

Assessment of *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Trichomonas vaginalis* prevalence using a molecular Point of Care: Findings from a respondent driven sampling study among MSM

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SUMMARY

Objectives: Due to the limited data available within the Moroccan context, the aim of the study was therefore to estimate the prevalence of *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG) and *Trichomonas vaginalis* (TV) infection and co-infection among men who have sex with men (MSM) as well as to update the behavioral indicators for this population.

Methods: During the period of November 2020 to January 2021, 275 and 303 MSM in Agadir and Fes respectively, were recruited by using respondent-driven sampling protocol (RDS). Eligibility criteria for participants included men identified as having anal sex with another man in the last 6 months, aged 18 years or older and residing in either Agadir or Fes, regardless of their nationality, for the past 6 months.

Anal swabs were collected from 445 respondents for molecular investigation of CT, NG, and TV. GeneXpert (Cepheid, USA) was used to test all samples. A survey on the socio-demographic, and risk behavior was then administered to participants.

Results: Most MSM subjects were identified as being young, and homosexual. CT prevalence was 11.3% (95%CI, 7.2 to 15.4) and 12.5% (95%CI, 7.5 to 17.5) in Agadir and Fes respectively; NG was 13.3% (95%CI, 8.5 to 18.1) in Agadir and 5.5% (95%CI, 1.9 to 9.2) in Fes. Meanwhile, TV prevalence was 0.4% (95%CI, 0 to 1.1) in Agadir and 0.2% (95%CI, -0.2 to 0.6) in Fes. A CT/NG co-infection was found in 4.5% (95%CI, 3.5 to 5.9) of cases in Agadir and 2.7% (95%CI, 1.9 to 3.9), in Fes.

Conclusion: It follows that a regular risk assessment and Sexually Transmitted Infectious (STIs) screening should be administered in these two cities as part of a global strategy to enhance the sexual health of the key populations in question.

Keywords: *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, MSM, RDS.

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■ INTRODUCTION

It was proven that sexually transmitted infections (STIs) are among the most common acute diseases that have a profound impact on sexual and reproductive health worldwide. In 2020, the World Health Organization (WHO) showed that *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), and *Trichomonas vaginalis* (TV) represent the most prevalent pathogens that cause STIs worldwide. The estimated new cases each year are 129 million for *C. trachomatis*, 82 million for *N. gonorrhoeae*, and 156 million for *T. vaginalis* infections globally [1]. These infections are frequently asymptomatic for extended periods of time and can lead to significant complications if left untreated [2].

C. trachomatis and *N. gonorrhoeae* infections are common among MSM and have been associated with a higher risk of acquisition and transmission of *Human Immunodeficiency Virus* (HIV) infection [3-5]. The anorectal infection prevalence of NG and CT among African MSM, ranges from 1% to 15%, and most infections are asymptomatic [6-8].

Across Middle East and North African (MENA) countries, some surveys indicate that MSM engage in a variety of high-risk behaviors, including condomless sex, multiple and concurrent sexual partnerships, and intercourse with female partners [9, 10]. However, the WHO recommended that asymptomatic MSM having unprotected receptive anal intercourse and either multiple sex partners or a sex partner with a STI in the previous 6 months should be treated for rectal NG and CT infections [11].

Within the Moroccan context, the National AIDS Program (NAP) identifies MSM as a vulnerable risk group with a higher STI burden. A study conducted in Marrakech in 2017, showed a prevalence of CT and NG among MSM of 9.24% and 8.40% respectively. Whereas, a CT/NG co-infection was found in 3.36% of cases [12].

Furthermore, *T. vaginalis* is considered to be the most common pathogen after viral STIs in Africa [13]. According to a study from Johannesburg, South Africa, it is the etiological cause of male urethritis syndrome in 6.1 % of cases [14]. It has also been shown to increase the risk of HIV sexual transmission [15-17]. Even though, *T. vaginalis* is a prevalent cause of urethral infection in males with urethral discharge, the occurrence of rectal *T. vagi-*

nalis infection is still not documented in African MSM [18].

The adoption of molecular diagnostic tests is an important step in the fight against the burden of STIs, particularly CT, NG, and TV. The recently developed Point of care (POC): Xpert CT/NG and Xpert TV tests allow detection of these STIs in resource-limited settings [19, 20].

In response to this challenge, the specific aim of the current study is then to investigate the prevalence of CT, NG, and TV among men having sex with men in Morocco as well as to assess behavioral indicators of STIs among this population.

■ PATIENTS AND METHODS

Study design and setting RDS

This cross-sectional study was carried out from November 2020 to January 2021, in two Moroccan cities, namely Agadir and Fes and involving 578 MSM.

To this end, Integrated biobehavioral surveys (IBBS) based on respondent-driven sampling (RDS) methods were used to recruit MSM on a casual or regular basis. They include the different categories: gay, bisexual, transvestite, etc. It is worth mentioning that this is the first time an IBBS survey has been conducted among MSM in Fes.

The interview and bioassay sites were defined in consultation with the field teams (Ministry of Health regional services and Non-Governmental Organization (NGOs).

Study participants

For accurate results, eligibility criteria for participants included:

(1) Being 18 years of age or older (2) Identifying as MSM (3) Having had penetrative sex with a man in the past 6 months (4) Being of Moroccan or foreign nationality (5) Residing at the study site for 6 months and (6) being able to understand the study and provide informed consent to participate. Accordingly, individuals who did not meet the above requirement were automatically excluded from the study, as were the case of:

- A person who has previously participated in this study
- Individuals who do not have a coupon
- Individuals who have an expired coupon
- Persons who refuse to complete all steps of the study

The RDS method was chosen because it allows for the recruitment of hidden populations such as MSM, drug users and sex workers, and has been used in 120 studies in over 20 cities and with over 32,000 people [21-25]. Having learned the objectives and process of the study and signing the consent form, eligible men then completed a face-to-face interview in Darija (Moroccan dialect) or in French. After completing the survey, they received up to three uniquely coded coupons to use to recruit their peers.

This study was approved by Casablanca Biomedical Research Ethics Committee in the Faculty of Medicine and Pharmacy of Casablanca at the Hassan II University. All procedures performed in this study were in accordance with the ethics standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethics standards.

Sample and data collection

A doctor conducted pre-test counselling for STI, and then a clinical examination was carried. The clinicians took a sample of the anus and sent it to the laboratories where it was stored before being sent again to National Institute of Hygiene (NIH). The anal swab was collected by using the Polymerase Chain Reaction (PCR) kit swab and then soaked in a tube with transportation media, and stored at +4°C. After that, it was sent to the National Reference Laboratory (NRL) for STIs at the NIH, Rabat.

Each participant completed a questionnaire that included sociodemographic information, risk behaviors, reproductive problems, history of drug use, and awareness about HIV, risk perception, service use as well as clinical manifestations. The questionnaire took approximately 45 min to complete.

Laboratory methods

Out of 578 MSM, only 245 and 200 from Agadir and Fes, respectively, were sampled. All samples were tested using a nucleic acid amplification-based point of care: GeneXpert (Cepheid, USA), for detection of CT/NG and TV.

The entire system requires the use of single-use disposable cartridges that contain the PCR reagents and host the PCR process. Cross-contamination between cartridges during the testing pro-

cess is minimized, as they are self-contained. In less than 90 minutes, the results were obtained and confirmed as positive or negative by the computer. As for the platform, it incorporates the following sample purification, nucleic acid amplification and target sequence detection in simple or complex samples through real-time PCR.

Data analysis

Data analysis and processing were performed using software adapted to the RDS technique: the RDS Analyst (www.hpmmrg.org), using the successive sampling estimator and a priori populations size estimates. Adjusted population estimates and 95% confidence intervals (CI) will be presented.

■ RESULTS

Socio-demographic characteristics

Age, education, and employment

It was observed that few MSM are over 40 years old, while most of them are between 20 and 24 years old. In both cities surveyed, almost all MSM reported being Moroccan, and having attended school. Just a little less than 50% of them had higher education. However, not many MSM reported attending Koranic school or vocational training.

In our study, 17.2% and 15.8% of MSM in Agadir and Fes, respectively, have never worked, while 19.9% in Agadir and less than 9% in Fes are not currently working. Slightly more than 50% and 38% of MSM in Agadir and Fes respectively, have a full-time job in the private sector whereas almost 4% of MSM in Agadir and 7% in Fes have a full-time job in the public sector (Table 1).

Sexual behaviors

Identities among MSM

It was found that few MSM were identified as heterosexual, while most in Agadir (62%) and in Fes (48%) were identified as homosexual/gay.

First male sexual partner

In both cities, little over half of MSM confessed that their first male sexual partner was 20 years or older than they were.

Passive and active male sex partners, group sex, and usage of a condom at last anal sex During the previous six months, between 36% (Agadir) and 55% (Fes) of MSM had passive anal intercourse with a

Table 1 - Age, education, and employment of MSM, Morocco, 2020.

	Agadir N = 275		Fes N = 303	
	n	%, (95% CIs)	n	%, (95% CIs)
<i>Age</i>				
<20	55	20.6 (15.3, 25.9)	86	30.3 (23.0, 37.5)
20-24	109	45.2 (39.0, 51.3)	127	39.9 (32.4, 47.3)
25-29	44	14.9 (10.5, 19.2)	41	12.5 (7.1, 17.8)
30-34	28	8.1 (4.8, 11.3)	19	5.7 (2.0, 9.4)
35-39	16	3.8 (1.7, 5.9)	11	3.9 (0.9, 6.8)
40-44	6	2.1 (0.4, 3.8)	7	2.6 (0.2, 5.4)
45+	17	5.5 (2.6, 8.2)	12	5.2 (1.6, 8.8)
<i>Nationality</i>				
Moroccan	275	100	303	100
<i>Ever attended school</i>				
No	12	3.8 (1.2, 6.5)	24	8.0 (3.6, 12.5)
Yes	263	96.2 (93.5, 98.8)	279	92.0 (87.5, 96.4)
<i>Education level (among those who ever attended school)</i>				
Koranic	21	7.6 (3.9, 11.4)	37	9.9 (5.8, 14.1)
Primary	28	10.1 (6.0, 14.2)	55	16.7 (11.7, 21.7)
Secondary	66	27.3 (21.3, 33.2)	55	20.4 (14.4, 26.4)
Vocational	18	7.0 (3.1, 10.8)	10	5.6 (1.8, 9.4)
Higher	127	48.1 (41.6, 54.5)	124	47.4 (40.3, 54.6)
<i>Employment*</i>				
Never worked	48	17.2 (12.7, 21.8)	54	15.8 (10.7, 20.9)
Not working now	56	19.9 (14.7, 25.1)	25	8.7 (4.9, 12.6)
Part-time	13	4.4 (2.0, 6.9)	34	10.8 (6.5, 15.1)
Full-time (Public sector)	12	3.7 (1.3, 6.1)	18	7.4 (3.4, 11.5)
Full-time (Private sector)	142	54.2 (48.1, 60.3)	113	38.3 (31.9, 44.7)
Other	0	–	57	19.0 (13.6, 24.2)

*No responses for students/ Some data are missing; Information was not always available for all the participants.

male partner. However, data from 44.1% in Agadir and 16.3% in Fes of respondent were not available. Across all sites, one-fifth of the MSM had ever had group sex, most of whom practiced it occasionally in the last six months.

Most MSM in Fes and 48% in Agadir used a condom at their most recent anal sex (Table 2).

Molecular microbiological findings

The overall prevalence of CT, NG, and TV in Agadir was 11.3%, 13.3%, and 0.4%, respectively. The prevalence of two simultaneous infections was 4.5% for CT/NG.

In Fes, the prevalence of CT, NG, and TV was 12.5%, 5.5%, and 0.2%, respectively. For CT/NG, the prevalence of two concurrent infections was 2.7% (Table 3).

DISCUSSION

On this study we report the prevalence of CT, NG, and TV among MSM and their risky sexual behaviors in both Moroccan cities, Agadir, and Fes. Accordingly, both CT and NG infections were considered as being common among MSM. The prevalence of CT in Agadir (11.3%) was significantly

similar to that found in Fes (12.5%), whereas NG prevalence in Agadir (13.3%) was more than double that in Fes (5.5%). A lower prevalence of *C. trachomatis* and *T. vaginalis* of “9.2% and 8.4% respectively”, was reported among MSM in a study conducted in 2017 in the city of Marrakech [12].

The study at hand also showed a low prevalence of CT/NG coinfection in both cities. It is worth mentioning that these results were consistent with those obtained in 2017 [12]. However, the prevalence of TV was almost the same in Agadir and Fes but at a low rate.

Table 2 - Sexual behaviours among MSM, Morocco, 2020.

	Agadir N = 275		Fes N = 303	
	n	%, (95% CIs)	n	%, (95% CIs)
<i>Sexual orientation</i>				
Homosexual/gay	184	62.4 (56.4, 68.5)	165	47.9 (39.7, 56.1)
Bisexual	88	37.3 (31.3, 43.3)	131	49.8 (41.7, 57.8)
Heterosexual	1	0.3 (0.1, 0.7)	1	2.3 (0.4, 4.3)
<i>Age of first male sexual partner</i>				
<15	19	7.5 (4.0, 11.1)	18	6.0 (2.4, 9.5)
15-19	91	34.6 (27.6, 41.6)	96	35.7 (27.9, 43.5)
≥20	164	57.9 (51.1, 64.7)	189	58.3 (50.6, 66.1)
<i>Passive anal sex in past 6 months</i>				
No	65	18.6 (13.6, 23.7)	93	28.1 (20.5, 35.9)
Yes	94	36.1 (29.6, 42.5)	150	55.5 (46.4, 64.6)
NA	111	44.1 (37.1, 51.2)	59	16.3 (11.3, 21.4)
<i>Ever had group sex</i>				
Yes	51	13.9 (10.3, 17.5)	60	13.0 (9.1, 16.9)
<i>Frequency of group sex in the past six months</i>				
Sometime	47	98.8 (98.4, 99.9)	55	98.7 (98.3, 99.3)
Always	3	1.2 (0.1, 1.6)	1	1.3 (0.7, 1.7)
<i>Used a condom at last anal sex</i>				
Always	103	37.5 (29.1, 41.2)	59	19.6 (15.2, 23.1)
Sometimes	131	47.9 (45.1, 50.3)	166	55 (51.2, 61.01)
Never	40	14.6 (12.1, 15.9)	76	25.4 (21.01, 26.1)

Some data are missing; Information was not always available for all the participants.

Table 3 - Prevalence of CT, NG, and TV co-infection, Morocco, 2020.

	Agadir N = 245		Fes N = 200	
	n	%, (95% CIs)	n	%, (95% CIs)
<i>Prevalence of CT, NG, and TV</i>				
Chlamydia trachomatis	24	11.3% (7.2, 15.4)	26	12.5% (7.5, 17.5)
Neisseria gonorrhoeae	29	13.3% (8.5, 18.1)	14	5.5% (1.8, 9.2)
Trichomonas vaginalis	1	0.4% (0, 1.1)	1	0.2% (-0.2, 0.6)
<i>Prevalence of CT/NG co-infection</i>				
CT/NG co-infection	10	4.5% (3.5, 5.9)	6	2.7% (1.9, 3.9)

Several studies exploring the prevalence of STIs among MSM reported varying estimates: ranging from 1.9-8.4% for CT and 0.4-11.1% for NG [26-29]. This variability had several roots: the presence or absence of symptoms at the time of specimen collection, and also the site of sampling: urethral, rectal, or pharyngeal. In this paper, we have privileged a site of extra genital contact (rectal), to seek possible infectious nesting sites.

Previous RDS studies among MSM in Morocco, reveal a self-reported anal risk behavior [30]. Nevertheless, our study suggests the formal possibility of including STI testing in the oropharyngeal matrix in future studies of RDS among MSM.

Strategies to reduce the burden of STIs among MSM in northern countries also have recommended screening at sites of sexual contact based on reported risk behavior [31].

Taking into account the site of sampling (Rectal), the prevalence of CT/NG among MSMs was comparable to that found amongst the same group in Peru and Haiti [32, 33].

With regard to TV infection, despite the low prevalence in our group (0.4%), there is evidence of a possible association between TV rectal colonization and proctitis [18]. Proctitis has been associated with an increased risk of HIV transmission [34]. A fact confirmed in South Africa where the incidence of HIV is one of the highest in the world, the estimated prevalence of TV within MSMs is around 3.8% [18].

Compared to other regions of Africa, a study in Côte d'Ivoire, where *T. vaginalis* infection is more common in the population, found that 2/94 (2.1%) of male sex workers tested positive for *T. vaginalis* rectal swab [35].

Given that STIs can facilitate HIV infection and transmission, recent recommendations for MSM STI management have advocated for regular STI screening (urethral and rectal CT/NG), including in those who are asymptomatic [36]. In Morocco, the national STI control strategy adopts the syndromic approach as recommended by the WHO [30].

MSM in Agadir and Fes share common risky sexual behaviors. Most of them are young (20-24 years), employed and having attended school. In a survey, among MSM from two cities in Morocco, namely Agadir and Marrakech, more than 67% reported bisexual experiences, whereas in our study they were predominantly homosexual [37].

Furthermore, our results show that the majority of MSM had their first sexual intercourse with a male

partner at an early age. This matches the results of previous surveys [38].

Between 36% and 55% of MSM in Agadir and Fes respectively, had passive anal sex with a male partner in the past six months. However, another study conducted in Egypt reported that almost 65.8% of MSM took both active and passive roles in sexual acts [39].

Our study also shows that the participants from both cities who had anal sex in the previous 6 months frequently used condoms. In contrast to previous studies in the Middle East region, suggesting that sex without a condom was significantly more common in the MSM community [40-45].

Our analysis is not an exhaustive one and it has some limitations. A considerable number of participants declined anal swabbing, this of course, may hinder the detection of certain infections such as CT, NG, and TV. In addition, the key population size was limited due to MSM's reluctance to participate, their potential choice to remain hidden, or their fear of criminalization or recognition since sex between men is illegal and punishable by jail time and fines.

Therefore, our findings in these two locations are not applicable to a large portion of MSM population, since the study sample was largely young, homosexuals/gay and educated. Thus, our results may not represent the experiences of older and heterosexually identified, including respondent with different or less educational levels.

■ CONCLUSIONS

To sum up, the data at hand show that CT, NG infections are relatively common among MSM. They therefore urge the necessity for taking quick countermeasures for early diagnosis and treatment. Though, the prevalence of TV among this key population was low. Furthermore, in both cities, co-infection with two CT/NG infections was comparable.

The findings on sexual risk behaviors underscore the importance of STIs awareness for MSM, especially those who are infected. To address the high prevalence of undetected and asymptomatic CT and NG infections among sexually active MSM, a regular risk assessment, periodic screening programs, and low-cost technological advances in health services will be required to better improve the sexual health of this key population.

Competing interests

The authors declare that they have no competing interests.

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Informed consent statement

Informed consent was obtained from all subjects involved in the study.

Contributors

HO, AH, IK, HR, AK, KA, LG and MY designed the study and implemented the study. RA, BB, SJ, CR, RC, and HA collected the data. Laboratory investigations performed by RA, BB, SJ, CR, and HA. Validation of analysis was done by HO and AH. RA wrote the first draft of the manuscript. All authors critically revised the manuscript for important intellectual content and gave final approval of the version to be published. RA, HO, AH and HR have full access to all of the data and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors read and approved the final manuscript.

Data availability statement

The datasets generated and/or analyzed during the current study are not publicly available due to the agreement with the Ethical Commission and in line with the national Law No. 09-08 of February 18, 2009, on the protection of individuals with regard to the processing of personal data, but are available from the corresponding author on reasonable request upon the authorization of The National Commission for the Control of the Protection of Personal Data (CNDP).

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