial resistance in MSM.

The present study also shows that asymptomatic MG infections are prevalent, but that most of them (81.5%) presented with RAMs for macrolides or fluoroquinolones. One of the most important criteria to screen and treat patients with asymptomatic infectious disease is the availability of an effective treatment to decrease disease prevalence. These results add up to the evidence that screening for MG and treatment of asymptomatic MG cases should be discouraged.

Treatment options for symptomatic infections with possible combined macrolide-resistant and fluoroquinolone-resistant MG are extremely limited. Pristinamycin is listed as third-line treatment in the International Union against STI (IUSTI) treatment guidelines, however, this treatment is difficult to obtain in Belgium. The current European IUSTI MG treatment guidelines advocate azithromycin as first-line therapy. The high prevalence of macrolide RAM in Belgium suggests that this approach is suboptimal. An alternative approach would be to follow the Melbourne protocol of treating MG infections with doxycycline for 7 days while awaiting test results for macrolide resistance. In the absence of macrolide resistance, azithromycin is administered, but if macrolide resistance is found then sitafloxacin/ moxifloxacin is given.

While this protocol resulted in high cure rates and very low risk of genesis of macrolide resistance, our study results show that almost all MSM had a mutation conferring macrolide resistance, which is in line with other results. Therefore, the detection of fluoroquinolone RAMs seems more relevant than the detection of macrolide resistance in this population.

In contrast to the commercial availability of several assays detecting macrolide RAMs in MG, assays detecting fluoroquinolone RAMs are sparse. There are different mutations in the quinolone resistance determining region of the parC and gyrA gene that have been associated with fluoroquinolone resistance. In our study, only seven samples presented with a Gyrase mutation and five of them coincided with a ParC mutation at amino acid position 83, which seems to lead to high-level resistance. The two other alterations found (M69I and A79V) have not yet been correlated with clinical resistance. Due to this low number of mutations, the role of mutations in Gyrase seems of less importance in MG. The ParC gene is more susceptible for mutations; in our study, most of the mutations were found at position 83 and 87, the ‘hot spots’ for fluoroquinolone resistance (n=55/83; 66.2%). Besides these two positions, an alteration at position 62 (P62S) in the ParC was detected in 32.9% of the samples with fluoroquinolone RAMs. This alteration has been previously described, however, clinical resistance has not yet been determined.

Our study has several limitations. First, the sampling methodologies may have introduced biases towards populations at higher risk for antimicrobial resistance, such as MSM with higher consumption of antimicrobials. Second, microbiological cure with moxifloxacin despite mutations at position 83/87 are documented, therefore the degree of fluoroquinolone resistance may be overestimated. Third, the estimation of antimicrobial resistance of MG has been made on new MG episodes only. Yet, we document that patients could become infected within 6 months with another MG genotype, which may be either explained by a true re-infection with another MG strain or by a mixed infection with multiple MG strains. Therefore, prevalence mentioned here may be inaccurate. Fourth, clinical data including antimicrobial treatment were lacking for many of the surveillance cases, and finally, no test-of-cure within 14 days of treatment was performed. It is therefore unclear whether the infection was new or persistent. However, we tried to correct for this bias, by only including new MG infections that are detected 6 months after the previous MG infection.

In conclusion, we found high rates of macrolide and possible fluoroquinolone RAMs in MG. These results provide further motivation to promote antimicrobial stewardship in the general population, but particularly among high STI prevalence populations such as PrEP cohorts. New treatment guidelines are urgently required that incorporate genotypic resistance testing for macrolides and fluoroquinolones. Finally, because of the high number of multiresistant cases found in this study, new

Key messages
► Our study reports an alarmingly high prevalence of Mycoplasma genitalium (MG) antimicrobial resistance to either macrolides or fluoroquinolones in Belgium (71.3%).
► Multiresistant MG was found in almost 20% of cases, however, the prevalence of multiresistant MG was remarkably higher in men (21.6% vs 3.0%).
► The number of MG-resistant cases was much higher in men who have sex with men compared with heterosexual men and women.
► These results highlight the need to improve antimicrobial stewardship in Belgium.
antimicrobials are urgently needed to treat patients with MG resistance to both macrolides and fluoroquinolones.

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Ethics approval The Be-PrEP-ared study was approved by the Ethics Committee of the University of Antwerp and the Institutional Review Board of the ITM. All individuals provided informed consent. Supplementary ethics approval was obtained for the detection of RAMs in MG-positive samples for both the Be-PrEP-ared study (988/15 and 15/25/255) and the surveillance samples (1374/20).

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Data availability statement Data are available on reasonable request. The data supporting the findings of this publication are retained at the Institute of Tropical Medicine (ITM), Antwerp and will not be made openly accessible due to ethical and privacy concerns. According to the ITM research data sharing policy, only fully anonymised data can be shared publicly. The surveillance data are de-identified (using a unique patient code) but not fully anonymised and it is not possible to fully anonymise them due to the longitudinal nature of the data. Data can however be made available after approval of a motivated and written request to the ITM at ITM recherche/dataaccess@itmit.be. The ITM data access committee will verify if the dataset is suitable for obtaining the study objective and will assure that confidentiality and ethical requirements are in place.

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