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Mycoplasma genitalium infection in Eswatini amid syndromic case management: prevalence, coinfections, diagnostic challenges and treatment gaps

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Abstract

Background The global epidemic of Mycoplasma genitalium (MG) is marked by its widespread prevalence, varied resistance patterns, and significant impact on sexual health. This study aimed to understand the prevalence and interaction of MG infections with other sexually transmitted infections (STIs) in a low-resource setting, as well as the implications for routine STIs care.

Methods This nested cross-sectional study was conducted from July 2022 to April 2023 across six outpatient care sites in Shiselweni, Eswatini. Participants completed a self-questionnaire, underwent syndromic case management, and provided urine samples for parallel molecular-based testing using the Cepheid GeneXpert® platform for MG, Chlamydia trachomatis (CT), Neisseria gonorrhoeae (NG), and Trichomonas vaginalis (TV). The proportion of MG mono-infection and coinfections were calculated. Multivariable logistic regression models identified predictors of symptomatic MG mono-infections, which could be used to streamline at-risk patients for MG testing.

Results Among 735 participants, the median age was 27 (interquartile range 23—34) years, 65.9% were women, and 9.5% were HIV-positive. MG infection was detected in 10.5% ($n=77$) of clients, with 45.5% ($n=35$) coinfected with any of CT/NG/TV, and one case (0.1%) showing macrolide resistance. Among women with vaginal discharge syndrome (28.1%, $n=136$), 0.7% ($n=1$) had MG mono-infection, and 10.3% ($n=14$) had MG and CT/NG/TV coinfections. Among men with male urethral syndrome (31.9%, $n=80$), 3.8% ($n=3$) had MG mono-infection, and 2.5% ($n=2$) had MG and CT/NG/TV coinfections. Most MG-positive cases (66.2%, $n=51$) did not receive antibiotic therapy, despite 68.6% ($n=35$) reporting symptoms of STIs. Of treated cases, 26.0% ($n=20$) received azithromycin monotherapy, 6.5% ($n=5$) doxycycline monotherapy, and 1.3% ($n=1$) both drugs. Of 305 individuals reporting STIs symptoms but tested negative for CT/NG/TV, 23 (7.5%) had symptomatic MG mono-infections. Unemployment and never having been tested for HIV were identified as risk factors. Streamlining 108/305 (35.4%) at-risk individuals

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for molecular-based MG testing would identify 14.8% (16/108) as positive, capturing 69.6% (16/23) of all symptomatic MG mono-infections.

Conclusions MG was common among outpatients and frequently co-occurred with CT, NG, and TV infections. Syndromic case management often misclassified MG infections, leading to ineffective treatment. Expanding molecular-based MG testing could enhance antibiotic stewardship, crucial for preventing the spread of drug-resistant strains.

Keywords Africa, STI, AMR, MG, Coinfections

Background

The global epidemic of Mycoplasma genitalium (MG) is characterized by its widespread prevalence, varied resistance patterns, and its impact on sexual health. MG infection may present with mild symptoms but can cause clinical manifestations such as urethritis and proctitis in men, as well as cervicitis and pelvic inflammatory disease in women [1–4]. MG infection is often combined with other bacterial (e.g., Chlamydia trachomatis [CT], Neisseria gonorrhoeae [NG), protozoan (Trichomonas vaginalis [TV]), and viral (e.g., Human papillomavirus) infections and it increases the risk of HIV acquisition [1, 5].

In Southern Africa, the prevalence of MG infection varies from 4.7% to 12.5% in populations with various risk factors and disease presentations, including HIV co-infection, and often presents with other infections such as NG [6]. Notably, MG prevalence estimates were higher (23.5%) in men who have sex with men attending pre-exposure prophylaxis services in West Africa [7]. Additionally, there is growing concern of antimicrobial resistance (AMR) to macrolides and fluoroquinolones [6], with azithromycin resistance in South Africa ranging from as low as 1.1% to as high as 9.8% [6, 8], and comparable low rates of 0.6% observed among men who have sex with men in West Africa [7]. AMR as a public health threat is specifically relevant for settings applying syndromic case management for sexually transmitted infections (STIs). It identifies the main STIs based on symptoms and signs as recognized by clinicians, enabling immediate therapy without laboratory confirmation [9]. Among recommended treatment options in case of male urethral syndrome (MUS) and vaginal discharge syndrome (VDS) are a combination of single-dose cefixime or intramuscular ceftriaxone, azithromycin, and metronidazole [9]. However, single-dose azithromycin showed decreasing cure rates in patients with MG infection, increasing the risk of AMR [10]. The recommended therapy of macrolide sensitive MG infections consists of doxycycline for 7 days, followed by azithromycin for 4 days [11].

A deeper understanding of the prevalence and interaction of MG infections with other STIs is needed to improve diagnostic and treatment recommendations in resource-poor settings lacking access to MG testing. The widespread use of azithromycin during COVID-19 may also have contributed to AMR [12, 13]. This is the first study from Eswatini to examine MG, related AMR, and its interaction with other curable STIs, with implications for case management.

Methods

Setting

Eswatini has a high HIV prevalence of 24.8% in adults aged ≥ 15 years [14]. The HIV epidemic has been intersecting with a high prevalence of other STIs, with 19.4% of women in reproductive age presenting with any of CT, NG, TV, syphilis infections or genital warts at outpatient care services [15, 16].

This study was conducted in Eswatini's rural Shiselweni region, with approximately 124,000 inhabitants aged ≥ 15 years [17]. Integrated HIV and STIs policies and care guidelines stipulate that patients with STIs should be routinely offered HIV testing, and patients with an HIV diagnosis should undergo STIs screening. STI screening based on risk factors was primarily conducted by HIV testing counsellors, while nurses used the syndromic case management approach.

Study design

This nested cross-sectional study is part of a larger investigation of outpatients aged ≥ 18 years, who underwent syndromic screening for STIs at six clinics in Shiselweni, along with simultaneous laboratory-based molecular testing between July 2022 and April 2023.

Procedures

Patients accessing routine HIV testing, HIV treatment refills, and other preventive and curative outpatient services were invited to participate in the study. After obtaining written informed consent, a paper or electronic self-questionnaire in English or SiSwati was administered to assess socio-economic and behavioural factors, and symptoms suggestive of STIs (see Supplementary File, Table S1). Self-collected urine samples were shipped to a nearby laboratory for molecular-based testing on the Cepheid GeneXpert® platform using cartridges for CT/NG (Xpert®) and TV (Xpert®), and the ResistancePlus®

MG FleXible (SpeeDx, Sydney, Australia) cartridge for MG and macrolide resistance detection. Due to costs, the number of available MG cartridges was limited, thus not all clients of the larger cross-sectional sample underwent MG testing. Testing was primarily targeted at clients deemed by clinicians to be at higher risk of bacterial STIs. Leukocyte esterase (LE) urine strip testing was performed on-site. Same-day standard triple single-dose therapy for CT/NG/TV was initiated based on syndromic screening results (VDS, MUS), as Xpert test results were delayed.

Main definitions

An infection with CT, NG, TV, and MG was confirmed if detected by Xpert. Combined CT/NG/TV infections were defined as the presence of one or more of these pathogens. Symptomatic STIs were defined as patients reporting at least one of the following symptoms, as assessed in the self-questionnaire: genital itchiness, genital discharge, pain when urinating, pain during sexual intercourse, abdominal pain in women, and scrotal swelling in men. An asymptomatic infection was defined as the absence of these symptoms but the molecular detection of a pathogen. VDS and MUS were confirmed based on the clinician's diagnosis using the syndromic case management approach [18].

Statistical analyses

All analyses were performed using Stata 18, and graphs were plotted in Microsoft Excel. Baseline characteristics were described using frequency statistics and proportions. First, predictors for clients undergoing MG testing were assessed using multivariable logistic regression models. All factors known at baseline to the clinician, and thus potentially influencing access to MG testing, were included in the model. Second, the proportion of MG infection among clients with available MG test results was calculated overall and disaggregated by baseline factors. Third, we calculated the proportion of MG coinfection among CT/NG/TV (both combined and individually for each pathogen) and examined the distribution of MG infection by sex based on LE test results. Fourth, we assessed the outcomes of syndromic screening (VDS, MUS) in relation to molecularly confirmed MG and CT/NG/TV infections and exposure to azithromycin and doxycycline therapy among cases with MG infection. Finally, we limited the sample to clients who tested negative for CT/NG/TV. Univariate and multivariable penalised logistic regression models were then used to identify risk factors for MG infection to hypothetically streamline CT/NG/TV-negative at-risk cases for molecular-based MG testing.

The extent of missing data is reported in the tables, and no imputation or adjustments were applied in the analysis.

Ethics

This study was approved by the Médecins Sans Frontières Ethics Review Board (ID:2154) and the Eswatini Health and Human Research Review Board (EHHRRB096/2021).

Results

Predictor of molecular MG testing

A total of 1396 clients had test results available for CT/NG/TV. Of these, 735 (52.7%) also had test results available for MG infection. Predictors increasing the odds of MG testing were two Médecins Sans Frontières supported health care sites, HIV testing at enrolment, and having a reactive or missing LE test result (see Supplementary File, Table S2). The odds were lower among clients accessing tuberculosis services, family planning, or partner notification services.

Prevalence of MG infection

Baseline characteristics and the proportion of clients undergoing MG and CT/NG/TV testing ($n=735$) are presented in Table 1. The median age was 27 years (interquartile range 23—34), 65.9% ($n=484$) were women, and 9.5% ($n=70$) were known HIV-positive.

Overall, 77 clients (10.5%) tested positive for MG, with proportions ranging from 7 to 13% across most factors. No pathogens were detected among clients who reported no recent sex, had no partners, or were unaware of their pregnancy at enrolment. Infections were low for clients uncertain about their STI risk (4.1%) or HIV risk (6.6%), whose partner had an STI in the last 6 months (4.7%), those uncertain about engaging in partner-notification services (4.3%), and when the LE test result was unknown (5.6%). Higher infection rates were observed in clients who were HIV-positive (14.3%) or had used HIV post-exposure prophylaxis in the past 6 months (20.0%), had never been tested for HIV (15.4%), or whose last HIV test was 4 to 6 months ago (15.2%). Additionally, higher rates were seen in those who reported sex under the influence of alcohol in the past 6 months (15.4%), used injectable drugs (22.2%), engaged in transactional sex (15.4%), had a primary partner with an age difference of ≥ 10 years (15.9%), or had missing LE test results (13.5%).

Table 1 Baseline characteristics of participants and prevalence of Mycoplasma genitalium infection among those tested with molecular diagnostics ($n = 735$)

(Missing: N; %)[a]	Undergoing MG testing N	MG-positive N, (row %)
Total	735	77 (10.5)
Facilities, (0; 0)		
Nhlangano Health Center	209	23 (11.0)
FTM	107	14 (13.1)
Zombodze	87	7 (8.0)
Gege	70	6 (8.6)
Nhlangano fixed site	124	11 (8.9)
Lavumisa fixed site	138	16 (11.6)
HIV status/ testing, (2; 0.3)		
Known HIV +	70	9 (12.9)
Newly diagnosed—established HIV	21	3 (14.3)
Newly diagnosed—acute/early HIV	9	1 (11.1)
HIV negative	633	63 (10.0)
Age category, years, (0; 0)		
18–29	442	56 (12.7)
30–39	204	14 (6.9)
40–49	65	5 (7.7)
≥ 50	24	2 (8.3)
Sex & Reproductive status, (0; 0)		
Men	251	22 (8.8)
Non-pregnant women	385	46 (11.9)
Pregnant women – unaware of pregnancy	12	0 (0.0)
Pregnant women – aware of pregnancy	41	5 (12.2)
Breastfeeding women	46	4 (8.7)
Education level completed, (5; 0.7)		
No formal education	15	2 (13.3)
Primary	78	6 (7.7)
Secondary	175	20 (11.4)
High school	334	37 (11.1)
Tertiary	128	10 (7.8)
Employment status, (5; 0.7)		
Employed	257	29 (11.3)
Self-employed	72	4 (5.6)
Casual worker	45	4 (8.9)
Unemployed	356	40 (11.2)
In a relationship, (17; 2.3)		
No	53	6 (11.3)
Yes	665	69 (10.4)
Wish for a child, (4; 0.5)		
No	498	54 (10.8)
Yes	197	20 (10.2)
Uncertain	36	3 (8.3)
Last sexual intercourse (past 6 months), (4; 0.5)		
No intercourse	29	0 (0.0)
< 1 month	578	68 (11.8)
≥ 1 month	124	9 (7.3)
Number of sexual partners (past 6 months), (1; 0.1)		
0	27	0 (0.0)

Table 1 (continued)

(Missing: N; %)[a]	Undergoing MG testing N	MG-positive N, (row %)
1	474	53 (11.2)
2	169	21 (12.4)
≥ 3	64	3 (4.7)
Age difference with main partner, years, (68; 9.3)		
< 5 yrs	362	36 (9.9)
5-9yrs	236	26 (11.0)
≥ 10 years	69	11 (15.9)
Sex under influence of alcohol (past 6 months), (36; 4.9)		
No	569	53 (9.3)
Yes	130	20 (15.4)
Injectable drug use since last HIV testing, (31; 4.2)		
No	695	70 (10.1)
Yes	9	2 (22.2)
Provided goods for sex, (42; 5.7)		
No	662	72 (10.9)
Yes	31	3 (9.7)
Received goods for sex, (42; 5.7)		
No	654	69 (10.6)
Yes	39	6 (15.4)
Condomless sex (past 6 months), (33; 4.5)		
No	175	14 (8.0)
Yes	527	63 (12.0)
Anal sex (past 6 months), (38; 5.2)		
No	661	73 (11.0)
Yes	36	3 (8.3)
Exposure to body fluids (past 6 months), (19; 2.6)		
No	360	40 (11.1)
Yes	356	35 (9.8)
Perceived risk of STIs (past 6 months), (15; 2.0)		
No	244	26 (10.7)
Yes	402	48 (11.9)
Uncertain	74	3 (4.1)
STIs diagnosed (past 6 months), (2; 0.3)		
No	547	62 (11.3)
Yes	186	14 (7.5)
Sexual partner had STIs (last 6 months), (0; 0)		
No	414	47 (11.4)
Yes	106	5 (4.7)
Unknown	215	25 (11.6)
Considering partner notification if STIs positive, (23; 3.1)		
No	80	8 (10.0)
Yes	609	67 (11.0)
Uncertain	23	77 (10.5)
Timing of last HIV test, (11; 1.5)		
Never tested	26	4 (15.4)
0 to < 2 months	188	19 (10.1)
2 to < 4 months	194	19 (9.8)
4 to < 6 months	125	19 (15.2)
≥ 6 months	191	16 (8.4)

Table 1 (continued)

(Missing: N; %)[a]	Undergoing MG testing N	MG-positive N, (row %)
Perceived risk of HIV (past 6 months), (42; 5.7)		
No	378	40 (10.6)
Yes	254	27 (10.6)
Uncertain	61	4 (6.6)
Used PREP (past 6 months), (2; 0.3)		
No	667	69 (10.3)
Yes	66	8 (12.1)
Used PEP (past 6 months), (2; 0.3)		
No	698	70 (10.0)
Yes	35	7 (20.0)
Considering PREP in future, (1; 0.1)		
No	362	37 (10.2)
Yes	291	32 (11.0)
Uncertain	81	8 (9.9)
LE testing outcomes, (0; 0)		
Negative	393	35 (8.9)
Reactive	288	39 (13.5)
Unknown	54	3 (5.6)

LE Leukocyte esterase, *MG* Mycoplasma genitalium, *N* Number, *PEP* Post-exposure prophylaxis for HIV, *PREP* Pre-exposure prophylaxis for HIV, *STIs* Sexually transmitted infections, % percentage

[a] Values in parentheses indicate the number and percentage of missing data for each variable

AMR

One case (0.1%) with a 23S rRNA mutation was detected, indicating AMR to macrolides.

MG coinfections with CT/NG/TV

Figure 1 presents a breakdown of MG and CT/NG/TV coinfections. More than half of the clients ($n=429$, 58.4%) did not have any pathogen detected, and almost one-third ($n=229$, 31.2%) had CT/NG/TV infection only.

A total of 77 (10.5%) cases had MG infections, with 33/77 (42.9%) cases presenting as MG mono-infection and 44/77 (57.1%) as CT/NG/TV coinfections. Disaggregated by pathogen, 29/77 (37.7%) had CT, 15/77 (19.5%) had NG, and 18/77 (23.4%) had TV coinfections respectively.

Among 154 cases with CT pathogens detected, 18.8% ($n=29$) had MG coinfection; the rate was 15.2% ($n=15$) in 99 cases with NG infection and 20.2% ($n=18$) among 89 individuals with TV infection.

MG and coinfections by LE testing results

A total of 465 LE test results (96.1%) were available for women, and 216 (86.1%) for men. Figure 2 presents the distribution of MG and CT/NG/TV infections by LE test result.

In men, MG mono-infection was seen only in negative to weak LE test results, with the highest occurrence in negative LE test results. In women, MG mono-infection appeared across all LE test results but was also most prominent in negative LE test results. In men, most moderate (60.0%) and strong (83.3%) LE test results were due to CT/NG/TV infections only, while this association was less pronounced in women (moderate LE: 24.2%, strong LE: 42.3%).

Syndromic case management and MG coinfection

Antibiotic therapy

In summary, of the 77 cases with MG infection, the majority ($n=51$, 66.2%) received no therapy, and 35/51 (68.6%) of these reported symptoms of STIs. Of the remaining cases, 20 (26.0%) were treated with azithromycin monotherapy, 5 (6.5%) with doxycycline monotherapy, and only 1 (1.3%) with both drugs.

Syndromic screening outcomes and therapy

Figure 3 presents a breakdown of the pathogen status by the outcome of syndromic screening, separately for women (Fig. 3A) and men (Fig. 3B).

In women ($n=484$), 28.1% ($n=136$) presented with VDS, of whom 1 (0.7%) had MG mono-infection, and 14 (10.3%) had MG and CT/NG/TV coinfections. Of these 15 clients with MG infection, 12 received azithromycin

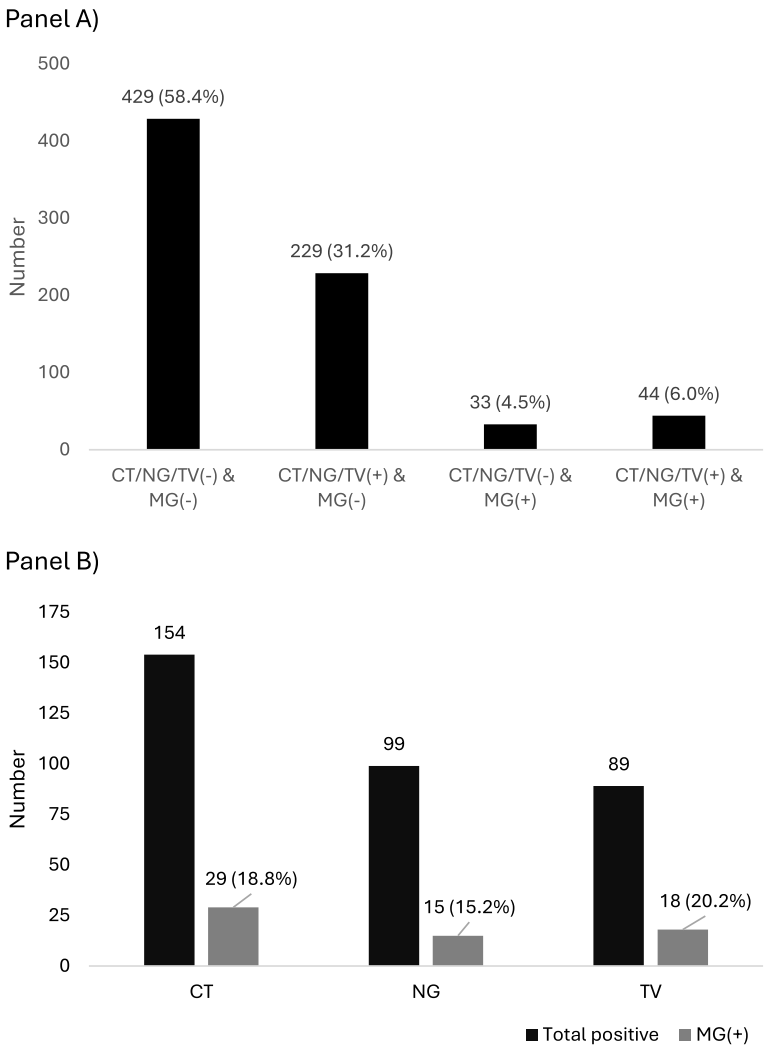

Fig. 1 Distribution of Mycoplasma genitalium mono-infections and coinfections with combined Chlamydia trachomatis, Neisseria gonorrhoeae, and Trichomonas vaginalis (Panel **A**), and with each individual pathogen (Panel **B**). CT, Chlamydia trachomatis; LE, Leukocyte esterase; MG, Mycoplasma genitalium, NG, Neisseria gonorrhoeae; TV, Trichomonas vaginalis; (-), negative; (+), positive. Footnote: *The denominator is the total number of infections with either CT, NG or TV. For Panel **A**, percentages were calculated over the entire number of tests performed (*n*=735). For Panel **B**, percentages of MG coinfection were calculated separately for each of CT, NG and TV

without doxycycline, and 1 received doxycycline only. Among those without VDS ($n=348$, 71.9%), 17 (4.9%) had MG mono-infection, and 23 (6.6%) had MG and CT/NG/TV coinfections. Of these 40 MG cases, 29 (72.5%) reported symptoms of STIs, 4 received azithromycin without doxycycline, and 1 received both azithromycin and doxycycline. The single case of AMR occurred in a woman who reported symptoms and had a CT coinfection, but was neither diagnosed with VDS nor treated.

In men ($n=251$), 31.9% ($n=80$) presented with MUS, of whom 3 (3.8%) had MG mono-infection, and 2 (2.5%) had MG and CT/NG/TV coinfections. Of these 5 clients with MG infection, 4 received azithromycin without doxycycline. Among those without MUS ($n=171$, 68.1%), 12 (7.0%) had MG mono-infection, and 5 (2.9%) had MG and CT/NG/TV coinfections. Among all 17 MG cases, 10 (58.9%) reported symptoms of STIs; none received azithromycin therapy, and 2 received doxycycline only.

Symptomatic MG mono-infection in CT/NG/TV-negative cases

Of 305 individuals that reported symptoms of STIs but tested negative for CT, NG and TV, 23 (7.5%) had MG mono-infections (Table 2). The adjusted odds of symptomatic MG mono-infection were lower for unemployed individuals (adjusted odds ratio: 0.28, 95% confidence

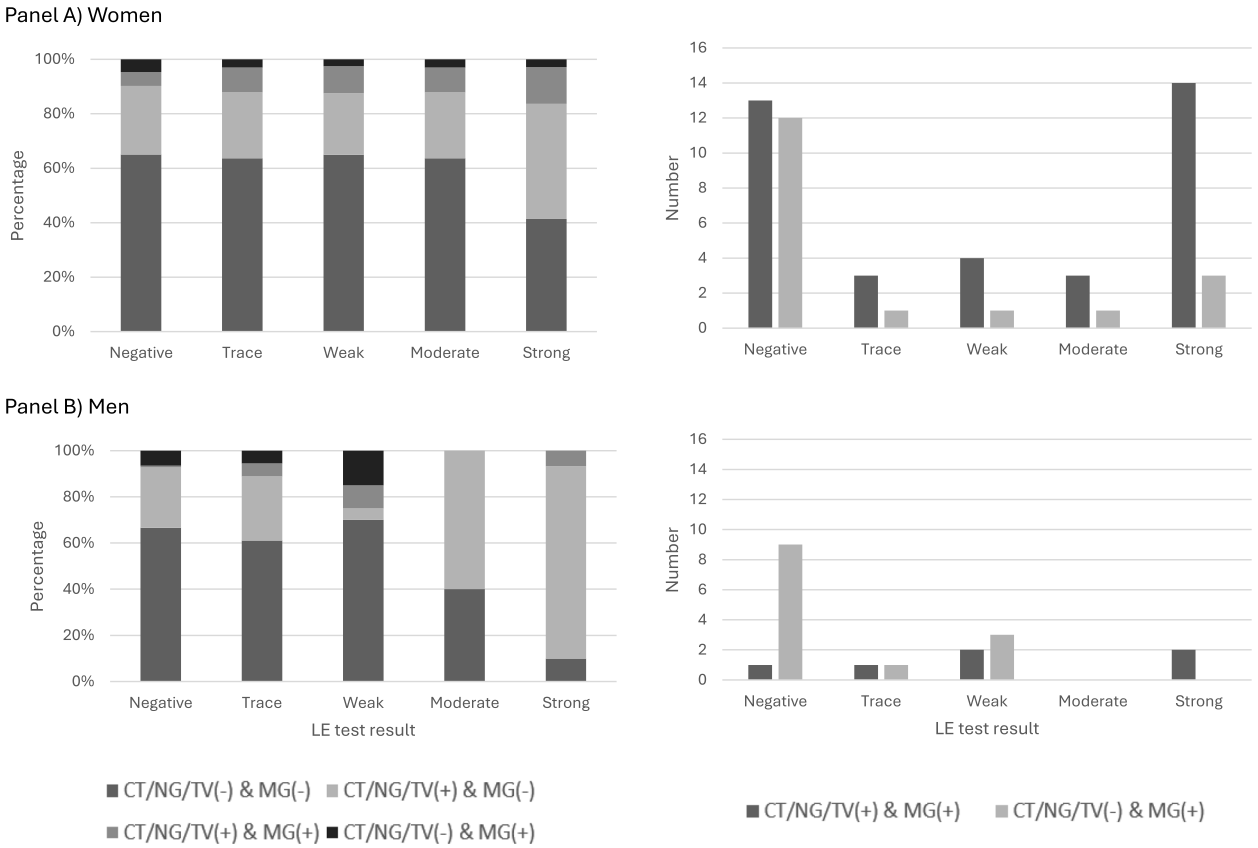

Fig. 2 Distribution of Mycoplasma genitalium and combined infections with Chlamydia trachomatis, Neisseria gonorrhoeae, and Trichomonas vaginalis by leukocyte esterase (LE) test result, with Panel **A** showing results for women and Panel **B** for men. CT, Chlamydia trachomatis; LE, Leukocyte esterase; MG, Mycoplasma genitalium, NG, Neisseria gonorrhoeae; TV, Trichomonas vaginalis; (-), negative; (+), positive

interval: 0.10–0.74) vs employed, and for those who had previously undergone HIV testing ($p=0.026$, Wald test) compared to those never tested (Table 2). By targeting MG testing towards at-risk individuals who were employed and/or never tested for HIV, 35.4% (108/305) of the sample would be tested, with 14.8% (16/108) testing positive for MG, capturing 69.6% (16/23) of all symptomatic MG mono-infections.

Discussion

This is the first study in Eswatini to estimate the prevalence of MG infection. We observed a relatively high MG prevalence among general outpatients, with low macrolide resistance. Coinfections with other curable STIs were common. The syndromic case management approach, complicated by bacterial and protozoan coinfections and overlapping symptoms in clients with MG mono-infections and coinfections, resulted in suboptimal MG identification and treatment.

Interpretation of findings

Prevalence of MG infection

The overall MG prevalence (10.5%) among patients accessing HIV testing, HIV care, and outpatient care aligns with estimates from Southern Africa [6]. The prevalence of MG infection was generally consistent across most baseline factors, though it was higher among individuals engaging in higher-risk behaviours including sex under the influence of alcohol, significant age differences between sexual partners, and transactional sex.

CT/NG/TV coinfections

MG and other curable STIs interacted in various ways. For instance, among patients with detectable CT/NG/TV infection, 15–20% also had MG detected. And, among clients with MG infections, there was a high probability (57.1%) of coinfection with CT/NG/TV. Symptoms of CT/NG/TV infections overlapped with those of MG, making aetiological diagnosis impossible without testing. This challenge extended to distinguishing between asymptomatic and symptomatic MG infections. Many

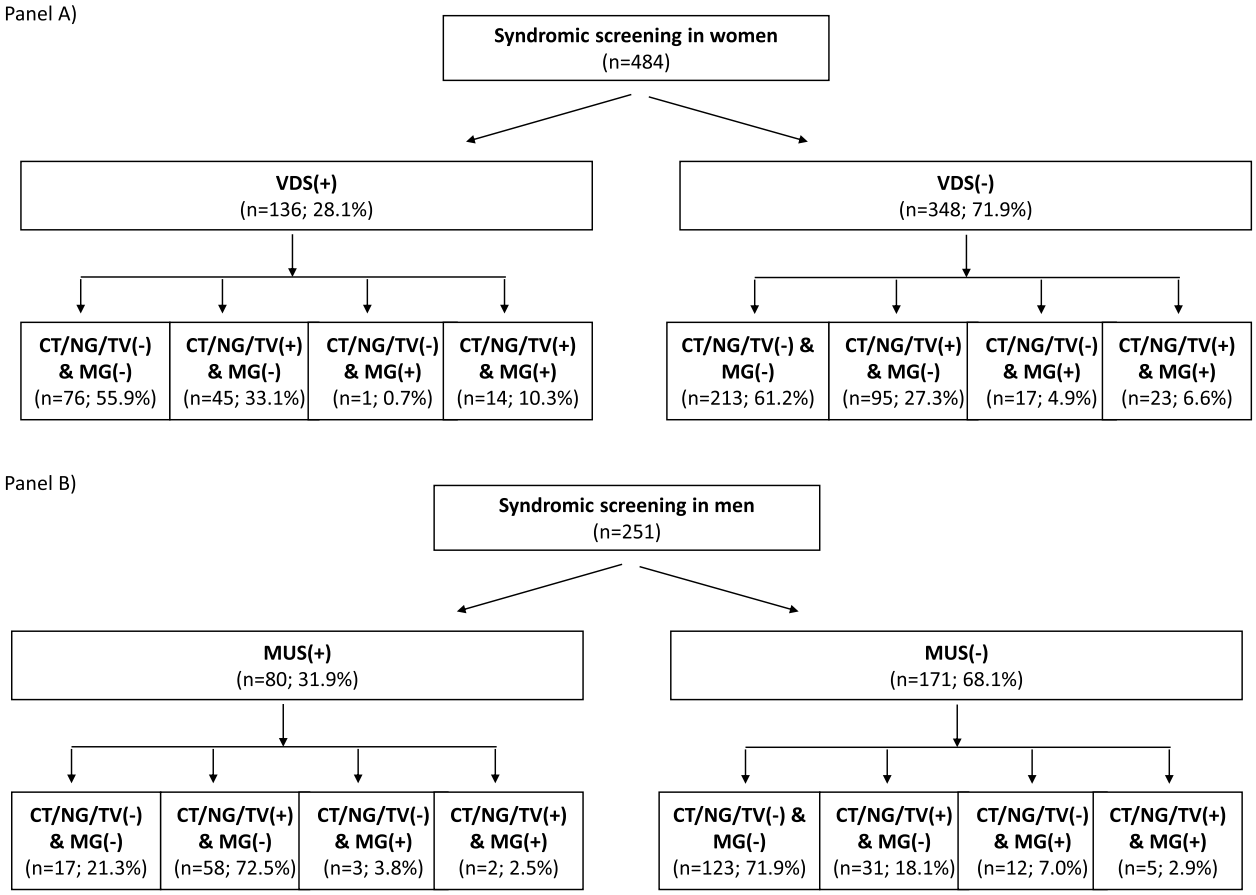

Fig. 3 Pathogen status by syndromic case management diagnosis of vaginal discharge syndrome in women (Panel **A**) and male urethral syndrome in men (Panel **B**), showing detection of Mycoplasma genitalium, and combined infections with Chlamydia trachomatis, Neisseria gonorrhoeae, and Trichomonas vaginalis. CT, Chlamydia trachomatis; MG, Mycoplasma genitalium; MUS, male urethral syndrome; n, number; NG, Neisseria gonorrhoeae; TV, Trichomonas vaginalis; VDS, vaginal discharge syndrome; (-), negative; (+), positive

clients with MG mono-infection reported symptoms of STIs. Given the coinfections, symptom overlap, and high proportion of symptomatic MG mono-infections, routine testing for asymptomatic MG infection in this setting was resource-intensive and probably not advisable. For instance, the US Centers for Disease Control and Prevention does also not recommend screening for asymptomatic MG infections [11].

It was suggested that LE urine testing could be used as a screening method for MG, helping to streamline patients for further diagnostic tests [19]. In this setting, adding LE testing appeared of limited usefulness for differentiation between symptomatic and asymptomatic MG infections, as MG pathogens were detected across all levels of LE test results, most prominently in women. While there was a tendency for fewer MG infections and a higher proportion of CT/NG/TV infections at higher LE reactivity in men, the clinical implications of this finding may be limited.

Antimicrobial resistance

Some high-income settings and high-risk populations in Western countries reported a high proportion of macrolide resistance with population-based estimates ranging from 16% in the UK, while resistance in more focused samples from STI clinics can reach up to 55% in high-risk populations in Belgium [20, 21] and may be even higher in certain clinical settings. In this setting, AMR to macrolides remained low at 0.1%, even post-COVID-19.

This aligns with the lower range of estimates from South Africa prior to COVID-19 (ranging from 1.1% to 9.8%) [6], low resistance levels among men who have sex with men in West Africa (0.6%) [7], and the absence of resistance among pregnant women in KwaZulu-Natal (South Africa) [22] which borders the study setting. Of note, azithromycin was widely used during the COVID-19 pandemic for respiratory infections and remains a key component of syndromic case management in Eswatini, where it is routinely prescribed in primary care clinics for VDS, MUS, and suspected infectious cervicitis or lower

Table 2 Baseline characteristics and predictors of symptomatic Mycoplasma genitalium mono-infection among participants who tested negative for combined infections with Chlamydia trachomatis, Neisseria gonorrhoeae, and Trichomonas vaginalis ($n = 305$)

	Baseline characteristics						Predictors of symptomatic MG infection[a]			
	Total tested	MG test result					Univariate analysis		Multivariable analysis ($n = 297$)	
(Missing: N; %)[*]		MG-negative N (%)		MG-positive N (%)		*p*-value[b]	cOR	95% CI	aOR	95% CI
Facilities, (0; 0)										
Nhlangano Health Center	83	78	(94.0)	5	(6.0)	0.572	1			
FTM	42	37	(88.1)	5	(11.9)		2.09	(0.60—7.26)		
Zombodze	33	32	(97.0)	1	(3.0)		0.66	(0.10—4.19)		
Gege	27	26	(96.3)	1	(3.7)		0.81	(0.13—5.18)		
Nhlangano fixed site	66	59	(89.4)	7	(10.6)		1.8	(0.57—5.69)		
Lavumisa fixed site	54	50	(92.6)	4	(7.4)		1.27	(0.35—4.64)		
HIV status/ testing, (1; 0.3)										
Known HIV +	18	16	(88.9)	2	(11.1)	0.747	1			
Newly diagnosed—established HIV	7	7	(100.0)	0	(0.0)		0.44	(0.02—10.34)		
Newly diagnosed—acute/early HIV	4	4	(100.0)	0	(0.0)		0.73	(0.03—18.18)		
HIV negative	275	254	(92.4)	21	(7.6)		0.56	(0.14—2.26)		
Age category, years, (0; 0)										
18–29	176	162	(92.0)	14	(8.0)	0.567	1			
30–39	91	86	(94.5)	5	(5.5)		0.71	(0.26—1.97)		
40–49	26	24	(92.3)	2	(7.7)		1.14	(0.28—4.67)		
≥ 50	12	10	(83.3)	2	(16.7)		2.67	(0.61—11.72)		
Sex & Reproductive status, (0; 0)										
Men	81	70	(86.4)	11	(13.6)	0.138	1			
Non-pregnant women	176	165	(93.8)	11	(6.3)		0.43	(0.18—1.01)		
Pregnant women – unaware of pregnancy	7	7	(100.0)	0	(0.0)		0.41	(0.02—7.65)		
Pregnant women – aware of pregnancy	15	15	(100.0)	0	(0.0)		0.2	(0.01—3.54)		
Breastfeeding women	26	25	(96.2)	1	(3.8)		0.36	(0.06—2.10)		
Education level completed, (3; 1)										
No formal education	5	5	(100.0)	0	(0.0)	0.703	1			
Primary	26	24	(92.3)	2	(7.7)		1.12	(0.05—26.83)		
Secondary	67	61	(91.0)	6	(9.0)		1.16	(0.06—23.49)		
High school	133	126	(94.7)	7	(5.3)		0.65	(0.03—12.93)		
Tertiary	71	64	(90.1)	7	(9.9)		1.28	(0.06—25.49)		
Employment status, (3; 1.0)										
Employed	104	89	(85.6)	15	(14.4)	0.014	1		1	
Self-employed	36	35	(97.2)	1	(2.8)		0.24	(0.04—1.36)	0.32	(0.06—1.81)
Casual worker	19	18	(94.7)	1	(5.3)		0.47	(0.08—2.69)	0.57	(0.10—3.37)
Unemployed	143	137	(95.8)	6	(4.2)		0.27	(0.11—0.71)	0.28	(0.10—0.74)
In a relationship, (9; 3.0)										
No	22	19	(86.4)	3	(13.6)	0.285	1			
Yes	274	254	(92.7)	20	(7.3)		0.45	(0.13—1.53)		
Wish for a child, (2; 0.7)										
No	206	188	(91.3)	18	(8.7)	0.339	1			
Yes	77	72	(93.5)	5	(6.5)		0.77	(0.29—2.08)		
Uncertain	20	20	(100.0)	0	(0.0)		0.25	(0.01—4.28)		
Last sexual intercourse (past 6 months), (0; 0)										
No intercourse	13	13	(100.0)	0	(0.0)	0.560	1			
< 1 month	236	217	(91.9)	19	(8.1)		2.42	(0.14—42.29)		

Table 2 (continued)

	Baseline characteristics						Predictors of symptomatic MG infection[a]			
	Total tested	MG test result					Univariate analysis		Multivariable analysis ($n=297$)	
(Missing: N; %)*		MG-negative N (%)		MG-positive N (%)		*p*-value[b]	cOR	95% CI	aOR	95% CI
≥ 1 month	56	52	(92.9)	4	(7.1)		2.31	(0.12—45.67)		
Number of sexual partners (past 6 months), (1; 0.3)										
0	14	14	(100.0)	0	(0.0)	0.466	1			
1	216	201	(93.1)	15	(6.9)		2.23	(0.13—39.19)		
2	59	53	(89.8)	6	(10.2)		3.52	(0.19—66.28)		
≥ 3	15	13	(86.7)	2	(13.3)		5.37	(0.24—122.30)		
Age difference with main partner, years, (29; 9.5)										
< 5 yrs	155	143	(92.3)	12	(7.7)	0.937	1			
5-9yrs	91	85	(93.4)	6	(6.6)		0.87	(0.33—2.34)		
≥ 10 years	30	28	(93.3)	2	(6.7)		1.01	(0.24—4.15)		
Sex under influence of alcohol (past 6 months), (13; 4.3)										
No	250	230	(92.0)	20	(8.0)	0.849	1			
Yes	42	39	(92.9)	3	(7.1)		1	(0.31—3.25)		
Injectable drug use since last HIV testing, (12; 3.9)										
No	289	267	(92.4)	22	(7.6)	0.199	1			
Yes	4	3	(75.0)	1	(25.0)		5.1	(0.72—36.19)		
Provided goods for sex, (18; 5.9)										
No	277	254	(91.7)	23	(8.3)	0.342	1			
Yes	10	10	(100.0)	0	(0.0)		0.52	(0.03—9.08)		
Received goods for sex, (18; 5.9)										
No	272	250	(91.9)	22	(8.1)	0.844	1			
Yes	15	14	(93.3)	1	(6.7)		1.15	(0.20—6.53)		
Condomless sex (past 6 months), (16; 5.2)										
No	64	61	(95.3)	3	(4.7)	0.273	1			
Yes	225	205	(91.1)	20	(8.9)		1.75	(0.54—5.64)		
Anal sex (past 6 months), (21; 6.9)										
No	266	243	(91.4)	23	(8.6)	0.193	1			
Yes	18	18	(100.0)	0	(0.0)		0.28	(0.02—4.80)		
Exposure to body fluids (past 6 months), (6; 2.0)										
No	133	121	(91.0)	12	(9.0)	0.440	1			
Yes	166	155	(93.4)	11	(6.6)		0.72	(0.31—1.66)		
Perceived risk of STIs (past 6 months), (4; 1.3)										
No	95	91	(95.8)	4	(4.2)	0.313	1			
Yes	183	166	(90.7)	17	(9.3)		2.14	(0.74—6.21)		
Uncertain	23	21	(91.3)	2	(8.7)		2.36	(0.47—11.90)		
STIs diagnosed (past 6 months), (2; 0.7)										
No	208	191	(91.8)	17	(8.2)	0.365	1			
Yes	95	90	(94.7)	5	(5.3)		0.67	(0.25—1.79)		
Sexual partner had STIs (last 6 months), (0; 0)										
No	162	150	(92.6)	12	(7.4)	0.418	1			
Yes	52	50	(96.2)	2	(3.8)		0.6	(0.15—2.40)		
Unknown	91	82	(90.1)	9	(9.9)		1.39	(0.57—3.36)		
Considering partner notification if STIs positive, (9; 3.0)										
No	24	23	(95.8)	1	(4.2)	0.497	1			
Yes	261	240	(92.0)	21	(8.0)		1.4	(0.25—7.73)		

Table 2 (continued)

	Baseline characteristics						Predictors of symptomatic MG infection[a]			
	Total tested	MG test result					Univariate analysis		Multivariable analysis ($n=297$)	
(Missing: N; %)*		MG-negative N (%)		MG-positive N (%)		*p*-value[b]	cOR	95% CI	aOR	95% CI
Uncertain	11	11	(100.0)	0	(0.0)		0.68	(0.03—18.06)		
Timing of last HIV test, (5; 1.6)										
Never tested	7	4	(57.1)	3	(42.9)	0.002	1		1[c]	
0 to < 2 months	85	80	(94.1)	5	(5.9)		0.09	(0.02—0.45)	0.09	(0.02—0.54)
2 to < 4 months	77	73	(94.8)	4	(5.2)		0.08	(0.01—0.43)	0.08	(0.01—0.47)
4 to < 6 months	59	51	(86.4)	8	(13.6)		0.21	(0.04—1.02)	0.19	(0.03—0.99)
≥ 6 months	72	69	(95.8)	3	(4.2)		0.06	(0.01—0.38)	0.07	(0.01—0.42)
Perceived risk of HIV (past 6 months), (18; 5.9)										
No	162	151	(93.2)	11	(6.8)	0.791	1			
Yes	105	96	(91.4)	9	(8.6)		1.3	(0.53—3.18)		
Uncertain	20	19	(95.0)	1	(5.0)		1.01	(0.17—5.92)		
Used PREP (past 6 months), (1; 0.3)										
No	277	256	(92.4)	21	(7.6)	0.974	1			
Yes	27	25	(92.6)	2	(7.4)		1.17	(0.30—4.61)		
Used PEP (past 6 months), (1; 0.3)										
No	288	266	(92.4)	22	(7.6)	0.838	1			
Yes	16	15	(93.8)	1	(6.3)		1.15	(0.20—6.47)		
Considering PREP in future, (0; 0)										
No	132	123	(93.2)	9	(6.8)	0.767	1			
Yes	138	126	(91.3)	12	(8.7)		1.28	(0.53—3.09)		
Uncertain	35	33	(94.3)	2	(5.7)		0.97	(0.23—4.11)		
LE testing outcomes, (0; 0)										
Negative	183	169	(92.3)	14	(7.7)	0.927	1			
Reactive	103	95	(92.2)	8	(7.8)		1.04	(0.43—2.52)		
Unknown	19	18	(94.7)	1	(5.3)		0.95	(0.17—5.44)		

aOR adjusted odds ratio, *CI* Confidence interval, *cOR* crude odds ratio, *LE* Leukocyte esterase, *MG* Mycoplasma genitalium, *N* Number, *PEP* Post-exposure prophylaxis for HIV, *PREP* Pre-exposure prophylaxis for HIV, *STIs* Sexually transmitted infections, *%* percentage

[a] All factors with a *p*-value < 0.20 in univariate analysis were included in one multivariable model. Variables with the largest *p*-value were removed stepwise until only factors with a *p*-value < 0.05 remained in the final model

[b] Differences across categories were assessed with the Pearson's chi-squared test

[C] The Wald test, used to assess the joint significance of categorical variable levels in multivariable analysis, indicated an overall statistically significant difference between clients who had never been tested for HIV and those who had previously tested, irrespective of timing ($p=0.026$)

[*] Values in parentheses indicate the number and percentage of missing data for each variable

abdominal pain syndrome in women. Given this extensive use, higher macrolide resistance rates might have been expected. The disparity in resistance levels compared to high-income settings could reflect underdetection due to the limited number of molecular-based AMR studies in Sub-Saharan Africa. Additionally, while systematic testing errors are a possible factor, this is unlikely, as routine quality controls did not indicate any issues. MG resistance may also be less common in this setting due to unidentified population-level differences in STIs epidemiology and differentiated risk behaviours, as well as differences in populations targeted. This study primarily served a general outpatient population, whereas STI-focused clinics in high-income Western settings may attract individuals with persistent or recurrent STIs, increasing the likelihood of AMR detection. Nevertheless, STI programs should invest in antibiotic surveillance and increase the rational and targeted prescription of antibiotics to prevent AMR and potential failure of second-line therapies. This is particularly important in settings where curable STIs and MG are highly prevalent,

likely leading to increased antibiotic exposure among sexually active populations.

Case management

Consideration of MG is critical in diagnostic algorithms and treatment strategies [23]. In this setting, several patients diagnosed with VDS or MUS received standard three-drug single-dose therapy for suspected CT/NG/TV infections, including single-dose azithromycin, which is generally ineffective for MG infection [24] and may increase the risk of developing macrolide resistance. In contrast, clients who were not diagnosed with VDS or MUS but had MG detected and reported symptoms of STIs may have needed MG-specific treatment but did not receive it. Notably, the single case with AMR to macrolides and CT coinfection did not receive any therapy, which could potentially contribute to the spread of resistant strains.

We also evaluated the practical implications of a sequential follow-up test for detecting MG in symptomatic clients who tested negative for CT/NG/TV. By focusing testing on at-risk clients for symptomatic MG mono-infection, approximately one-third would require testing, detecting two-thirds of symptomatic MG cases. This two-step approach could reduce resource allocation for molecular-based MG testing, and enable quicker identification of clients needing sequential MG monotreatment, thereby lowering the risk of developing AMR. However, some individuals with symptomatic MG mono-infection were missed, and two-thirds of symptomatic clients would still require close monitoring after a negative CT/NG/TV test result. Although the two-step approach has advantages, only universal MG testing for symptomatic but CT/NG/TV-negative cases would fully benefit all individuals.

Wider considerations

While the number of clients exposed to single-dose azithromycin may seem small, repeated misdiagnosis and ineffective treatment could drive AMR, allowing resistant strains to spread unnoticed. Timely and accurate MG diagnosis is crucial, but the high cost of molecular-based testing raises affordability concerns in resource-limited settings. Affordable tests with macrolide resistance detection are needed. Cost-effectiveness analyses could help assess the broader public health impact of different testing approaches.

Treatment for MG is guided by clinical presentation, with antibiotics prescribed for symptomatic infections to relieve symptoms and prevent complications. In contrast, asymptomatic infections (colonization) may clear on their own without treatment [25]. Due to rising AMR and limited alternative treatments, treating asymptomatic cases with complex treatment regimens could cause more harm than the infection itself [25]. The World Health Organization and other global health authorities, including the British Association for Sexual Health and HIV and the US Centers for Disease Control and Prevention, do not recommend routine screening for MG in asymptomatic individuals but advocate for targeted testing only in symptomatic patients, particularly in cases of persistent or recurrent urethritis, cervicitis, pelvic inflammatory disease, or treatment failure in other STIs [11, 26–28] Thus, monitoring without immediate intervention is recommended to manage potential risks effectively, while screening for asymptomatic cases is discouraged [11, 25]. However, in resource-limited settings where these recommendations may be challenging to implement (e.g. resource constraints, suboptimal follow-up care), there is a need to understand the clinical and public health implications of untreated MG infections.

Recent advancements in near-patient multiplex molecular testing hold promise for improving STI diagnosis and treatment in decentralized and resource-limited settings, but their implementation requires careful oversight. Multiplex platforms should meet high sensitivity and specificity standards and should overall not be used indiscriminately [27–29]. In addition, expanding diagnostic capacity must be accompanied by health worker training to ensure appropriate treatment indication and provision, as well as adherence to international recommendations and national guidelines [27, 29, 30].

Limitations and strengths

This study does not provide estimates of MG infection in the general population but reflects an outpatient population attending routine care in Southern Africa, including HIV testing services, HIV treatment refills, and general outpatient consultations. Therefore, findings are generalizable to similar predominantly rural populations accessing primary and secondary care facilities.

This study did not investigate AMR to fluoroquinolones, which are recommended in a two-step treatment regimen in the presence of macrolide resistance. Data on fluoroquinolone-related resistance are limited, although indications suggest it is uncommon in Southern Africa but reaches 11.3% in high-risk clients using HIV pre-exposure prophylaxis in West Africa [6, 7]. More research is needed to understand the extent of fluoroquinolone resistance.

A main strength of this study is that it is the first in Eswatini to examine the interaction between MG infections and other curable STIs, not only from an epidemiological perspective but also from a case management viewpoint. This study also adds valuable insights to the limited data on MG in Sub-Saharan Africa and can

inform treatment guidelines and policies in settings with no or limited access to molecular-based testing for the confirmation of STIs.

Conclusions

MG infections were common and often co-occurred with CT, NG, and TV. Syndromic case management frequently misclassified these infections, potentially resulting in ineffective azithromycin treatment for MG. A two-step approach for targeted MG testing in at-risk patients could improve antibiotic stewardship. Although macrolide resistance was low, affordable MG testing is essential to prevent the spread of drug-resistant strains.

Abbreviations

AMR	Antimicrobial resistance
COVID	Coronavirus Disease
CT	Chlamydia trachomatis
HIV	Human Immunodeficiency Virus
LE	Leukocyte Esterase
MG	Mycoplasma genitalium
MUS	Male Urethral Syndrome
NG	Neisseria gonorrhoeae
STI	Sexually Transmitted Infection
TV	Trichomonas vaginalis
VDS	Vaginal Discharge Syndrome

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12879-025-10944-0.

Additional file 1: .Table S1. Self-administered patient questionnaire assessing socio-economic and behavioural factors and symptoms suggestive of sexually transmitted infections. Table S2. Baseline characteristics and predictors of undergoing molecular-based Mycoplasma genitalium testing among eligible study participants (n = 1396), based on univariate and multivariable logistic regression.

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Authors' contributions

BK, NN, EM, SD, MM, SM, NS, AC, LTT, and IC conceptualized the study. BK conducted the data analysis. All authors contributed significantly to the study protocol, methodology, and interpretation of the findings. BK drafted the manuscript with substantial input from IC. All authors critically reviewed and approved the final version of the manuscript. BK and IC take final responsibility for the content. Study implementation was supervised by EM and BK.

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Data availability

The datasets generated and/or analysed during the current study are not publicly available because data sharing was not mentioned in the consent form for study participation but are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This study was conducted in full compliance with the ethical principles outlined in the Declaration of Helsinki. Additionally, it adhered to all applicable national guidelines, including the approval of the Eswatini Health and Human Research Review Board (EHHRRB096/2021) and the Médecins Sans Frontières (MSF) Ethics Review Board (ID:2154). Written informed consent was obtained from all participants prior to study enrolment.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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