

PREVALENCE OF *NEISSERIA* *GONORRHOEAE* AND *MYCOPLASMA* *GENITALIUM* IN BULGARIAN POPULATION OF REPRODUCTIVE AGE FROM SOFIA MUNICIPALITY, 2018-2019

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ABSTRACT

Background: *N. gonorrhoeae* and *M. genitalium* infections remain public health concern due to emerging antimicrobial resistance which compromise effective treatment and infection prevention and control.

Objective: To estimate the prevalence of *N. gonorrhoeae* and *M. genitalium* infections and identify demographic and sexual behavioral risk factors in Bulgarian population of reproductive age from Sofia municipality.

Material and methods: Participants were sexually active 443 men and 198 women aged 18-49 years. They answered questionnaires on sexual behavior and provided samples for *N. gonorrhoeae* and *M. genitalium* testing.

Results: The prevalence of *N. gonorrhoeae* infection was 3.28% (21/641) and with *M. genitalium* infection it was 2.49% (16/641). For both infections the presence of urogenital

symptoms was associated with a greater risk of being infected. Among gonococcal infections an increased number of lifetime partners and partners during the last 6 months, previously diagnosed STIs and use of contraception other than condoms were associated risk factors.

Conclusions: The prevalence of *N. gonorrhoeae* and *M. genitalium* infections in Bulgarian population of reproductive age from Sofia municipality was relatively low but due to the significant disease burden data on prevalence and identified risk factors should help the development of future control strategies.

Keywords: *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, prevalence, risk factors, Bulgaria

INTRODUCTION

Bacterial sexually transmitted infections (STIs) remain a global public health concern. In 2016, the World Health Organization (WHO) estimated 376 million new cases worldwide of four curable non-viral STIs. Among the estimated bacterial STIs, *Neisseria gonorrhoeae* was one of the most frequent infections (87 million cases) (1). Additionally, STIs caused by *Mycoplasma genitalium*, which are not estimated by the WHO, are exceedingly prevalent (2,3). The emergence of antimicrobial resistance in *N. gonorrhoeae* and *M. genitalium* is a major concern, as it has significantly compromised treatment effectiveness and infection prevention and control. The development of antimicrobial resistance would not only result in an increased prevalence of these bacterial infections but also in a considerably increasing number of severe complications affecting reproductive health (4). STIs are considered a priority in Bulgarian public health system and gonorrhea is a nationally notifiable disease (5). However, as in other settings the reporting of *M. genitalium* is not mandatory and the reporting of gonorrhea cases is likely to be incomplete (6). For these reasons and because of the scarce published studies on *N. gonorrhoeae* and *M. genitalium* infections in recent years

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(7–9), supplemental data on gonorrhea and genital mycoplasma prevalence would be very useful in assessing the burden of disease in Bulgarian population. The main objective of this study was to estimate the prevalence of *N. gonorrhoeae* and *M. genitalium* infections and the association with possible risk factors in Bulgarian population of reproductive age (18–49 years) from Sofia municipality.

MATERIALS AND METHODS

Study population

This study was performed on specimens and questionnaires obtained in the period 2018–2019 from a population of 18–49-year-old sexually active individuals living in Sofia municipality. Sofia Municipality has a population of 1 291 591 inhabitants (comprising 17.6% of the Bulgarian population) and houses the largest city in Bulgaria, i.e. Sofia, with 1 202 761 inhabitants. A total of 500 men and 500 women were randomly invited to participate, and 443 men and 198 women submitted specimens to be tested for *N. gonorrhoeae* and *M. genitalium* infection. A total of 835 specimens from 641 patients were tested for *N. gonorrhoeae* and *M. genitalium* in the 2-year study period. One hundred and ninety-four specimens were excluded from statistical analysis because they originated from a follow-up study of positive patients (n=39) and from patients who had more than one specimen taken on the same date (n=155). In the event of a discrepant result (negative/positive) from samples taken on the same day, the negative specimen was excluded, leading to a registration of the patient as a positive one. Among the specimens with discrepancy between test results, the vast majority of patients (80.6%) were positive for the urogenital specimen and negative for the specimen from an extragenital site.

Detection of *N. gonorrhoeae* and *M. genitalium*

In 2018 and 2019, 680 urogenital (446 first void urine samples from men and 234 vaginal

swabs from women) and 155 extragenital specimens were tested for *N. gonorrhoeae* and *M. genitalium* infections at the National Center of Infectious and Parasitic Diseases, Bulgaria. *N. gonorrhoeae* and *M. genitalium* were detected by AmpliSens multiplex real-time PCR assay (InterLabService, St. Petersburg), according to manufacturer's instructions (10). All positive results were confirmed by a duplex PCR targeting the gonococcal *porA* pseudogene and multicopy *opa* genes (11) and PCR detecting the *MgPa* adhesion gene (12) for gonorrhea and genital mycoplasma, respectively.

Demographic and behavioral characteristics

The demographic and behavioral characteristics were collected as follows: assigned sex at birth (female/male), age (in full years and grouped as 18–20, 21–30, 31–40 and >40 years), urogenital symptoms (yes/no), number of partners during the last 6 months (grouped as 0–1, 2–3 and >3 partners), number of lifetime partners (grouped as 1–3, 4–10 and >10 partners), use of contraception (use of condoms/occasional use of condoms/other), HIV status known (yes/no), previously diagnosed with other STIs, including *N. gonorrhoeae* and *M. genitalium* (yes/no).

Statistical analyses

First, the prevalence of *N. gonorrhoeae* and *M. genitalium* infection with 95% confidence intervals (CI) was calculated, then a bivariate logistic regression analysis with demographic and behavioral characteristics as independent variables and *N. gonorrhoeae* or *M. genitalium* diagnosis as the dependent variable was performed (13). Bivariate odds ratios (ORs) and 95% CIs were reported. In the statistical analysis, $p < 0.05$ was considered significant (14).

Ethics and informed consent

Written informed consents were obtained from all participants for the demographic data collection and sample testing as required by the National law and the Ethics Committee at the National Center for Infectious and Parasitic Diseases, Sofia, Bulgaria.

RESULTS**Demographic characteristics and sexual behavior**

Among the 1000 randomly invited sexually active people living in Sofia municipality, 35.9% did not submit any specimen for microbiological testing, neither filled the questionnaire and were excluded. In total, 641 participants were

included in the study (30.89% females and 69.11% males). Demographic and behavioral characteristics of participants are summarized in Table 1. The mean age was 35 ± 4.9 years. The majority of participants were asymptomatic (85.8%) and the median number of partners was 6 (IQ25-75: 4-10) in total and 2 (2-4) during the last 6 months.

Table 1. Distribution of demographic and behavioral characteristics in 18–49-year-old sexually active people (n=641) from Sofia municipality, 2018-2019

	N	%
Sex		
Female	198	30.89
Male	443	69.11
Age		
18-20	35	5.46
21-30	178	27.77
31-40	262	40.87
>40	166	25.90
Urogenital symptoms		
Yes	91	14.20
No	550	85.80
Number of partners during the last 6 months		
0-1	510	79.56
2-3	63	9.83
>3	68	10.61
Number of lifetime partners		
1-3	422	65.84
4-10	87	13.57
>10	132	20.59
Use of contraception		
Use of condoms	75	21.53
Occasional use of condoms	63	
Other [#]	503	78.47
HIV status known		
Yes	215	33.54
No	426	66.46
Previously diagnosed with other STIs, incl. N. gonorrhoeae and M. genitalium		
Yes	116	18.10
No	525	81.90

[#] Oral contraception, intra uterine devices, fertility awareness, coitus interruptus

Prevalence

The prevalence of *N. gonorrhoeae* and *M. genitalium* infection in Bulgarian population of reproductive age (18-49 years) from Sofia municipality was 3.28% (95% CI 2.58% to 3.98%) and 2.49% (95% CI 1.29% to 3.69%), respectively as shown in Table

2. Among the positive cases 19 men and 2 women (mean age 32 and 30 years, respectively) had a gonococcal infection, whereas 15 men (mean age 28 years) and 1 woman (age 27) were infected with *M. genitalium*. No *N. gonorrhoeae* and *M. genitalium* co-infection was identified.

Table 2. Prevalence of *N. gonorrhoeae* and *M. genitalium* in 18–49-year-old sexually active people (*n*=641) from Sofia municipality, 2018-2019.

	N	%	95% CI
<i>N. gonorrhoeae</i> infection	21	3.28	2.58 - 3.98
<i>M. genitalium</i> infection	16	2.49	1.29 – 3.69

Risk factors

In the bivariate analysis associated with risk factors with *N. gonorrhoeae*, it was found that increasing the number of partners was associated with a greater risk of being infected (Table 3). Thus, subjects with >3 partners during the past 6 months had an OR of 7.99 (95% CI 1.0- 63.65) of being infected compared with those having 0-1 partner; subjects with 2-3 partners during the past 6 months had an OR of 8.81 (CI 95% 1.12-68.91) compared with those having 0-1 partner; and subjects with >10 lifetime partners had an OR of 3.61 (1.01-12.87) compared with those having 1-3 partners. Also, the use of contraception influenced the *N. gonorrhoeae* risk and using oral contraception, intra uterine devices, fertility awareness and coitus interruptus significantly increased the

risk of the infection compared with those who use condoms (OR=5.05; CI 95% 1.1- 23.0). Having urogenital symptoms and previously being diagnosed with other STIs (including *N. gonorrhoeae* and *M. genitalium* infection) was significantly associated with a gonococcal infection (OR=11.42; CI 95 % 2.63- 49.46 and OR=11.31; CI 95% 4.29- 29.78, respectively). Known HIV status was not associated with being infected.

For infections with *M. genitalium* having urogenital symptoms was associated with a greater risk of being infected (OR=5.21; CI 95% 1.47- 18.46) but neither the number of partners (lifetime and during the last 6 months), previously diagnosed STIs, nor contraception habits were significantly associated with the presence of the infection.

Table 3. Statistical analysis of possible risk factors for *N. gonorrhoeae* and *M. genitalium* infections.

	<i>N.gonorrhoeae</i> (%)	OR (95% CI)	P-value	<i>M.genitalium</i> (%)	OR (95% CI)	P-value
Urogenital symptoms						
Yes	19 (6.53%)	11.42 (2.63- 49.46)	0.0011*	13 (4.47%)	5.21 (1.47- 18.46)	0.0105*
No	2 (0.57%)	1		3 (0.85%)	1	
Number of partners during the last 6 months						
0-1	1 (0.53%)	1		4 (2.1%)	1	
2-3	11 (4.64%)	8.81 (1.12- 68.91)	0.0380*	5 (2.11%)	1.02 (0.26- 3.78)	0.0810

	<i>N.gonorrhoeae</i> (%)	OR (95% CI)	P-value	<i>M.genitalium</i> (%)	OR (95% CI)	P-value
>3	9 (4.21%)	7.99 (1.0-63.65)	0.0497*	7 (3.27%)	1.55 (0.44-5.39)	0.4875
Number of lifetime partners						
1-3	3 (1.61%)	1		4 (2.15%)	1	
4-10	5 (2.16%)	1.33 (0.31-5.66)	0.6941	6 (2.59%)	1.2 (0.33-4.32)	0.7775
>10	13 (5.83%)	3.61 (1.01-12.87)	0.0474*	6 (2.69%)	1.25 (0.34-4.5)	0.7316
Use of contraception						
Use of condoms	2 (0.99%)	1		2 (0.99%)	1	
Occasional use of condoms	8 (3.69%)	3.74 (0.78-17.83)	0.0976	5 (2.3%)	2.33 (0.44-12.18)	0.3132
Other[#]	11(4.98%)	5.05 (1.1-23.0)	0.0366*	9 (4.07%)	4.13 (0.88-19.35)	0.0716
HIV status known						
Yes	10 (3.17%)	1		7 (2.22%)	1	
No	11(3.37%)	1.06 (0.44-2.53)	0.8908	9 (2.76%)	1.24 (0.45-3.37)	0.6706
Previously diagnosed with other STIs						
Yes	15 (12.93%)	11.31 (4.29-29.78)	< 0.0001*	4 (3.45%)	1.5 (0.47-4.76)	0.4832
No	6 (1.14%)	1		12 (2.29%)	1	

* Significant P-value

[#] Oral contraception, intra uterine devices, fertility awareness, coitus interruptus

DISCUSSION

In this study, the prevalence of *N. gonorrhoeae* and *M. genitalium* infections in Bulgarian population of reproductive age from Sofia municipality was estimated and possible risk factors for these infections were identified. The prevalence of *N. gonorrhoeae* infection was 3.28% (95% CI 2.58% to 3.98%). The associated risk factors were an increasing number of lifetime partners and during the last 6 months, presence of urogenital symptoms, previously diagnosed STIs and using contraception methods other than

condoms (i.e., oral contraception, intra uterine devices, fertility awareness, coitus interruptus). The prevalence of *M. genitalium* infection was 2.49% (95% CI 1.29% to 3.69%) and the presence of urogenital symptoms was associated with a greater risk of infection. In Bulgaria, available data for *N. gonorrhoeae* infections in the general population are reported to The European Surveillance System (TESSy) and are accessible through ECDC Annual Epidemiological Reports (15). The prevalence of *N. gonorrhoeae* infection identified in this

study is in concordance with those reported by the Bulgarian Ministry of Health. The identified sexual behavioral risk factors significantly associated with *N. gonorrhoeae* infection are well established for the sexual mode of the gonococcal transmission (16–19).

Regarding *M. genitalium*, studies in Bulgaria remain very scarce. The WHO has no global infection estimates and *M. genitalium* infection is not mandatory reported internationally (2,4). Nevertheless, numerous prevalence studies worldwide have identified rates between 1.3% and 3.9% in the general population (20–22). In the present study, prevalence of *M. genitalium* infection was 2.49%. Other Bulgarian studies have reported rates from 0.29% to 2.45% (7–9). In addition, the only identified risk factor significantly associated with *M. genitalium*, was the presence of urogenital symptoms. In contrast to *N. gonorrhoeae*, *M. genitalium* infection was not characterized by high-risk behavior, suggesting that circulation of gonorrhea and genital mycoplasma take place in distinct sexual networks despite the common modes of transmission (23). The identified nonconformity between risk factors for *N. gonorrhoeae* and *M. genitalium* infections suggest that broad-based testing for *M. genitalium*, similar to chlamydial control strategies, may be more effective than strategies designed to identify hard to reach high-risk populations such as those employed in the control of gonorrhea.

The main limitation of the present study is related to the analysis of risk factors as the low prevalence of *N. gonorrhoeae* and *M. genitalium* infections resulted in a low number of cases available for the statistical analysis. However, the present study is one of the first studies to evaluate risk factors in Bulgarian population and would mainly constitute probable parameters to select in future research.

In conclusion, the prevalence of *N. gonorrhoeae* and *M. genitalium* infections in Bulgarian population of reproductive age from Sofia municipality was relatively low but the burden of

these infections should not be underestimated. Due to emergence of antimicrobial resistance, infections with these bacteria have become exceedingly difficult to treat and could result in adverse sequelae for the reproductive health. Therefore, it is essential to develop control strategies to limit the transmission chains, avoid future complications and reduce the health and cost burden on the Bulgarian population. Prevalence and risk factors identified here and from other studies may help for such future developments.

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