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

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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

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, gonorrhoia, 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 gonorrhea were among the most common infections with 127 and 87 million 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 gonorrhea 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

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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 ratios (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](image)
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 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) |

**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

**DISCUSSION**

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.

AKNOWLEDGEMENTS

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

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PREVALENCE OF PHARYNGEAL AND RECTAL CHLAMYDIA TRACHOMATIS...
PREVALENCE OF PHARYNGEAL AND RECTAL CHLAMYDIA TRACHOMATIS INFECTIONS

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