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MAJOR ARTICLE

High Rates of Asymptomatic *Mycoplasma genitalium* Infections With High Proportion of Genotypic Resistance to First-Line Macrolide Treatment Among Men Who Have Sex With Men Enrolled in the Zurich Primary HIV Infection Study

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Background. *Mycoplasma genitalium* (*Mg*) is an emerging sexually transmitted pathogen among men who have sex with men (MSM). Resistance to recommended antimicrobial agents are of public health concern. Few data exist on *Mg* infections in MSM diagnosed with human immunodeficiency virus (HIV) during primary HIV infection.

Methods. Participants of the Zurich Primary HIV Study (ClinicalTrials.gov Identifier NCT 00537966) were systematically offered screening for sexually transmitted infections (STIs) between April 2019 and September 2020. Screening was performed using an in-house polymerase chain reaction panel comprising *Mg* including genotypic resistance testing for macrolides and quinolones, *Chlamydia trachomatis* including *serovars* L1-L3, *Neisseria gonorrhoeae*, *Treponema pallidum*, and *Hemophilus ducreyi*.

Results. We screened 148 of 266 (55.6%) participants, with an overall total of 415 follow-up visits. Ninety-one percent were MSM. The incidence rate for all STIs was 47.0 (95% confidence interval [CI], 32.2–68.6) per 100 person-years. *Mycoplasma genitalium* was the most frequently detected pathogen: 30 participants (20%) presented with at least 1 *Mg* infection, corresponding to a period prevalence of 20.3% and incidence rate of 19.5 *Mg* infections (95% CI, 11.8–32.4). Most *Mg* infections (93%) were asymptomatic, and 9 (30%) participants showed spontaneous clearance. We detected high rates of antibiotic resistance: 73.3% to macrolides, 3.3% to quinolones, and 13.3% resistance to both antibiotics.

Conclusions. The high prevalence of mostly asymptomatic *Mg* infections and high rate of spontaneous clearance support cautious initiation for treatment. The high proportion of macrolide-resistant strains suggests that a genotypic determination of resistance should be standard of care. Moxifloxacin should be the preferred treatment option for symptomatic *Mg* infections among MSM if resistance testing is unavailable.

Keywords. antibiotic resistance; *Mycoplasma genitalium*; primary HIV-1-infection; screening; sexually transmitted infection.

Numbers of *Mycoplasma genitalium* (*Mg*) infections have sharply risen over the past decade [1]. Infections are often asymptomatic [2, 3] but can cause a range of symptoms and sequelae, ranging from urethritis [4] and cervicitis to pelvic inflammatory syndrome, miscarriages, and infertility [5, 6]. Treatment is challenging due to intrinsic resistance of *Mg* towards beta-lactams. Although doxycycline and azithromycin were historically the standard of care, resistance to various classes of antibiotic agents (ie, macrolides and quinolones) are rising at an alarming rate [7].

Molecular approaches are the preferred method of *Mg* detection, and resistance is determined genotypically. Distinct 23S ribosomal ribonucleic acid (rRNA) mutations are associated with macrolide resistance and azithromycin treatment failures, whereas fluoroquinolone resistance is mainly associated with mutations in the quinolone resistance-determining region of the genes *parC* and *gyrA*. In men who have sex with men (MSM), including those receiving pre-exposure prophylaxis, resistance of *Mg* to first-line treatment azithromycin or second-line treatment moxifloxacin is prevalent in up to 84.2% and 33.3% of cases [8–11]. A correlation between the consumption of macrolides and levels of resistance exists [12].

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Although it is widespread, in most cases, *Mg* infection does not seem to be associated with active infectious processes, and the natural history of the disease is currently not well understood. Considering the low pathogenicity of *Mg* and the complexity of treatment, current guidelines do not recommend routine screening and treatment of asymptomatic individuals. Among individuals with active disease, treatment recommendations in Europe consist of a 5-day course of azithromycin or a 7- to 10-day course of moxifloxacin [13]. The British Association for Sexual Health and HIV (BASHH) recently proposed resistance-guided treatment with lead-in doxycycline followed by either azithromycin or moxifloxacin based on the results of genotypic resistance testing [14].

Overall, prospective data on the prevalence and incidence of *Mg* infections and antibiotic resistance patterns in MSM with human immunodeficiency virus (HIV) are sparse [15, 16]. We have previously published data from the Zurich Primary HIV Infection Study (ZPHI) that show a high incidence of STI in MSM enrolled in the ZPHI study [17]. In the current study, we investigated incidence and associated risk factors for *Mg* infection based on regular pooled sexually transmitted infection (STI) swabs, by applying a multipathogen STI polymerase chain reaction (PCR) assay. We investigated risk factors associated with rates of infection during an 18-month study period as well as prevalence and factors associated with resistance to macrolides and quinolones.

METHODS

Patient Consent Statement

All participants gave their written consent, and the study was approved by the local ethics committee of the Kanton Zurich. The design of the work has been approved by local ethical committees, and it conforms to the standards currently applied in Switzerland of origin and includes the name of the authorizing body, which should be stated in the paper.

Study Population

All participants are part of the ongoing ZPHI study, a multicentric observational study (ClinicalTrials.gov Identifier NCT00537966), and most of them participate in the Swiss Cohort Study (www.shcs.ch), a large prospective study [18, 19]. Participants enrolled in the ZPHI study have a documented primary HIV infection and are followed longitudinally with clinical visits every 3 months. Primary HIV-1 infection was defined as published elsewhere [17]. Within the studies, detailed clinical, laboratory, and socioeconomical data, including sexual behavior, as well as detailed treatment information are recorded.

Within the data collection period between April 2019 and September 2020, all participants were offered STI screenings during their regular ZPHI clinical visits or during additional visits due to suspicion of symptomatic STI infection or reported prior sexual risk exposure. Participants had to be screened at least once to be included in the study. Swabs were taken from the oropharynx, urethral, and anal site and pooled for PCR testing as described elsewhere [18]. In specific cases (eg, urethral discharge), the different sites were analyzed individually. Test results were communicated to the participants immediately after becoming available (usually the next day). Microbiological cure was defined as the elimination of the putative pathogen after repeated testing of the site of infection. Clinical cure was defined as the physician's assessment of clinical response and absence of symptoms of STI. Participants were also asked to complete a detailed questionnaire documenting behavioral characteristics or symptoms related to a possible STI infection (see Supplementary Material) every 3 months (or between 2 visits if longer than 3 months). Sexual contact was defined as any form of sexual contact within the past 3 months. Chemsex included the use of the following substances to enhance sexual activity: cocaine, 3,4-methylendioxy-*N*-methylamphetamin (MDMA), alkyl nitrites (known as poppers), γ-hydroxybutyric acid (GHB), mephedrone, methamphetamine, and ketamine [20]. Higher education was defined as education equal to or above bachelors' level.

Laboratory Assays

Patient samples were analyzed batchwise once per week. Samples were stored at 4°C until further processing. Automated deoxyribonucleic acid extraction from clinical specimens and PCR setup was performed using the QIAsymphony platform. Samples were analyzed on a LightCycler 480-II by a custom-made multiplex LightMix reverse transcription-PCR assay targeting *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *C trachomatis* serovar L1–L3 (*Lymphogranuloma venereum*), *Treponema pallidum*, *Haemophilus ducreyi*, and *Mg*. *Mycoplasma genitalium* macrolide resistance was assessed by subsequent melting curve analysis of a 115-base pair (bp) fragment of the 23S rRNA gene (melting temperature [Tm] $\geq$ 65°C, wild type; <63°C, resistant). Possible resistance of *Mg* to quinolones was assessed by PCR amplification and sequencing of a 214-bp fragment of the *parC* quinolone resistance-determining region (QRDR), comprising codons 81, 83, 87, and 97 (*Escherichia coli* numbering). Point mutations within the QRDR were identified by comparing the obtained sequences with the respective wild-type sequences of *M genitalium* G37. In addition, fragments of *gyrA* (231 bp; comprising codons 95, 99, and 108 *E coli* numbering), *gyrB* (410 bp; comprising codons 464 and 483), and *parE* (302 bp; comprising codon 466) were amplified and analyzed by sequencing because mutations in the indicated codons may occur alongside with *parC* mutations.

Statistical Analysis

We calculated bivariate *P* values using the Fisher's exact test for categorical variables and Wilcoxon test for continues variables. We used Benjamini-Hochberg procedure to adjust for multiple

testing. Time-to-event survival analysis was applied to estimate the incidence rate of STIs. We fitted univariable and multivariable generalized mixed-effects logistic models to assess the risk factors associated with STI events during the study period and Cox proportional-hazard models to assess the risk factors associated with time to STI events. Based on previous studies and clinical opinion, we evaluated the following variables in the univariable and multivariable analysis: number of sexual partners, anal intercourse, partnership (stable partner [sP], protected sex with nonsteady partners [nsPs], and unprotected sex with nsP), CD4 cell count at STI screening, chemsex, and previous STI. Square root transformation was performed on the CD4 cell counts to fulfill model assumptions [21]. We used complete-case analyses in the fitted models. We defined previous antibiotic treatment as follows: macrolide (azithromycin and clarithromycin) and quinolone (moxifloxacin, ciprofloxacin, norfloxacin, and levofloxacin) treatment. We tested for associations between previous exposure and resistant *Mg* infection using Fisher's exact test. Antibiotic treatment was ascertained by abstraction from medical records. We performed all analyses in R, version 4.0.0 (R Foundation for Statistical Computing, Vienna, Austria). We used lme4 package in R to perform generalized mixed-effect logistic regression and the survival package in R to perform time-to-event survival analysis and fit Cox-regression models.

RESULTS

Visits, Participants, and Period Prevalence of Sexually Transmitted Infections

During the 18-month study period (April 2019 to September 2020), 266 ZPHI participants were offered an STI screen, 148 (55.6%) of whom agreed to be screened at least once, leading to a total of 415 follow-up visits and valid screens (Figure 1). Reasons to refuse testing of the 118 remaining ZPHI participants were as follows: 63 participants had no sexual contact during the study period, 46 participants had sexual contact only with their steady partner, and 9 participants had no reason recorded. Overall, 61 of 148 participants (41.2%) reported at least 1 STI event during the study period, 16 (26.2%) of which were symptomatic (Table 1). A total of 30 of 148 participants (20.3%) had at least 1 *Mg* infection during the study period, corresponding to an incidence rate of 19.5 cases per 100 person-years (py) (95% confidence interval [CI], 11.8–32.4). The distribution of infection sites was as follows: 6 urethral, 4 rectal, and the remaining were pooled. It is notable that only 2 of these 30 participants (6.7%) tested positive for *Mg* and they were symptomatic. Across all STIs screened, *Mg* had the highest period prevalence of 20.3 cases/100 participants. The incidence rate and period prevalence for the remaining STIs were as follows (Table 1): *C trachomatis* 17.0 cases per 100 py (95% CI, 9.8–29.2) and 16.2%; *LGV* 3.3 per 100 py (95% CI, 1.1–10.4) and 2.0%; *N gonorrhoeae* 22.0 per 100 py (95% CI, 13.7–35.4) and 16.9%; and *T pallidum* 14.5 per 100 py (95% CI, 8.2–25.5) and 13.5%. We did not detect any cases of *H ducreyi*.

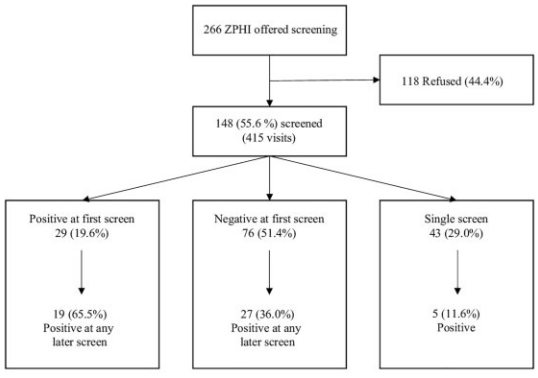

Figure 1. Flow chart of the study population, study design, and prevalence of *Mycoplasma genitalium* infections. ZPHI, Zurich Primary HIV Infection Cohort.

Factors Correlating With Any Sexually Transmitted Infection Detection and *Mycoplasma genitalium* Infection Specifically

In a bivariate analysis, compared with visits without STI detection, detection of an STI during a visit was significantly associated with time since HIV diagnosis (median, 6.1 years [interquartile range {IQR}, 3.8–9.4] vs 7.1 years [IQR, 4.7–11.4]; $P = .003$), year of HIV diagnosis (median, 2013.5 [2010–2016] vs 2012 [2008–2014]; $P = .002$), sP (median, 75 [33.2%] vs 90 [47.6%]; $P = .002$), stable partnership (75 [33.2%] vs 90 [47.6%]; $P = .002$), number of sexual partners in the preceding 3 months (5 [IQR, 3–8] vs 3 [IQR, 2–5]; $P < .001$), condomless sex (147 [65.0%] vs 95 [50.3%]; $P < .001$), and number of sex partners (5 [IQR, 3–8] vs 3 [IQR, 2–5]; $P < .001$) (Table 2).

Compared with visits without STI detection, detection of *Mg* during a visit was significantly associated with sP (37 [30.3%] vs 90 [47.6%]; $P = .002$), number of partners (5 [IQR, 3–10] vs 3 [IQR, 2–5]; $P = .001$], condomless sex (84 [68.85%] vs 95 [50.26%]; $P < .001$), and chemsex (34 [27.87%] vs 29 [15.34%]; $P = .009$) (Table 2).

In univariable generalized mixed-effects logistic models, the number of sexual partners (risk ratio [RR], 1.13; 95% CI, 1.03–1.25) and partnership (unprotected sex with nsP vs sP) (RR, 7.29; 95% CI, 1.79–29.72) were significantly associated with STIs (Figure 2A). The latter remained significant in a multivariable model (RR, 7.33; 95% CI, 1.70–31.58).

In univariable Cox-regression models, number of sexual partners (hazards ratio [HR], 1.19; 95% CI, 1.1–1.3), partnership (unprotected sex with nsPs vs sPs) (HR, 6.11; 95% CI, 1.46–25.56), and chemsex (HR, 1.89; 95% CI, 1.04–3.46) were significantly associated with STIs (Figure 2B). In multivariable analysis, number of sexual partners (HR, 1.14; 95% CI, 1.05–1.24)

Table 1. Sexually Transmitted Infections Detected and Stratified by STI Symptoms

Detected Pathogen	Participants With STI	Participants With Symptoms	Incidence Rate (per 100 py); n, 95% CI	1.5-Year Period Prevalence, %
Mycoplasma genitalium	30	2 (6.7%)	19.5 (11.8–32.4)	20.3
Neisseria gonorrhoeae	25	10 (40.0%)	22.0 (13.7–35.4)	16.9
Chlamydia trachomatis	24	5 (20.8%)	17.0 (9.8–29.2)	16.2
Lymphogranuloma venereum	3	2 (66.7%)	3.3 (1.1–10.4)	2.0
Treponema pallidum	20	7 (35%)	14.5 (8.2–25.5)	13.5
Haemophilus ducreyi	0	0 (–)	0.0	0.0
Any STI	61	16 (26.2%)	47.0 (32.2–68.6)	41.2

Abbreviations: CI, confidence interval; py, patient years; STI, sexually transmitted infection.

and unprotected sex with nonsteady partners (HR, 5.72; 95% CI, 1.11–29.43) remained significantly associated with STIs.

Participants With *Mycoplasma genitalium* Infection Stratified by Disease Characteristics

Figure 3 shows symptoms, treatment, and resistance for all participants with *Mg* infection (n = 30) during the study period. Disease characteristics of participants with any STI (n = 61) are depicted in the Supplementary Figure 1. Of the 30 participants with *Mg* infection, 22 (73.3%) had macrolide-resistant *Mg* infections, 1 (3.3%) had quinolone-resistant infection, and 4 (13.3%) had resistance to both macrolide and quinolones (Table 3 and Figure 3). We did not find any significant association between previous macrolide treatment and macrolide-resistant *Mg* infection ($P = .35$). We also did not find any significant association between previous quinolone treatment and quinolone-resistant *Mg* infection ($P = 1.0$).

Treatment guided by genotypic resistance testing was initiated in 2 participants (1 symptomatic, 1 asymptomatic—treatment requested by patient) with doxycycline 2 × 100 mg for 7

Table 2. Association of Selected Factors With Any Sexually Transmitted Infection Versus No STI, and Cases With Symptomatic *Mycoplasma genitalium* Versus All *Mg* Infections

Factor	All	No STI	Any STI	*P* Value	All *Mg*	*P* Value	Symptomatic *Mg*	*P* Value
Participants	148	87	61		30		2	
Visits	415	189	226		122		9	
Age at STI diagnosis	40.84 (34.15–48.11)	42.86 (33.51–48.21)	39.93 (34.41–47.55)	.344	40.5 (38.02–47.06)	.69	38.56 (38.05–48.9)	.947
Male sex (%)	414 (99.76)	188 (99.47)	226 (100)	.455	122 (100)	1	9 (100)	1
Time since HIV diagnosis	6.57 (4.02–10.37)	7.07 (4.74–11.35)	6.09 (3.78–9.42)	**.003**	7.35 (4.02–10.65)	.2	7.34 (6.83–12.39)	.566
Year of HIV diagnosis	2013 (2009–2015)	2012 (2008–2014)	2013.5 (2010–2016)	**.002**	2012 (2009–2015)	.207	2012 (2007–2012)	.412
White ethnicity (%)	381 (91.81)	172 (91.01)	209 (92.48)	.595	114 (93.44)	.525	9 (100)	1
Higher education (%)	145 (34.94)	65 (34.39)	80 (35.4)	.837	40 (32.79)	.807	0 (0)	**.032**
MSM (%)	381 (91.81)	171 (90.48)	210 (92.92)	.375	109 (89.34)	.847	9 (100)	1
Stable partnership, yes[a] (%)	165 (39.76)	90 (47.62)	75 (33.19)	**.002**	37 (30.33)	**.002**	0 (0)	.064
Sexual contact, yes[b] (%)	324 (78.07)	150 (79.37)	174 (76.99)	**.022**	92 (75.41)	.161	3 (33.33)	1
Anal intercourse, yes[c] (%)	296 (71.33)	133 (70.37)	163 (72.12)	**0.036**	86 (70.49)	.139	3 (33.33)	1
Oral sex only[d] (%)	13 (3.13)	9 (4.76)	4 (1.77)	.153	2 (1.64)	.214	0 (0)	1
Number of sex partners	4 (2–6)	3 (2–5)	5 (3–8)	<**.001**	5 (3–10)	**.001**	5 (4.5–6)	.344
Condomless sex, yes[e] (%)	242 (58.31)	95 (50.26)	147 (65.04)	<**.001**	84 (68.85)	<**.001**	3 (33.33)	.554
Self-reported chemsex	85 (20.48)	29 (15.34)	56 (24.78)	**.02**	34 (27.87)	**.009**	0 (0)	.362
Symptoms attributed to STI (%)	47 (11.33)	21 (11.11)	26 (11.5)	1	13 (10.66)	1	4 (44.44)	–
CD4 cell count	767 (637.5–959.5)	747.18 (607–915)	796.5 (651–966.93)	.609	836 (667.25–978)	.111	1252.7 (844–1420.4)	**.009**
CD4/CD8 ratio	1.17 (0.9–1.54)	1.12 (0.88–1.37)	1.26 (0.93–1.62)	**.031**	1.24 (0.92–1.67)	**.012**	1.16 (1.05–1.79)	.314
HIV-RNA <50 copies/mL	398 (95.9)	178 (94.18)	220 (97.35)	.136	117 (95.9)	.605	8 (88.89)	.437

Abbreviations: CD4, cluster of differentiation 4; HIV, human immunodeficiency virus; *Mg*, *Mycoplasma genitalium*; MSM, men who have sex with men; RNA, ribonucleic acid; STI, sexually transmitted infection.

NOTES: All *P* values are with respect to no STI infection. Bold = significant *P* values. Numbers for visits, male sex, HIV diagnosis, white ethnicity, higher education MSM, stable partners, sexual contact, anal intercourse, oral sex only, number of sex partners, condomless sex, symptoms, and housing represent total occurrences based on the number of visits; and age at STI diagnosis and time since HIV diagnosis represent the median. The missing values are listed below.

[a]N = 95.

[b]N = 86.

[c]N = 104.

[d]N = 104.

[e]N = 113.

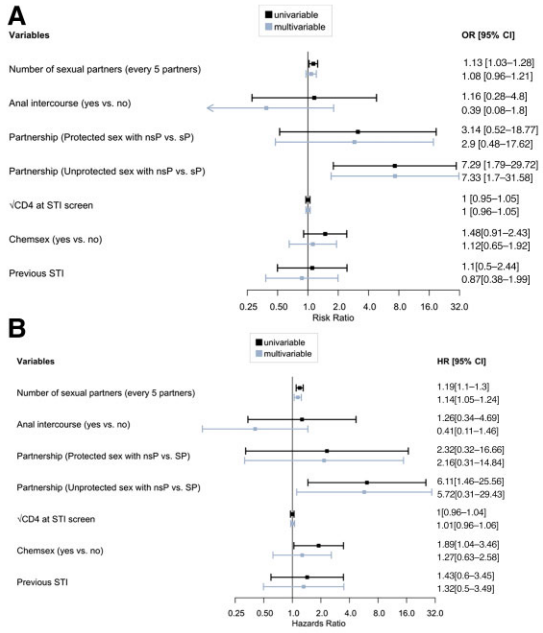

Figure 2. Factors associated with any sexually transmitted infection (STI). (*A*) Generalized logistic mixed effect model. (*B*) Cox proportional hazard model. CD4, cluster of differentiation 4; HR, hazards ratio; nsP, nonsteady partner; RR, risk ratio; sP, steady partners.

days followed by moxifloxacin 1 × 400 mg for 14 days (100% cure rate). One symptomatic and 7 asymptomatic participants were treated for co-occurring STIs. All individuals with any antibiotic treatment and available follow-up data (n = 7, 23.3%) tested negative for *Mg* at the subsequent visit posttreatment. A total of 9 (30%) asymptomatic participants showed spontaneous clearance of their *Mg* infection without any treatment initiation.

DISCUSSION

In our prospective, longitudinal study within the ZPHI study, we found a high prevalence (20%) of *Mg* infections among MSM initially diagnosed with primary HIV infection. In fact, *Mg* had the highest prevalence and the second highest incidence rate after *N gonorrhea* among a total of 6 different STIs screened. All but 2 of detected *Mg* infections were asymptomatic, and 30% of patients with *Mg* infection showed spontaneous clearance of their *Mg* infection. Regarding the resistance patterns of *Mg*, we observed a very high rate (72%) of antibiotic resistance to the recommended first-line treatment azithromycin.

Our findings are in accordance with recent reports on rising rates in both *Mg* infection and antibiotic resistance in the general population and in particular among MSM [11, 22–24].

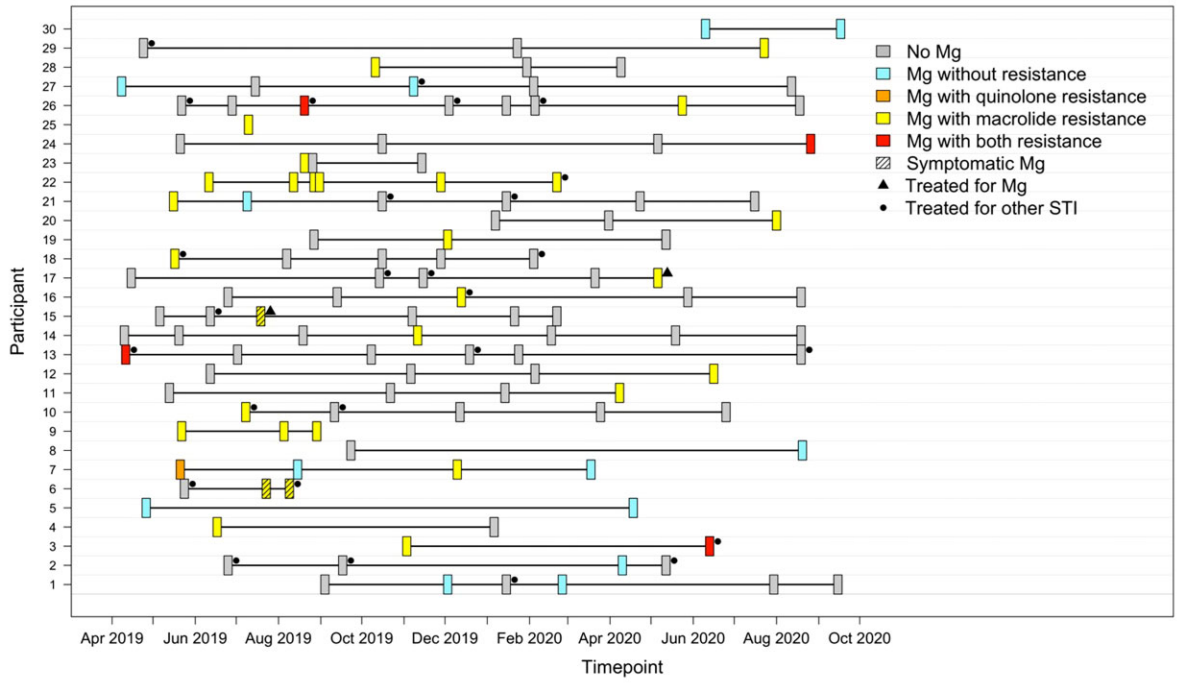

Figure 3. Follow-up timeline of detected *Mycoplasma genitalium* (*Mg*) infection per participant and visit. Participants are stratified by disease characteristics (symptoms, treatment, resistance).

Table 3. Antibiotic Resistance in *Mg* Infection Detected in the Study Cohort

Total number of participants with *Mg*	30
Macrolide only resistant cases	22 (78%)
Quinolone only resistant cases	1 (2.4%)
Macrolide- and quinoline-resistant cases	4 (13.3%)
Symptomatic	2 (6.7%)
Total tests	122
Pooled	111 (91.0%)
Pharyngeal	1 (0.8%)
Urethral	6 (4.9%)
Rectal	4 (3.3%)
Positive test	48
Pooled	40 (83.3%)
Urethral	4 (8.3%)
Rectal	4 (8.3%)

Abbreviations: Mg, Mycoplasma genitalium.

The Centers for Disease Control and Prevention list of antibiotic-resistant bacteria and fungi places *Mg* in the watch list category. In the analysis presented here, 73.3%, 3.3%, and 13.3% of the isolates displayed resistance mutations related to macrolides, quinolones, or both antibiotics. The correlation of mutations in the 23S rRNA encoding gene at position 2058 or 2058 (*E coli* numbering) and macrolide treatment failure is well established [25, 26]. Resistance to quinolones has been mainly associated with the Ser83Ile mutation in ParC [27]. However, the presence of this distinct mutation does not necessarily result in treatment failure [28, 29]. Nevertheless, given the high resistance rates and the possible clinical impact, genotypic resistance testing for macrolides should be standard of care in symptomatic patients. Various PCR approaches have been described in literature or are commercially available, which allow for the simultaneous detection of *M genitalium* and macrolide resistance-related mutations in clinical samples. These tests can be established at relatively low cost in a routine diagnostics laboratory equipped with a standard molecular diagnostic setup. Quinolone resistance is still relatively rare but shows a rather strong association with macrolide resistance [30]. Thus, patients tested positive for macrolide-resistant *M genitalium* should be additionally tested for *parC* mutations before initiation of treatment in settings with appropriate resources.

Major STI guidelines advise against routine screening for *Mg* infections in asymptomatic persons [13, 14, 31]. Our systematic screening revealed a very small proportion of symptomatic individuals and no occurrence of *Mg*-related medical complications during follow-up visits without treatment initiation. Based on our findings, we conclude that indications for testing should be either based on clinical symptoms or based on risk factors in asymptomatic individuals (eg, ongoing sexual contacts of persons treated for *Mg* infection or with symptomatic *Mg* infections), in line with the most recent major STI guidelines [13, 14].

Previous studies showed high clearance rates (41%–55%) in at-risk females [32, 33]. Our study showed for the first time that spontaneous clearance of *Mg* infections also occurs at a high rate in MSM (30%). In our study, MSM with *Mg* infection were rescreened during their next clinical visit, which occurred in most cases 3 months after the first detection of *Mg*. One important factor associated with persistent *Mg* infection described in literature are people with HIV with CD4 count $<350/mm^3$ [33]. In our study, the median CD4 cell count of MSM with *Mg* infections was 837 cells/μL; hence, this negative predictor for persistence was not present. It is notable that several MSM with *Mg* infection (13.3%) were treated for co-occurring STIs, including *N gonorrhoeae* and syphilis, using standard antibiotic regiments (ceftriaxone, penicillin) and tested negative for *Mg* at their subsequent follow-up visit. However, it appears unlikely that these inhibitors of cell wall synthesis would influence viability because *Mg* does not possess a cell wall. Although a correlation between the consumption of macrolides and levels of resistance is well described in literature, in our cohort we did not detect a correlation between preceding azithromycin treatment and azithromycin-resistant strains [8, 12, 34]. It is likely that the numbers assessed in this study were too small to detect any significant correlation.

We found that higher numbers of sex partners, use of chemsex, and stable partnership with increased condomless sex were associated with both increased rates of *Mg* infections and STIs in general. All of these factors have previously been shown to be proxies for high-risk sexual behavior and correlated with a higher likelihood to acquire STIs [35, 36]. Chemsex is an emerging global problem and public health concern, particularly among MSM, with increasing numbers being observed also in Switzerland [20].

One strength of our study is the prospective and longitudinal design of the ZPHI study, including well described participants defined by applying very strict primary HIV infection criteria. This rich and rigorous data resource allowed us to test for correlations of STIs such as *Mg* with other factors in a large MSM population with primary HVI infection, shedding light on a sparsely investigated, yet highly relevant area of epidemiological research and public health. One potential limitation of the study is the relatively small number of symptomatic *Mg* cases, which did not allow us to assess the efficacy of the various treatment regimens and limited the statistical power to use regression models. On the other hand, we gained insights on the persistence rates of asymptomatic *Mg* infections among MSM with HIV and a preserved immune state. Another limitation could be that a relatively high number of participants refused screening, which limits our ability to comprehensively determine incidence and prevalence of *Mg* infection. However, most of the participants who refused to get an STI screen had lower risk for acquiring an STI. Performing mostly pooled swabs further limits the resolution of our analysis regarding the exact anatomical site of infection. Nevertheless, follow-up swabs were

performed based on symptoms and likely anatomical site. We believe this strategy represents an efficient, cost-saving, and feasible approach to diagnose STIs.

CONCLUSIONS

In conclusion, we found a very high prevalence and incidence of asymptomatic *Mg* infections in patients who initially presented with a primary HIV-1 infection, almost all of whom were treated with suppressive antiretroviral therapy during the study. The very high rate of macrolide-resistant *Mg* strains, the spontaneous clearance in one third of *Mg*-infected individuals, as well as the mostly asymptomatic course of disease (93.3% of all participants tested *Mg* positive) argues for a differentiated evaluation and identification of patients in need for treatment. Where treatment is indicated, the choice of antibiotic regimen should be carefully considered, warranting genomic resistance testing.

Overall, considering the increasing need for antibiotic stewardship, future research will specifically need to address the question of whether or when treatment is needed or whether safer sex and spontaneous clearance could play a major part in the eradication of *Mg* infections. Other important unanswered questions to date are the incubation period of *Mg*, how often transmission occurs per episode of unprotected sexual intercourse, and how the risk of transmission differs between different types of sex acts.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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