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Prevalence and macrolide resistance of *Mycoplasma genitalium* from patients seeking sexual health care in Southern Ghana

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Abstract

Background *Mycoplasma genitalium* (MG), a sexually transmitted infection (STI), has emerged as a common cause of non-gonococcal urethritis and cervicitis worldwide, with documented resistance to commonly used antibiotics including doxycycline and azithromycin. Data in Ghana regarding the prevalence of MG is limited.

Methods This retrospective study investigated MG presence and macrolide resistance among patients who previously reported to selected clinics for STI symptoms between December 2012 and June 2020. Samples were screened for MG and mutations associated with azithromycin resistance were investigated using Nucleic Acid Amplification Testing (NAAT) including the Resistance Plus MG® kit from SpeeDx and the LightMix® kit for MG, combined with the Modular Mycoplasma Macrolide from TIB Molbiol.

Results A total of 1,015 samples were screened, out of which MG infection rate by TIB Molbiol and SpeeDx were 3.1% and 3.4%, respectively. The mutation responsible for macrolide resistance was detected in one MG positive sample by both assays. Both diagnostic tests revealed no significant association between MG infection and socio-demographic characteristics, clinical symptoms, gonorrhea, and chlamydia infection status. There was no significant difference in the mycoplasma percentage positivity rate detected using SpeeDx (3.4%) and TIB Molbiol (3.1%).

Conclusions While not commonly tested as a cause of STI symptoms, MG is widespread in Ghana, exhibiting symptoms and prevalence comparable to those in other countries and linked to antimicrobial resistance. Future research using various molecular techniques is essential to monitor resistance trends and guide future antibiotic choices.

Keywords Mycoplasma genitalium, Prevalence, Macrolide resistance, Nucleic Acid Amplification Test (NAAT), Sexually Transmitted Infections (STI)

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Introduction

Mycoplasma genitalium (MG), a fastidious bacterium responsible for non-chlamydial non-gonococcal urethritis (NCNGU), is a sexually transmitted infection (STI) in both men and women [1]. Infections can lead to pelvic inflammatory disease (PID) [2] tubal factor infertility, preterm birth, and spontaneous abortion as well as increased transmission rates among HIV positive patients [1, 3]. Some of the symptoms associated with the disease include discharge, pruritis, dysuria, abdominal/pelvic pain, and dyspareunia [4].

Coinfection with N*eisseria gonorrhoeae* (NG), *Chlamydia trachomatis* (CT), or *Trichomonas vaginalis* (TV) [5] has been observed in MG infections, which have also been reported as co-infecting agents in various STI studies conducted globally [1, 2]. Risk factors associated with acquiring MG are age<25 years old, increased sexual partners and high-risk sexual behaviours [5, 6].

Although MG is the second most prevalent cause of non-gonococcal urethritis (NGU) [7] after CT, its prevalence varies widely in different countries. A West African study conducted in 2002 involving Ghana and Benin established a 23.3% prevalence of MG among female sex workers (FSW) from Accra, Ghana [8]. Varying prevalence rates of MG infection was reported among FSW and non-FSW (4.7% and 2%, respectively, in one study) and an overall percentage of 3.3% among 300 women attending an STI clinic in Kumasi, Ghana [9]. A similar study also conducted in Kumasi reported a prevalence of 4.5% among 200 symptomatic and asymptomatic women attending an STI clinic in 2014, using multiplex PCR [10].

Owing to the slow and poor growth of MG in culture, its detection and diagnosis is almost exclusively performed using NAAT [11]. Over the years, several kits targeting *16 S rRNA*, MgPa encoding the adhesion protein, *P115*, or the *gap* genes have been used for gene-based detection of MG via various conventional and real-time PCR methods [3, 12–14].

Due to MG resistance to macrolides, the Center for Disease Control (CDC) recommends doxycycline (100 mg twice daily for 7 days) as the first line treatment option for MG [13, 15, 16], whereas Ghana's standard treatment guidelines recommend the use of doxycycline, tetracycline, erythromycin, or azithromycin to treat patients with symptoms of NGU and cervicitis [17]. Unfortunately, macrolide resistance, first reported in Australia in 2006 has gradually increased in countries where macrolides are the recommended empiric treatment of choice for NGU and cervicitis [13]. The resistance mechanism involves the alteration of ribosomal proteins, preventing macrolides from binding to the ribosomes and causing the inhibition of bacterial protein synthesis [3, 13]. Mutations identified in the V region of the *23 S rRNA* gene occurring at positions A2058G, A2058T, A2058C, A2059G, or A2059C (*E. coli* numbering) corresponding to position 2063/2064 in Mycoplasma are responsible for macrolide resistance [16, 18]. Macrolide resistance has been documented in the United States, South Africa, and Japan with fewer reports from West Africa, including Ghana [3, 18, 19].

The objective of this study was to detect the presence of MG and macrolide resistance in archived samples. Additionally, two commonly used diagnostic assays from TIB Molbiol and SpeeDx were compared. Results of this study provided baseline information on current MG and macrolide resistance status among STI patients in Southern Ghana.

Methods

Sample processing from stored data

In a prior investigation on surveillance, specimens obtained from patients diagnosed with sexually transmitted infections (STIs) were analyzed for the presence of NG and CT. Among male samples, 47.6% showed negative results for both NG and CT, while among female samples, this percentage was 76% [20]. Men were included if they exhibited symptoms such as urethral discharge or dysuria, while women were included if they displayed symptoms like vaginal discharge, intermenstrual bleeding, or abdominal pain. The study utilized 1,349 archived urine samples from patients stored in a freezer at -80 °C, along with completed interviewer-administered questionnaires, spanning from June 2012 to December 2020 [21]. For the present investigation, 1,015 patients who had provided consent for future use of their samples and had provided an adequate sample volume were screened for MG and macrolide resistance. Samples were collected from individuals attending five specific clinics: the three military clinics affiliated with 2 Garrison in Sekondi/Takoradi, as well as 37 Military Hospital and the Adabraka STI clinic in Ghana.

Specimen analysis and NAAT testing

DNA was extracted from urethral swabs and urine samples using the QIAamp DNA Mini kit and QIAamp Circulating kit, (QIAGEN, USA), respectively, according to manufacturer's instructions.

Two research-only approved assays were used in the study: Resistance Plus MG® kit from SpeeDx (Sydney, Australia), and the LightMix® kit for MG combined with the Modular Mycoplasma Macrolide from TIB Molbiol (Berlin, Germany).

NAAT was performed with the Roche LightCycler 480II instrument using two commercial kits [3, 12] The ResistancePlus™ MG kit from SpeeDx, Pty Ltd (Sydney, Australia) was used for the simultaneous detection of the MG *MgPa* gene and macrolide resistance markers comprising five mutations (A2058G, A2059G, A2058C,

A2059C and A2058T) [*E. coli* numbering] in the *23 S rRNA* gene. Interpretation of results was done with the ResistancePlus™ MG FastFinder™ Analysis Software. Each sample well was a qPCR multiplex that consisted of three read-outs shown on the software during analysis. The first read-out indicated the presence or absence of the MgPa adhesin gene, to determine MG positivity, the second showed the presence or absence of macrolide resistance via the detection of five mutations in the *23 S rRNA* gene, and the third read-out was an internal control to detect extraction efficiency and qPCR inhibition [16].

The LightMix® kit for MG detection was used to target the *gap* gene using the LightCycler 480II instruments and viewing results in channel 640. The LightMix Modular Mycoplasma Macrolide test kit from TIB Molbiol (Berlin, Germany) was also used for macrolide resistance testing targeting the five mutations in the *23 S rRNA* gene [12].

Ethical clearance

This study was approved by the institutional review boards (IRBs) of the Noguchi Memorial Institute for Medical Research (NMIMR) (approval number: NMIMR-IRB CPN 016/20–21) and the Naval Medical Research Command, Maryland (approval number: NAMRU-PJT-20-02).

Statistical analysis

Categorical variables were described using frequencies and percentages. Statistical tests from the bivariate analysis were performed using chi-square test. Mycoplasma percentage positivity results from the two assays were compared using exact McNamar's test. Agreement of the two assays was determined using Cohen kappa's (k) test, where a k value of 1 indicates complete agreement.

To estimate the infection proportion of MG, sensitivity, and specificity of the two assays in the absence of a gold standard and with assumption of conditional independence, the Bayesian latent class method (BLCM) was used. Uniform prior was assumed for mycoplasma prevalence and weak informative prior was assumed for sensitivity and specificity of the two assays. Model fitting was performed using Markov-chain Monte Carlo (MCMC) simulation with a Gibb's sampler. Analysis was performed using R statistical software. The statistical package, JAGS version 4.3.0 in the R package rjags was used [22].

Results

Socio-demographic characteristics of the study participants

A total of 1,015 archived samples were tested, with majority (61.1%) of the samples from females. Approximately, 71% of the samples were from patients under 32 years. The samples had a gonorrhea positivity of 24.2% (246) and chlamydia positivity of 9.9% (100). Positive results for MG did not have any significant association with socio-demographic characteristics, clinical symptoms, gonorrhea, and chlamydia infection status (Table 1).

There was no significant difference in the mycoplasma positivity detected by SpeeDx (3.4%) and TIB Molbiol (3.1%). The kappa value of 0.937 indicates that the two-test had an almost perfect agreement. (Table 2).

Both tests identified macrolide resistance in a single sample. Specifically, the SpeeDx assay detected macrolide resistance in 2.9% (95% CI: 0.4–19.2%) of cases, while Tibmolbiol identified it in 3.2% (95% CI: 0.4–21.6%) among the positive MG samples.

Coinfection status of Mycoplasma among patients with Gonorrhea and Chlamydia

In the Bayesian class model, MG positivity was 3.3[2.5–4.5] % (Table 3).

Among patients that were diagnosed as gonorrhea positive, MG positivity was 4.2[2.0-7.2] %. Regarding patients that tested positive for chlamydia, 3% had MG (Additional file 1). One patient tested positive for all three pathogens irrespective of the assay for testing MG.

Diagnostic performance of SpeeDx and TIB Molbiol using bayesian latent class model (BLCM)

To determine the diagnostic estimates of SpeeDx and TIB Molbiol, Bayesian latent class model was used because both tests are imperfect. Visual inspection of the trace plot for all the parameters indicates that the Markov Chain Monte Carlo (MCMC) chain converged (Additional file 2a & 2b). The sensitivity of SpeeDx was 84.1[74.3–92.0] %, this was slightly higher than the sensitivity of TIB Molbiol which was 83.8[74.0- 91.7] %. However, the specificity of SpeeDx, 98.3[97.6–99.0] %, was slightly lower than the specificity of TIB Molbiol; 98.9[98.3–99.4] % (Table 3).

Macrolide resistance

The only sample that showed macrolide resistance was collected from in 2014 from a 30-year-old male who took an unspecified antibiotic prior to his hospital visit. He was treated according to the standard of care at the health center which involved a 250 mg intramuscular injection of ceftriaxone and a 1 g of oral azithromycin. MG culture and subsequent phenotypic testing were not done; however, NAAT was performed using the TIB Molbiol and SpeeDx assays. Both assays detected mutations in the V region of the *23 S rRNA* gene of the sample, responsible for macrolide resistance.

Discussion

The study aimed to investigate the proportion of infection of MG and the presence of macrolide resistance among STI patients who presented at the five selected health

Table 1 Characteristics of patients whose archived samples were used in this study

Characteristics	Total *N* (%)	SpeeDx, + *n* (%)	*p*-value[e]	TIB Molbiol, +ve *n* (%)	*p*-value[e]
[a]Sex					
Male	393(38.9)	19(4.8)	0.058	16(4.1)	0.141
Female	617(61.1)	16(2.6)		15(2.4)	
[b]Age (years)					
18–24	301(29.9)	12(4.0)	0.295	12(4.0)	0.349
25–31	413(41.0)	12(2.9)		10(2.4)	
32–38	191(19.0)	10(5.2)		8(4.2)	
39–45	70(7.0)	0(0.0)		0(0.0)	
Above 46	32(3.2)	1(3.1)		1(3.1)	
[c]Education					
No schooling	36(3.6)	1(2.8)	0.779	1(2.8)	0.986
Basic	185(18.4)	8(4.3)		6(3.2)	
Secondary and above	782(78.0)	26(3.3)		24(3.1)	
Symptoms					
Dysuria and/or urethral discharge	961(94.7)	33(3.4)	0.916	29(3.0)	0.776
[d]Other symptoms	54(5.3)	2(3.7)		2(3.7)	
Gonorrhea infection					
Positive	246(24.2)	10(4.1)	0.542	9(3.7)	0.527
Negative	769(75.8)	25(3.3)		22(2.9)	
Chlamydia infection					
Positive	100(9.9)	3(3.0)	0.796	3(3.0)	0.974
Negative	915(90.1)	32(3.5)		28(3.1)	
Gonorrhea/Chlamydia Coinfection					
Yes	1,310(97.1)	1(3.1)	0.919	1(3.1)	0.981
No	39(2.9)	34(3.5)		30(3.1)	
Total	**1015**	**35(3.4)**		**31(3.1)**	

[a]5 patients' information on sex was missing

[b]8 patients' information on age was missing

[c]12 patients' information on education was missing

[d]itching, abdominal pain, pain when having sex, foul smell/odor from urine, bleeding from penis or vagina

[e]*p*-values were obtained using a Chi-square test

+ve: positive

Table 2 Results for *Mycoplasma genitalium* detection using SpeeDx and TIB Molbiol

		SpeeDx		Total
		Positive	**Negative**	
TIB Molbiol	Positive	31	0	31
	Negative	4	980	984
	Total	35	980	1015

Exact McNemar *p value*=0.125

Kappa=0.937

Agreement=99.6%

Table 3 Performance of SpeeDx and TIB Molbiol using bayesian latent class model (BLCM)

	Model with all Patients	Model with Patients diagnosed with Gonorrhea
Mycoplasma positivity (95% CrI)	3.3(2.5–4.5)	4.2(2.0–7.2)
SpeeDx		
Sensitivity (95% CrI)	84.1(74.3–92.0)	78.5(63.0–92.3)
Specificity (95% CrI)	98.3(97.6–99.0)	97.6(95.6–99.0)
TIB Molbiol		
Sensitivity (95% CrI)	83.8(74.0–91.7)	78.9(59.2–93.6)
Specificity (95% CrI)	98.9(98.3–99.4)	96.8(94.6–98.5)

CrI: Credible interval

centers in the southern part of Ghana. We used two different diagnostic assays, TIB Molbiol and SpeeDx, to compare and detect MG. Utilizing NAAT is a strength of the study as MG prevalence has not been well described previously in Ghana.

The MG proportion of infection (3.3%) (Table 3) among individuals presenting to sexual health clinics is similar to the estimate from Agyarko and colleagues' study conducted among 300 Female Sex Workers (FSW) at Suntreso Government Hospital in Kumasi [9]. Many studies have reported higher MG infection rates among specific population types such as FSW and patients

seeking gynecological, antenatal, and general healthcare [5, 8–10]. The FSW cohort belong to a higher-risk group. It is important to note that these higher prevalence rates do not necessarily mean that these population types are inherently more susceptible to the diseases. Instead, it may be due to a combination of social, economic, and environmental factors that increase their risk of infection [23].

Our study outcome showed a 4.2% positivity rate of MG among patients with NG (Table 3) while MG was 3% among patients who tested positive for CT (Additional file 1). This was comparable to the co-infection rates in a prospective study conducted in Barcelona (Spain) that tested 249 asymptomatic patients for MG using the SpeeDx assay. In that study, patients with NG had an MG positivity rate of 4.8%, while patients with CT had a co-infection rate with MG of 4.0% [24]. It is important to note that the prevalence of MG can vary among different populations and geographic locations, and the use of different diagnostic tests can also affect the results [5]. Nonetheless, these findings suggest that co-infection with MG, NG, CT and other STIs is not uncommon among patients attending STI screening services [3, 6].

Many studies have reported high rates of macrolide resistance among MG-positive cases [3, 16, 18, 19, 25]. In comparison, this study identified just one macrolide-resistant sample, relatively low number but highly significant as it represents the first reported case from mycoplasma research in Ghana. However, the low proportion of macrolide resistance is similar to other studies of MG in Africa [19, 26, 27]. The presence of macrolide resistance is of public health concern, as it can have implications for the effectiveness of treatment and the potential for further spread particularly in the setting of overprescribing of the antibiotics belonging to this class.

Socio-demographic characteristics (gender, age, and educational level), clinical symptoms, CT and NG infection had no association with MG infection. The lack of statistical association between MG infection status and potential risk factors in this study may be due to the study site preference and the low cases of infection identified during the study. It is possible that increased association of infection with certain socio-demographic factors, such as age and education, could be more pronounced in certain populations, such as female sex workers (FSW), where sexual behavior and the number of sexual partners may have a greater impact on the likelihood of getting an infection. This is further demonstrated in the study of Agyarko-Poku et al. in 2013, which was conducted among female sex workers in Kumasi where age was identified as the strongest predictor of MG infection [9]. Other studies among asymptomatic women and female sex workers have also found that age and education are associated with MG infection [5, 8].

Regarding the clinical symptoms (Table 1), there was no association found between MG infection and urethral discharge and/or dysuria, despite this symptom being commonly reported in other studies on STIs [8, 28]. Additionally, this study did not observe the commonly reported association between MG and other STIs such as NG and CT. High associations of MG (22.2%), NG (24.2%), and CT (32.7%) among patients with urethritis were found in a cross-sectional study among 290 men attending STD clinics in Baltimore city [29]. This study showed the importance of screening patients for mycoplasma as a possible cause of non-gonococcal urethritis. Other studies have reported such associations [6, 8].

The sensitivity and specificity of the TIB Molbiol and SpeeDx assays were compared to assess the variability and authenticity of both assays. In this study, the TIB Molbiol LightMix kit MG assay showed a sensitivity and specificity of 83.8% and 98.9%, respectively, which are different from results in an evaluation study of two commercial kits where sensitivity and specificity were reported as 92.6% and 100%, respectively [2, 12]. The SpeeDx assay, on the other hand showed similar sensitivity and specificity (84.1%; 98.3%) as shown by the TIB Molbiol LightMix kit MG assay results. Studies investigating the sensitivity and specificity of the SpeeDx assay in the presence of Mycoplasma and macrolide resistance have shown higher sensitivity and specificity results of 99.1%-97.4% and 98.5-100%, respectively [16, 25]. The main advantage of the SpeeDx assay is its ability to simultaneously detect MG and mutations associated with macrolide resistance, which can expedite treatment decisions. However, the TIB Molbiol assay requires an initial MG diagnostic test before macrolide testing, which can result in a delay in reporting the mutation status and thus a delay in administering appropriate treatment. This is an important consideration when selecting a diagnostic assay for MG infections, as timely treatment is essential for the effective management (timely cure) of this infection [25]. It is also worth noting that more sensitive assays may detect a higher number of positive cases, which could lead to the identification of more potential associations with various socio-demographic factors [30].

Conclusions

Overall, the study has shown the occurrence of MG and macrolide resistance, which has contributed to the understanding of STI epidemiology and can inform treatment regimen. Results from the study have shown that although MG is not often screened for as an etiology of STI symptoms, it is present in Ghana and shows similar symptoms as well as prevalence with other countries. To inform future antibiotic selection in Ghana, there is the need for the continuous monitoring of antimicrobial resistance in future STI studies.

Limitations

It is important to note that the long duration of storage of specimens used in this retrospective study (up to eight years) could have affected the results outcome, as previously reported by Murray and colleagues [31]. Since the study enrolled only patients who showed symptoms, mycoplasma prevalence in asymptomatic individuals is absent. The data presented in this study were from the southern parts of Ghana and is not representative of the entire Ghanaian population.

Abbreviations

BLCM	Bayesian latent class method
CDC	Center for Disease Control
CT	Chlamydia trachomatis
EURAFCENT	European, African, and Central command of the US Naval Medical Research
FSW	Female Sex Workers
GEIS	Global Emerging Infections Surveillance
IRB	Institutional Review Board
MCMC	Markov-chain Monte Carlo
MG	Mycoplasma genitalium
NAAT	Nucleic Acid Amplification Test
NCNGU	Non-Chlamydial Non-Gonococcal Urethritis
NG	Neisseria gonorrhoeae
NGU	Non-gonococcal Urethritis
NMIMR	Noguchi Memorial Institute for Medical Research
PID	Pelvic Inflammatory Disease
STI	Sexually Transmitted Infections
TV	Trichomonas vaginalis

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

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Author contributions

Study conception and design: NA, HD, and EB. Study execution: NA, HD, EB, KO, JNY. Data analysis: EB. Manuscript composition: HD, NA, EB, HM, AL, AF, TS, KKA. All authors reviewed and endorsed the final manuscript. The author assumes complete accountability for the study's execution and/or conduct, had access to the data, and had authority over the decision to publish. The corresponding author confirms that all named authors fulfill the criteria for authorship and that no other eligible contributors have been excluded.

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Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Approval for this study was granted by the institutional review boards (IRBs) of the Noguchi Memorial Institute for Medical Research (NMIMR) (approval number: NMIMR-IRB CPN 016/20–21) and the Naval Medical Research Command, Maryland (approval number: NAMRU-PJT-20-02). Eligible patients were made to provide a written informed consent before enrollment.

Consent for publication

Not applicable.

Conflict of interest

The authors declare no conflict of interest. Some authors are military service members or employees of the U.S. Government. This work was prepared as part of their official duties. Title 17, U.S.C., § 105 provides that copyright protection under this title is not available for any work of the U.S. Government. Title 17, U.S.C., § 101 defines a U.S. Government work as work prepared by a military service member or employee of the U.S. Government as part of that person's official duties. The funders had no role in the study's design, in the collection, analyses, or interpretation of data, in writing the manuscript, or in the decision to publish the results.

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