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Lower *mgpB* diversity in macrolide-resistant *Mycoplasma genitalium* infecting men visiting two sexually transmitted infection clinics in Montpellier, France

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Objectives: Men engaged in high-risk sexual behaviour, such as MSM, are likely to be infected by resistant *Mycoplasma genitalium* strains. Understanding the transmission dynamics is challenging. We aimed to investigate the molecular epidemiology of *M. genitalium* in men visiting sexually transmitted infection (STI) clinics.

Patients and methods: Between June 2017 and February 2018, 95 *M. genitalium*-positive specimens from 78 men, including 76.9% MSM, visiting two STI clinics in Montpellier, France, were analysed for SNPs in the *mgpB* adhesin gene and number of tandem repeats in the *MG_309* gene. Macrolide and fluoroquinolone resistance were determined. Typing results were compared with antibiotic resistance, sexual behaviour, sampling site, HIV pre-exposure prophylaxis (PrEP) usage and HIV status.

Results: Thirty-eight *mgpB* STs were identified, including 23 new STs, with ST4 being most prevalent. The *mgpB*/*MG_309* typing method identified 52 genetic profiles, resulting in a discriminatory index of 0.979. Macrolide and fluoroquinolone resistance-associated mutations were detected in 58.3% and 10.8% of patients, respectively. The macrolide resistance rate was higher among MSM than among men who have sex with women only (68.4% versus 9.1%; adjusted OR, 1.57; 95% CI, 1.13–2.18; $P = 0.007$). A lower *mgpB* diversity of 0.870 was found among macrolide-resistant strains in comparison with 0.978 in macrolide-susceptible strains, with an over-representation of *mgpB* ST62 and ST153.

Conclusions: Although macrolide resistance spread appears polyclonal in *M. genitalium*, the lower diversity of *mgpB* types among macrolide-resistant strains may reflect the easier spread of a few specific *mgpB* types or the occurrence of sexual networks among MSM.

Introduction

Mycoplasma genitalium is a sexually transmitted pathogen that has recently been added to the 'watch list' of CDC antibiotic resistance threats because of a worrying increase in macrolide and fluoroquinolone resistance worldwide.[1–4] People who engage in high-risk sexual behaviour and who have a high exposure to antibiotics, such as MSM, HIV pre-exposure prophylaxis (PrEP) users and HIV-positive patients, are more likely to be infected by resistant *M. genitalium*.[4–7] In these populations, elucidating the transmission dynamics is challenging. Epidemiological studies of *M. genitalium* genotypes are mainly based on *mgpB* adhesin gene SNP typing analyses,[8,9] which have suitable discriminatory power, reproducibility and stability. Combination analysis with a variable-number tandem-repeat (VNTR) marker in the *MG_309* locus is useful to investigate transmission networks.[10–12] We investigated the molecular epidemiology of *M. genitalium* in men visiting sexually transmitted infection (STIs) clinics, using both the *mgpB* and *MG_309* typing methods, and compared the typing results with the occurrence of macrolide and fluoroquinolone resistance-associated mutations and the sexual behaviours of patients.

Patients and methods

This retrospective study included men who attended two STI clinics in Montpellier, France, between June 2017 and February 2018: the free information centre for screening and diagnosis of STI (CeGIDD) and the infectious and tropical disease department of University Hospital of Montpellier. Overall, 95 *M. genitalium*-positive samples from 78 men were studied, including 15 follow-up samples from 13 patients (Table 1) and two concurrent samples from two patients (Figure 1). Specimens included 60% (57/95) urines, 37.9% (36/95) rectal swabs and 2.1% (2/95) throat swabs. The population included 76.9% (60/78) MSM, 24.4% (19/78) HIV-positive patients and 20.5% (16/78) PrEP users (Figure 1).

Remnants of *M. genitalium*-positive specimens detected using the S-DiaMGTV kit (Diagenode Diagnostics, Belgium) were stored at −80°C. Macrolide and fluoroquinolone resistance-associated mutations were detected in the 23S rRNA and *par*C gene, respectively.[13] Molecular typing was performed by combining the characterization of SNPs in a 281 bp fragment of the *mgpB* adhesin gene[8–12] and VNTR in the putative lipoprotein gene *MG_309*.[10,14] Nucleotide sequences of the *mgpB* gene were analysed using MEGA (version 7.0) and compared with that of the reference *M. genitalium* G37 strain and with the 168 *mgpB* sequences previously described.[12,15] Genetic data were analysed using FSTAT software,[16] and the discriminatory index was calculated.[10] Age, sexual behaviour, sampling site, PrEP usage and HIV status were collected anonymously.

Results

Overall, 38 *mgpB* STs were identified (Figure 1), with a gene diversity of 0.927, and 23 new types were deposited in GenBank

Table 1. Comparison of the genetic and resistance profile of *M. genitalium* collected from 13 patients in first and follow-up samples

					Genetic characterization			Resistance profile	
Patient no.	Sample no.	Sampling site	Sampling date	Time between samples (days)	GenBank no. of *mgpB* type[a]	*mgpB* type	*MG_309* type[b]	*23S rRNA* mutation (*Escherichia coli* numbering)	*ParC* mutation (*M. genitalium* numbering)
232	232-T1-U	urine	28 July 2017	87	MN387732	153	15	A2058G	WT
	232-T2-U	urine	23 Oct 2017		MN387732	153	15	A2058G	WT
234	234-T1-U	urine	2 Aug 2017	92/90	GU226227	**25**	**10**	**WT**	**WT**
	234-T2-U	urine	2 Nov 2017		GU226206	**3**	**13**	**A2058G**	**Ser83Ile**
	234-T3-U	urine	31 Jan 2018		GU226206	3	13	A2058G	Ser83Ile
239	239-T1-U	urine	1 Aug 2017	85	GU226205	2	10	NA	Ser83Ile
	239-T2-U	urine	25 Oct 2017		GU226205	2	10	A2059G	WT
244	244-T1-A	rectum	4 Sept 2017	42	GU226207	4	NA	NA	nd
	244-T2-A	rectum	16 Oct 2017		GU226207	4	9	A2059G	WT
246	246-T1-A	rectum	28 Jun 2017	216	GU226207	4	14	A2059G	WT
	246-T2-A	rectum	30 Jan 2018		GU226207	4	14	A2059G	WT
255	255-T1-U	urine	22 Sept 2017	36/97	MT327190*	175	9	A2058G	WT
	255-T2-U	urine	28 Oct 2017		MT327190*	175	9	A2058G	WT
	255-T3-U	urine	2 Feb 2018		MT327190*	175	9	NA	nd
259	259-T1-U	urine	13 Jun 2017	170	MN387732	153	12	A2058G	WT
	259-T2-U	urine	30 Nov 2017		MN387732	153	12	A2058G	WT
263	263-T1-A	rectum	30 Oct 2017	93	MT327181*	166	8	A2059G	WT
	263-T2-A	rectum	2 Feb 2018		MT327181*	166	8	A2059G	WT
266	266-T1-U	urine	3 Jan 2018	42	MT327195*	180	13	NA	Nd
	266-T2-U	urine	14 Feb 2018		MT327195*	180	13	A2059G	Ala119Pro[c]
278	278-T1-U	urine	27 Sept 2017	112	MT327184*	169	12	A2059G	WT
	278-T2-U	urine	17 Jan 2018		MT327184*	169	12	A2059G	WT
283	283-T1-U	urine	5 July 2017	29	KU856547	108	13	A2059G	Ser83Ile
	283-T2-U	urine	3 Aug 2017		KU856547	108	13	A2059G	Ser83Ile
289	289-T1-U	urine	6 Jun 2017	65	FJ750828	**62**	**15**	A2059G	WT
	289-T2-U	urine	10 Aug 2017		FJ750828	**62**	**13**	A2059G	WT
82	82-T1-A	rectum	18 Oct 2017	105	GU226207	4	NA	A2059G	WT
	82-T2-A	rectum	31 Jan 2018		GU226207	4	NA	NA	nd

NA, no amplification; nd, not determined.
*New *mgpB* genotypes reported in this study were numbered from 161 to 183 (accession numbers MT327176 to MT327198).
Changes of genotype and/or resistance profile between first and follow-up samples from the same patient are in bold.
[a]GenBank accession numbers are those assigned to the corresponding *mgpB* adhesin gene fragment.
[b]Number of tandem repeats in MG_309.
[c]The Ala119Pro mutation is far from the QRDR and is probably not associated with fluoroquinolone resistance.

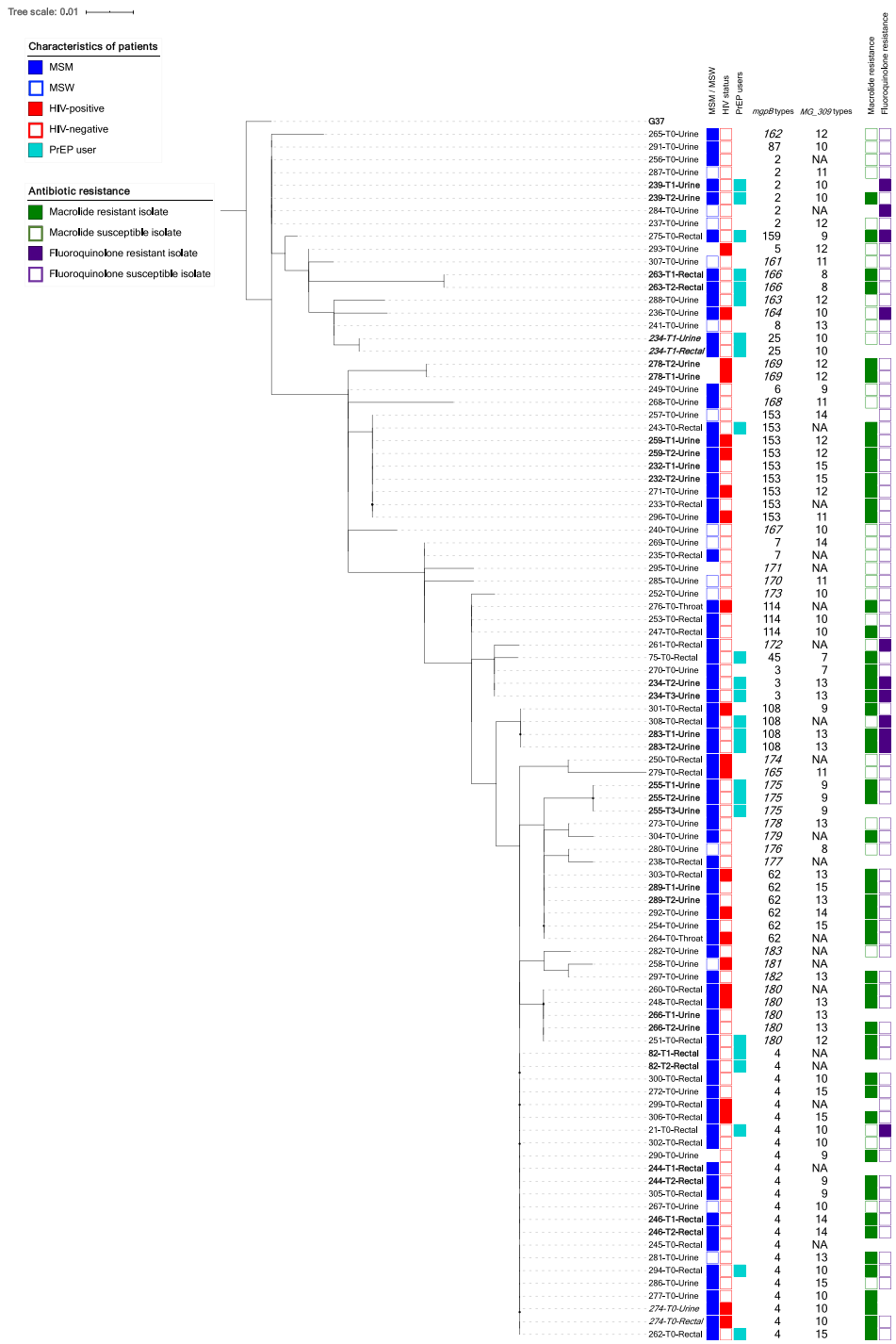

Figure 1. Maximum likelihood tree using the *mgpB*-based single-locus-sequence-based typing of 95 *M. genitalium* isolates from 78 infected men. The tree was constructed with IQ-TREE (version 1.6.11) software using an HKY+F + I model. Branch support values were generated from 1000 bootstrap replicates. The phylogenetic tree was annotated using iTOL (version 5). The *M. genitalium* G37 strain sequence was used as a reference sequence (accession number NC_000908.2). Sample numbers were composed of the patient number, the visit number and the anatomical collection site of the sample (T0 corresponds to a single visit, and T1, T2 and T3 correspond to subsequent visits). Single specimens are shown in black, first and follow-up specimens from the same patients are shown in bold, and concurrent specimens from Patients 234 and 274 are italicized. NA, no amplification. MSM are represented by blue squares, MSW are represented by open blue squares, HIV-positive patients are represented by red squares, HIV-negative patients are represented by open red squares, PrEP users are represented by light blue squares, macrolide- or/and fluoroquinolone-resistant isolates are represented by green or/and purple squares, respectively, WT isolates are represented by open green or/and purple squares, and no symbol means that data were unavailable. In this study, only one MSW was HIV positive and all PrEP users were MSM. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

(accession numbers MT327176–MT327198). Removing concurrent and subsequent samples, the most prevalent STs were ST4 (19/80; 23.8%), ST153 (7/80; 8.8%), ST62 (6/80; 7.5%), ST2 (5/80; 6.3%) and ST180 (4/80; 5%). Amplicons of the *MG_309* locus were obtained for 74 samples (77.9%) from 63 men. The *mgpB*/*MG_309* typing method identified 52 different genotypes from 74 *M. genitalium* strains, resulting in a discriminatory index of 0.979. Both concurrent specimens from Patients 234 and 274 harboured the same genotype (Figure 1). With regard to the 13 patients providing subsequent specimens, identical genotypes were found in 11 (84.6%) patients, suggesting the persistence of *M. genitalium* infection (Table 1). In Patient 289, only the *MG_309* type changed after 65 days, with no evolution of the resistance profile, but infection by a new strain could not be excluded. In Patient 234, both the *mgpB* and the *MG_309* types changed after 92 days, with a change in the resistance profile, suggesting a new *M. genitalium* infection.

Investigation of macrolide and fluoroquinolone resistance was successful for 72 (92.3%) and 74 (94.9%) patients, respectively (Table S1, available as Supplementary data at *JAC* Online). Macrolide and fluoroquinolone resistance-associated mutations were detected in 58.3% (42/72) and 10.8% (8/74) of patients, respectively. The A2059G and Ser83Ile mutations were the most frequent, in 66.7% (28/42) and 87.5% (7/8) of patients harbouring resistance-associated mutations, respectively. The rate of macrolide resistance was significantly higher among MSM than among men who have sex with women only (MSW) [68.4% versus 9.1%, adjusted OR (aOR), 1.57; 95% CI, 1.13–2.18; $P = 0.007$]. It also appeared higher among HIV-positive patients and PrEP users than among HIV-negative patients who did not use PrEP (76.5% and 80% versus 42.5%), although not statistically significant in a multivariate analysis (aOR, 1.20; 95% CI, 0.89–1.62; $P = 0.24$ and aOR, 1.17; 95% CI, 0.89–1.56; $P = 0.26$, respectively). Nevertheless, PrEP users had a higher rate of fluoroquinolone resistance than HIV-negative non-PrEP users (33.3% versus 4.9%; aOR, 1.32; 95% CI, 1.09–1.60; $P = 0.005$), but non-significantly higher rates were observed among MSM compared with MSW (12.3% versus 7.7%, $P = 0.63$) and among HIV-positive patients compared with HIV-negative non-PrEP users (5.6% versus 4.9%, $P = 0.91$). Three (4.2%) HIV-negative MSM patients harboured dual-resistant strains that belonged to three distinct genotypes.

We compared the *mgpB*-based genotype distribution in different groups in which the *mgpB* diversity was calculated: (i) MSM versus MSW; (ii) HIV-positive versus HIV-negative; (iii) PrEP users versus non-PrEP users; (iv) urine versus rectal samples; and (v) macrolide- and fluoroquinolone-resistant versus susceptible strains. The first three comparisons revealed similar *mgpB* diversity. Nevertheless, ST2 was significantly more frequent in MSW than in MSM (21.4% versus 3.2%, $P = 0.014$) and ST108 was more frequent in PrEP users than in non-PrEP users (12.5% versus 1.6%, $P = 0.039$). Rectal samples revealed a lower *mgpB* diversity compared with urine samples (0.804 versus 0.966, respectively), and ST4 was over-represented in rectal samples (43.8% versus 10.9%, $P = 0.0009$). Interestingly, although similar *mgpB* diversity was found among fluoroquinolone-resistant and -susceptible strains (0.964 versus 0.924, respectively), lower genetic diversity was found among macrolide-resistant strains (0.870) compared with macrolide-susceptible strains (0.978). Macrolide-resistant *M. genitalium* strains were assigned to only 15 *mgpB* types of the 38 types reported here (versus 25 in susceptible strains), with ST62 and ST153 more frequent than in susceptible strains (14% versus 0%, $P = 0.03$, for both STs). Additionally, all ST62 *M. genitalium* strains harboured an A2059G substitution, whereas all ST153 strains harboured an A2058G mutation (Table S1). Furthermore, 85.7% (6/7) and 100% (5/5) of men infected with ST62 and ST153, respectively, were MSM.

Discussion

Our results confirm the high discriminatory power of the *mgpB*/*MG_309* typing method.[10–12] Identical genotypes found between concurrent samples and most subsequent samples from the same patients confirm the stability over time (up to 216 days in this study) and the reproducibility of the method. Despite the high genetic heterogeneity, our results revealed predominant *mgpB* STs, mainly ST4 (23.8%), followed by ST153, ST62 and ST2. The high prevalence of ST4 has been reported previously in studies conducted in Germany[12] and France,[10] but not in Spain, where ST5 was more frequent.[9] Additionally, in our study ST4 was found in 43.8% of the rectal samples, suggesting that *M. genitalium* ST4 strains might be more easily transmitted by anal intercourse. Notably, ST2 was predominant in MSW, suggesting a possible different way of spreading for the *mgpB* STs in the MSM and MSW populations, likely in relation to sexual networks. Nevertheless, no significant difference in overall *mgpB* diversity was observed between MSM and MSW in the present study. The limited overall concordance between *M. genitalium* genotypes and sexual behaviours suggests that the spread of an *M. genitalium* genotype is not limited to a specific sexual network. Sexual network bridging may scramble the genotype distribution patterns.

In this study, the rate of macrolide and fluoroquinolone resistance was high compared with the prevalences of resistance measured in other European non-Nordic countries.[4] However, as previously reported in France and several other countries,[4–7,9] high rates of macrolide-resistant *M. genitalium* are commonly found among MSM and PrEP users, suggesting that specific *M. genitalium* infection management should be implemented in these populations. As in other studies,[9,11,12,15] a large distribution of *M. genitalium* genotypes was found among macrolide- and fluoroquinolone-resistant strains, supporting the hypothesis of a multiclonal spread of resistance in this species, likely associated with the consequences of antibiotic selection pressure on diverse independent *M. genitalium* strains. Such a polyclonal spread of macrolide-resistant strains has also been demonstrated for *Mycoplasma pneumoniae*, the closest species phylogenetically.[17] Nevertheless, our analysis revealed lower *mgpB* diversity within macrolide-resistant *M. genitalium* strains, associated with an over-representation of ST62 and ST153 in a population mostly composed of MSM. This finding may result from the combination of antimicrobial drug exposure[1] and close sexual transmission networks, as already reported for *Neisseria gonorrhoeae*.[18] However, the fitness cost of macrolide resistance may also be involved. Although antibiotic resistance generally confers reduction of strain fitness,[19] some macrolide-resistant *M. genitalium* genotypes may have a lower fitness cost or even increased fitness in relation to their own genetic background. Such increased fitness was previously reported in *Helicobacter pylori* isolates harbouring identical macrolide resistance-associated mutations.[20] Further studies are needed to assess this hypothesis. Overall, the lower *mgpB* diversity

observed will have to be confirmed by larger studies because the limited number of participants and clinics from which participants were recruited are limitations of the present study.

In conclusion, although macrolide resistance spread appears polyclonal in *M. genitalium*, the lower genetic diversity of *mgpB* types found among macrolide-resistant strains may reflect an easier spread of a few specific *mgpB* types or the occurrence of sexual networks among MSM.

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Transparency declarations

None to declare.

Supplementary data

Table S1 is available as Supplementary data at *JAC* Online.

References

1 Li Y, Su X, Le W *et al*. *Mycoplasma genitalium* in symptomatic male urethritis: macrolide use is associated with increased resistance. *Clin Infect Dis* 2020; **70**: 805–10.

2 Unemo M, Salado-Rasmussen K, Hansen M *et al*. Clinical and analytical evaluation of the new Aptima *Mycoplasma genitalium* assay, with data on *M. genitalium* prevalence and antimicrobial resistance in *M. genitalium* in Denmark, Norway and Sweden in 2016. *Clin Microbiol Infect* 2018; **24**: 533–9.

3 Murray GL, Bradshaw CS, Bissessor M *et al*. Increasing macrolide and fluoroquinolone resistance in *Mycoplasma genitalium*. *Emerg Infect Dis* 2017; **23**: 809–12.

4 Machalek DA, Tao Y, Shilling H *et al*. Prevalence of mutations associated with resistance to macrolides and fluoroquinolones in *Mycoplasma genitalium*: a systematic review and meta-analysis. *Lancet Infect Dis* 2020; doi: 10.1016/51473-3099(20)30154-7.

5 Deborde M, Pereyre S, Puges M *et al*. High prevalence of *Mycoplasma genitalium* infection and macrolide resistance in patients enrolled in HIV pre-exposure prophylaxis program. *Med Mal Infect* 2019; **49**: 347–9.

6 Ducours M, Alleman L, Puges M *et al*. Incidence of sexually transmitted infections during pre-exposure prophylaxis for HIV: a worrying outcome at 2 years! *Sex Transm Infect* 2019; **95**: 552.

7 McIver R, Jalocon D, McNulty A *et al*. Men who have sex with men with *Mycoplasma genitalium*-positive nongonococcal urethritis are more likely to have macrolide-resistant strains than men with only female partners: a prospective study. *Sex Transm Dis* 2019; **46**: 513–17.

8 Hjorth SV, Björnelius E, Lidbrink P *et al*. Sequence-based typing of *Mycoplasma genitalium* reveals sexual transmission. *J Clin Microbiol* 2006; **44**: 2078–83.

9 Fernández-Huerta M, Serra-Pladevall J, Esperalba J *et al*. Single-locus-sequence-based typing of the *mgpB* gene reveals transmission dynamics in *Mycoplasma genitalium*. *J Clin Microbiol* 2020; **58**: e1886–19.

10 Cazanave C, Charron A, Renaudin H *et al*. Method comparison for molecular typing of French and Tunisian *Mycoplasma genitalium*-positive specimens. *J Med Microbiol* 2012; **61**: 500–6.

11 Piñeiro L, Idigoras P, Cilla G. Molecular typing of *Mycoplasma genitalium*-positive specimens discriminates between persistent and recurrent infections in cases of treatment failure and supports contact tracing. *Microorganisms* 2019; **7**: 609.

12 Dumke R, Rust M, Glaunsinger T. *mgpB* types among *Mycoplasma genitalium* strains from men who have sex with men in Berlin, Germany, 2016–2018. *Pathogens* 2020; **9**: 12.

13 Le Roy C, Hénin N, Pereyre S *et al*. Fluoroquinolone-resistant *Mycoplasma genitalium*, Southwestern France. *Emerg Infect Dis* 2016; **22**: 1677–9.

14 Ma L, Taylor S, Jensen JS *et al*. Short tandem repeat sequences in the *Mycoplasma genitalium* genome and their use in a multilocus genotyping system. *BMC Microbiol* 2008; **8**: 130.

15 Laumen JGE, van Alphen LB, Maduna LD *et al*. Molecular epidemiological analysis of *Mycoplasma genitalium* shows low prevalence of azithromycin resistance and a well-established epidemic in South Africa. *Sex Transm Infect* 2020; doi:10-1136/sextrans-2019-054371.

16 Goudet J. FSTAT, A Program to Estimate and Test Gene Diversities and Fixation Indices (version 2.9.3). 2001. https://www2.unil.ch/popgen/softwares/fstat.htm.

17 Pereyre S, Charron A, Hidalgo-Grass C *et al*. The spread of *Mycoplasma pneumoniae* is polyclonal in both an endemic setting in France and in an epidemic setting in Israel. *PLoS One* 2012; **7**: e38585.

18 Kenyon CR, Schwartz IS. Effects of sexual network connectivity and antimicrobial drug use on antimicrobial resistance in *Neisseria gonorrhoeae*. *Emerg Infect Dis* 2018; **24**: 1195–203.

19 Andersson DI. The biological cost of mutational antibiotic resistance: any practical conclusions? *Curr Opin Microbiol* 2006; **9**: 461–5.

20 Björkholm B, Sjölund M, Falk PG *et al*. Mutation frequency and biological cost of antibiotic resistance in *Helicobacter pylori*. *Proc Natl Acad Sci USA* 2001; **98**: 14607–12.