PREVALENCE OF PHARYNGEAL AND RECTAL *CHLAMYDIA TRACHOMATIS* AND *NEISSERIA GONORRHOEAE* INFECTIONS AMONG MSM IN SOFIA, BULGARIA

Ivva Philipova[1], Elena Birindjieva[2], Alexander Milanov[2], Savina Stoitsova[1]

[1]National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria
[2]Center for Sexual Health, CheckPointSofia, Sofia, Bulgaria

ABSTRACT

Sexually transmitted infections (STIs) caused by *Chlamydia trachomatis* and *Neisseria gonorrhoeae* continue to be a major public health problem. Although they mainly affect the urogenital tract, *N. gonorrhoeae* and *C. trachomatis* can also be found in the pharynx and rectum. As data on extragenital chlamydia and gonorrhea in Bulgaria are still scarce, this study aimed to (1) determine the prevalence of pharyngeal and rectal infections with *C. trachomatis* and *N. gonorrhoeae* among men who have sex with men (MSMs) from Sofia and (2) to identify risk factors related to these infections to support screening recommendations based on scientific evidence. One hundred and fifteen MSM aged 16-50 were tested by systematic sampling during a visit to a sexual health center for voluntary and confidential HIV testing in Sofia. A questionnaire was used to collect demographics and risk factors, and clinical material from three anatomical sites: pharynx, rectum, and urogenital tract (first void urine or urethral swab) was examined to detect *C. trachomatis* and *N. gonorrhoeae* infections. The prevalence of *C. trachomatis* was 8.7% in the rectal samples tested, and the prevalence of *N. gonorrhoeae* was 0.9% and 5.2% in the pharyngeal and rectal samples, respectively. Local symptoms were reported in only 16.6% of rectal gonococcal infections and in 20% of *C. trachomatis* rectal infections. Patients reporting multiple partners had a significantly higher risk of being positive (OR = 3.8, 95% CI 1.03-14). The risk of HIV-positive MSM and those having unsafe sex was also higher (OR = 1.9 95% CI 0.19-20 and OR = 4.6 95% CI 0.98-21, respectively), but the findings were not statistically significant. Overall, more than 80% of extragenital infections would remain undetected and therefore transmissible if only symptomatic cases were investigated. These results suggest that in Bulgaria HIV-positive MSM and those having multiple sexual partners and unprotected sex would benefit from screening for extragenital STIs. Larger sample surveys could provide a better characterization of risk factors to guide screening choices.

Keywords: *extragenital infections, Chlamydia trachomatis, Neisseria gonorrhoeae, MSM*

ADDRESS FOR CORRESPONDENCE:
Ivva Philipova
National Center of Infectious and Parasitic Diseases
26, Yanko Sakazov Blvd.
1504 Sofia, Bulgaria
e-mail: ivva.philipova@gmail.com

INTRODUCTION

Bacterial sexually transmitted infections (STIs) remain a global public health concern. According to World Health Organization (WHO), the global prevalence of urogenital chlamydial infection, gonorrhoea, trichomoniasis and syphilis in adults of reproductive age (15-49 years) is high, with nearly one million newly diagnosed cases daily. In 2016, chlamydia and gonorrhoea were among the most common infections with 127 and 87 million m registered cases , respectively [1]. In addition to urogenital tract infections *N. gonorrhoeae* and *C. trachomatis* can also be detected in the pharynx and rectum [2]. In rectum gonorrhoea and chlamydia can cause proctitis and proctocolitis with symptoms such as rectal pain, bleeding, and discharge, etc. In pharynx, these infections can cause pharyngitis and lymphadenitis, but most often remain asymptomatic. An overview of existing literature shows over 80 studies published between 2000 and 2016 on the prevalence of pharyngeal and rectal infection with *N. gonorrhoeae* and *C. trachomatis* [3]. Most studies present evidence on the high prevalence of extragenital *N. gonorrhoeae* and *C. trachomatis* infections among MSM, the asymptomatic nature of these infections, as well as prevalence of extragenital infection without simultaneous urogenital

infection, supporting the need for further testing at extragenital sites. For that reason, European STIs treatment guidelines (The International Union Against Sexually Transmitted Infections (IUSTI) and the British Association for Sexual Health and HIV (BASHH)) provide recommendations for extragenital testing depending on history of exposure [4-7]. Further international guidelines (The Centers for Disease Control and Prevention (CDC) and Australian Sexual Health Alliance) additionally recommend prophylactic extragenital testing on yearly basis among specific high risk groups, such as MSM and commercial sex workers [8-10]. Given the fact that extragenital testing is not always a part of routine STIs screening, particularly in the absence of symptoms, many extragenital infections remain undiagnosed and untreated. Untreated extragenital infections could lead to more severe sequelae, such as increased HIV acquisition risk; autoinoculation from rectum to urogenital tract in women with subsequent pelvic inflammatory disease, ectopic pregnancy, infertility, disseminated gonococcal infection and sexually acquired reactive arthritis [11, 12]. As data on extragenital chlamydia and gonorrhea in Bulgaria are still limited, this study aimed to (1) determine the prevalence of pharyngeal and rectal infections with *C. trachomatis* and *N. gonorrhoeae* among men who have sex with men (MSMs) from Sofia and (2) to identify risk factors related to these infections to support screening recommendations based on scientific evidence.

MATERIALS AND METHODS

Study population

This study was performed on clinical specimens from three anatomical sites (pharynx, rectum, and urogenital tract (first void urine or urethral swabs)) and questionnaires obtained from a population of MSM visiting CheckPointSofia Sexual Health Center in Sofia for voluntary and confidential HIV testing. The CheckPointSofia Sexual Health Center in Sofia was established by the Health Without Borders Association, the successor to the Doctors Without Borders mission, and has been involved in voluntary and confidential HIV testing and counseling for more than 20 years. As this is a place for open dialogue, counseling and support for each individual regardless of sexual identity and orientation, more than 75% of annual male consultations comprise MSM.

Detection of *N. gonorrhoeae* and *C. trachomatis*

The gold standard for diagnosis of urogenital infection due to *N. gonorrhoeae* and *C. trachomatis* are the nucleic acid amplification tests (NAATs). The National Center of Infectious and Parasitic Diseases (NCIPD) has performed NAAT validation for detecting extragenital infections and offers this testing option since NAATs are the most sensitive tests for detecting *C. trachomatis* and *N. gonorrhoeae* and are recommended by CDC [10]. NAATs have demonstrated higher sensitivity but lower specificity as compared to cultivation for detecting extragenital infections [13-15]. The lower specificity of extragenital NAATs was overcome by confirmation of all positive samples with duplex PCR targeting the gonococcal *porA* pseudogene and multicopy *opa* genes [16] and PCR detecting the cryptic plasmid [17] for gonorrhea and chlamydia, respectively.

Demographic and behavioral characteristics

The demographic and behavioral characteristics were collected as follows: assigned sex at birth (male/female), sexual contacts in the last 3 months (male/female), age (in full years and grouped as 16-20, 21-30, 31-40 and >40 years), extragenital symptoms (yes/no), number of partners during the last 3 months (grouped as 0-1, 2-3 and >3 partners), having intercourse without condom in the last 3 months (yes/no) and HIV status (positive/negative).

Statistical analyses

The prevalence of extragenital *N. gonorrhoeae* and *C. trachomatis* infections with 95% confidence intervals (CI) was calculated, then a bivariate logistic regression analysis with demographic and behavioral characteristics as independent variables and extragenital *N. gonorrhoeae* and *C. trachomatis* diagnosis as the dependent variable was performed [13]. Bivariate odds rations (ORs) and 95% CIs were reported. In the statistical analysis, $p < 0.05$ was considered significant [19].

Ethics and informed consent

Written informed consents were obtained from all participants for demographic data collection and

sample testing as required by the National law and the Ethics Committee at the National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria.

RESULTS

In 2021, more than 1,120 MSM attended CheckPointSofia for voluntary and confidential HIV testing, and 115 MSM provided clinical materials from three anatomical locations and completed questionnaires. Demographic and behavioral characteristics of participants are summarized in Table 1. The mean age was 30 ± 7.8 years (age range 16-50). The majority of participants had not any extragenital symptoms (97.48%) and the median number of partners was 2 (IQ25-75: 2-3) during the last 3 months. More than two-thirds of the participants (66.1%) reported having sexual intercourse without condom in the last 3 months and 3.5% reported being HIV positive.

Table 1. *Distribution of demographic and behavioral characteristics in MSM (n=115) visiting CheckPointSofia for HIV testing in 2021*

	N	%
Age		
16-20	12	10.4
21-30	48	41.7
31-40	38	33
>40	17	14.9
Extragenital symptoms		
Yes	3	2.6
No	112	97.4
Number of partners during the last 3 months		
0-1	47	40.9
2-3	42	36.5
>3	26	22.6
Intercourse without condom in the last 3 months		
Yes	76	66.1
No	39	33.9
HIV status		
Positive	4	3.5
Negative	111	96.5

The prevalence of *N. gonorrhoeae* was 0.9% and 5.2% in the pharyngeal and rectal samples, respectively, and the prevalence of *C. trachomatis* was 8.7% in the rectal samples tested. No *C. trachomatis* was detected in any pharyngeal sample (Table 2.). The results comprised 14 (82.4%) cases of exclusively rectal infections and 1 (5.8%) exclusively pharyngeal infection, whereas 2 (11.8%) cases represented simultaneous extragenital and urogenital infection. Therefore, exclusively extragenital infections accounted for the majority of cases (n=15; 88.2%), whereas the remainder 2 (11.8%) corresponded to mixed extragenital and urogenital infections. Local symptoms were reported in 16.6% of rectal gonococcal infections and in 20% of *C. trachomatis* rectal infections which leaves the majority of extragenital gonorrhoea and chlamydia infections asymptomatic (Fig. 1.).

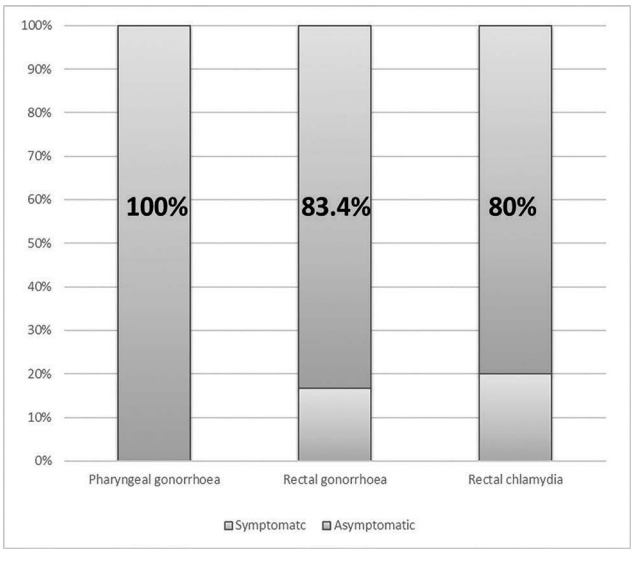

Figure 1. Distribution of symptomatic and asymptomatic extragenital infections in MSM (pharyngeal gonorrhoea n=1; rectal gonorrhoea n=6 and rectal chlamydia n=10) visiting CheckPointSofia for HIV testing in 2021

Table 2. *Prevalence of extragenital gonorrhoea and chlamydia in MSM (n=115) visiting CheckPointSofia for HIV testing in 2021*

	N	%
Pharyngeal gonorrhoea	1	0.9
Rectal gonorrhoea	6	5.2
Rectal chlamydia	10	8.7
Total extragenital infections	17	14.8 (95% CI 8.31-21)

Patients reporting multiple partners had a significantly higher risk of being positive (OR = 3.8, 95% CI 1.03-14). The risk of HIV-positive MSM and those having unsafe sex was also higher (OR = 1.9 95% CI 0.19-20 and OR = 4.6 95% CI 0.98-21, respectively), but the findings were not statistically significant (Table 3.).

Table 3. Statistical analysis of risk factors for extragenital *N. gonorrhoeae/C. trachomatis* infections

	Extragenital gonorrhoea and chlamydia (%)	OR 95% CI	P-value
Number of partners during the last 3 months			
0-1	3 (2.6%)	1	
≥2	14 (12.17%)	3.8 (1.03-14)	0.0455*
Intercourse without condom in the last 3 months			
Yes	15 (13.04%)	1	
No	2 (1.74%)	4.6 (0.98-21)	0.0524
HIV status			
Positive	1 (0.87%)	1	
Negative	16 (13.91%)	1.9 (0.19- 20)	0.5648

* Significant P-value

DISSCUSSION

To the best of authors' knowledge, this is the first Bulgarian study to investigate extragenital infections by *N. gonorrhoeae* and *C. trachomatis* among MSM. In this study, the prevalence of extragenital *N. gonorrhoeae* and *C. trachomatis* infections in MSM visiting CheckPointSofia for HIV testing in 2021 was estimated and possible risk factors for these infections were identified. The prevalence of extragenital gonorrhoea and chlamydia was 14.8% (95% CI 8.31% to 21%) and 82.3% of these infections were asymptomatic. The associated risk factors were an increasing number of partners during the last 3 months, having intercourse without condom and being HIV positive.

Exclusive extragenital gonorrhoea and chlamydia without concurrent urogenital infection accounted for the majority of cases. This means that a considerable number of extragenital infections were identified that would have been undiagnosed if urethral screening alone had been performed. Furthermore, in most cases (97.4%) the patients did not present with any extragenital symptoms. Considering that local complaints were found at extragenital sites only in 16.6% of the rectal gonorrhoea and in 20% of the rectal chlamydia infections, 83.4% of the gonococcal and 80% of the chlamydial infections would have been missed if only the patients reporting local symptoms had been tested for *N. gonorrhoeae* and *C. trachomatis* at these sites. In fact, it is known that extragenital gonococcal and chlamydial infections are usually asymptomatic [4,5,20]. According to other authors, anorectal symptoms like itching, pain, discharge and hemorrhage, are less common but more specific for infection, whereas oropharyngeal complaints, like sore throat, are more commonly reported but nonspecific [21]. Therefore, extragenital gonorrhoea and chlamydia may be missed unless they are actively investigated [22]. Undiagnosed and untreated extragenital infections can lead to

complications and constitute potential disease reservoirs [23], which may contribute to increasing transmission, incidence and antimicrobial resistance [24]. Of particular concern is the endemic spread among MSM of invasive serotypes *C. trachomatis* L1-L3 and the possible role of oropharyngeal infections in promoting resistance among Neisseria species [25,26]. The presented results confirm the relevance of testing for gonorrhoea and chlamydia at extragenital sites in MSM, regardless of local complaints.

Reducing the burden of extragenital infections by timely diagnosis and treatment is an important strategy to reduce gonorrhoea and chlamydia prevalence overall. To address this public health concern, several current international guidelines recommend testing for *N. gonorrhoeae* and *C. trachomatis* infection at extragenital sites, although different strategies have been proposed worldwide: IUSTI and BASHH recommend extragenital testing depending on the history of exposure and CDC recommends annual screening for MSM (every 3 – 6 months for high-risk MSM) at all sites, regardless of reported location of sexual contact [4-7, 10]. However, in our country, as in others [27], *N. gonorrhoeae* and *C. trachomatis* testing at extragenital sites is uncommonly performed, and thus many diagnoses and treatment opportunities may be missed. Also, unfortunately, there are currently no national guidelines specifically recommending extragenital gonorrhoea and chlamydia testing, which compromises routine and standardized screening in clinical settings. Implementation of appropriate screening programs may have substantial benefits for the public health but may find several limitations in Bulgaria, including limited institutional funding, insufficient training of physicians in addressing extragenital gonococcal and chlamydial infections and patients' lack of risk awareness and fear of judgement. Finally, larger sample surveys on this subject would be of value.

In conclusion, this study found a considerable burden of extragenital gonorrhoea and chlamydia among MSM visiting Sexual Health Center in Sofia in 2021. Exclusively extragenital infections without concurrent urogenital gonorrhoea and chlamydia accounted for the majority of cases, and most extragenital infections were asymptomatic. The presented findings reinforce the relevance of screening MSM for *N. gonorrhoeae* and *C. trachomatis* at extragenital sites, regardless of the existence of local complaints, in order to prevent these potential infection reservoirs from being underdiagnosed and untreated. Screening is of particular importance in HIV-positive MSM, and in those having multiple sexual partners and unprotected sex. This study might inform future guidelines and standardized practices concerning the screening of extragenital gonorrhoea and chlamydia among MSM in Bulgaria.

AKNOWELDGEMENTS

This work was supported by a research grant KP-06-M33/1-13.12.2019 "Investigating benefits of extragenital gonorrhea and chlamydia testing for public health" from Bulgarian National Science Fund

REFERENCES

1. Rowley J, Vander Hoorn S, Korenromp E, Low N, Unemo M, Abu-Raddad LJ, et al. *Chlamydia, gonorrhoea, trichomoniasis and syphilis: global prevalence and incidence estimates, 2016*. Bull World Health Organ. 2019;97(8):548.
2. Workowski KA. *Centers for Disease Control and Prevention sexually transmitted diseases treatment guidelines*. Clin Infect Dis. 2015;61(suppl_8):S759–62.
3. Chan PA, Robinette A, Montgomery M, Almonte A, Cu-Uvin S, Lonks JR, et al. *Extragenital infections caused by Chlamydia trachomatis and Neisseria gonorrhoeae: a review of the literature*. Infect Dis Obstet Gynecol. 2016;(2016): 129-145.
4. Lanjouw E, Ouburg S, De Vries HJ, Stary A, Radcliffe K, Unemo M. *2015 European guideline on the management of Chlamydia trachomatis infections*. Int J STD AIDS. 2016;27(5):333–48.
5. Unemo M, Ross JDC, Serwin AB, Gomberg M, Cusini M, Jensen JS. *Background review for the '2020 European guideline for the diagnosis and treatment of gonorrhoea in adults.'* Int J STD AIDS. 2021;32(2):108–26.
6. Fifer H, Saunders J, Soni S, Sadiq ST, FitzGerald M. *British Association for Sexual Health and HIV national guideline for the management of infection with Neisseria gonorrhoeae (2019)*. London: British Association for Sexual Health and HIV; 2019.
7. Fitzgerald N, Anderson ER, Apea V, Curtis H, Goodall L. *2019 British Association of Sexual Health and HIV National Clinical Audit of timelines to be seen, test results and treatment for Chlamydia trachomatis*. Int J STD AIDS. 2022;33(6):604–7.
8. Dukers-Muijrers NHTM, Schachter J, van Liere GAFS, Wolffs PFG, Hoebe CJPA. *What is needed to guide testing for anorectal and pharyngeal Chlamydia trachomatis and Neisseria gonorrhoeae in women and men? Evidence and opinion*. BMC Infect Dis. 2015;15(1):1–13.
9. Australian Sexual Health Alliance, *Australian STI Management Guidelines for Use in Primary Care.* Australian Government Department of Health, Darlinghurst, Australia, 2016.
10. Workowski KA, Bolan GA. *Sexually transmitted diseases treatment guidelines, 2015*. MMWR Recomm reports Morb Mortal Wkly report Recomm reports. 2015;64(RR-03):1.
11. Marcus JL, Bernstein KT, Kohn RP, Liska S, Philip SS. *Infections missed by urethral-only screening for chlamydia or gonorrhea detection among men who have sex with men*. Sex Transm Dis. 2011;38(10):922–4.
12. De Vries HJC, de Barbeyrac B, De Vrieze NHN, Viset JD, White JA, Vall-Mayans M, et al. *2019 European guideline on the management of lymphogranuloma venereum*. J Eur Acad Dermatol Venereol. 2019;33(10):1821–8.
13. Bachmann LH, Johnson RE, Cheng H, Markowitz L, Papp JR, Palella Jr FJ, et al. *Nucleic acid amplification tests for diagnosis*

of Neisseria gonorrhoeae and Chlamydia trachomatis rectal infections. J Clin Microbiol. 2010;48(5):1827–32.
14. Schachter J, Moncada J, Liska S, Shayevich C, Klausner JD. *Nucleic acid amplification tests in the diagnosis of chlamydial and gonococcal infections of the oropharynx and rectum in men who have sex with men*. Sex Transm Dis. 2008;637–42.
15. Bachmann LH, Johnson RE, Cheng H, Markowitz LE, Papp JR, Hook III EW. *Nucleic acid amplification tests for diagnosis of Neisseria gonorrhoeae oropharyngeal infections*. J Clin Microbiol. 2009;47(4):902–7.
16. Goire N, Nissen MD, LeCornec GM, Sloots TP, Whiley DM. *A duplex Neisseria gonorrhoeae real-time polymerase chain reaction assay targeting the gonococcal porA pseudogene and multicopy opa genes*. Diagn Microbiol Infect Dis. 2008;61(1):6–12.
17. Hokynar K, Rantakokko-Jalava K, Hakanen A, Havana M, Mannonen L, Jokela P, et al. *The Finnish new variant of Chlamydia trachomatis with a single nucleotide polymorphism in the 23S rRNA target escapes detection by the Aptima Combo 2 test*. Microorganisms. 2019;7(8):227.
18. Altman DG. *Practical statistics for medical research*. CRC press; 1990.
19. Sheskin DJ. *Handbook of parametric and nonparametric statistical procedures*. crc Press; 2020.
20. Tongtoyai J, Todd CS, Chonwattana W, Pattanasin S, Chaikummao S, Varangrat A, et al. *Prevalence and correlates of Chlamydia trachomatis and Neisseria gonorrhoeae by anatomic site among urban Thai men who have sex with men*. Sex Transm Dis. 2015;42(8):440–9.
21. Dudareva-Vizule S, Haar K, Sailer A, Wisplinghoff H, Wisplinghoff F, Marcus U, et al. *Prevalence of pharyngeal and rectal Chlamydia trachomatis and Neisseria gonorrhoeae infections among men who have sex with men in Germany*. Sex Transm Infect. 2014;90(1):46–51.
22. Lutz AR. *Screening for asymptomatic extragenital gonorrhea and chlamydia in men who have sex with men: significance, recommendations, and options for overcoming barriers to testing*. LGBT Heal. 2015;2(1):27–34.
23. . Hui B, Fairley CK, Chen M, Grulich A, Hocking J, Prestage G, et al. *Oral and anal sex are key to sustaining gonorrhoea at endemic levels in MSM populations: a mathematical model*. Sex Transm Infect. 2015;91(5):365–9.
24. Annan NT, Sullivan AK, Nori A, Naydenova P, Alexander S, McKenna A, et al. *Rectal chlamydia—a reservoir of undiagnosed infection in men who have sex with men*. Sex Transm Infect. 2009;85(3):176–9.
25. Barbee LA. *Preparing for an era of untreatable gonorrhea*. Curr Opin Infect Dis. 2014;27(3):282.
26. Cole MJ, Field N, Pitt R, Amato-Gauci AJ, Begovac J, French PD, et al. *Substantial underdiagnosis of lymphogranuloma venereum in men who have sex with men in Europe: preliminary findings from a multicentre surveillance pilot*. Sex Transm Infect. 2020;96(2):137–42.
27. den Heijer CDJ, Hoebe CJPA, van Liere GAFS, van Bergen JEAM, Cals JWL, Stals FS, et al. *A comprehensive overview of urogenital, anorectal and oropharyngeal Neisseria gonorrhoeae testing and diagnoses among different STI care providers: a cross-sectional study*. BMC Infect Dis. 2017;17(1):1–10.