

# Prevalence of four urogenital sexually transmitted infections in a dedicated clinic from Lisbon

## Prevalência de quatro infeções sexualmente transmissíveis curáveis numa clínica especializada em Lisboa

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### Abstract

**Background/Objectives:** To determine the prevalence of urogenital *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), *Trichomonas vaginalis* (TV), and *Mycoplasma genitalium* (MG) among attendees of an open and freely available sexually transmitted infections (STI) dedicated clinic in Lisbon, at Centro de Saúde da Lapa, during 1-year. **Methods:** Molecular testing for CT, NG, MG, and TV was performed on 1,062 urogenital specimens (one specimen per person). A descriptive, cross-sectional, observational study was conducted to evaluate the characteristics of infected persons. Statistical analysis was performed. **Results:** Around 237 infections were detected in 214 patients. CT was the most prevalent (11.6%), with a similar infection rate between men and women. NG was the second most frequently detected (7.3%), followed by MG and TV (2.9 and 0.5%, respectively). Statistically significant associations were found: 1) between younger age and CT and NG prevalence, where being < 25 years old constituted an increased risk factor; 2) between CT and NG prevalence and sexual orientation, where heterosexuals presented an increased risk for CT infections while men who have sex with men (MSM) had a higher risk for NG infections; and 3) between “having symptoms” and gonococcal infection. **Conclusions:** This study highlights the rising of CT and NG in contrast to a low rate of MG and to the scarceness of TV.

**Keywords:** Sexually transmitted infections. *Trichomonas vaginalis*. *Neisseria gonorrhoeae*. *Chlamydia trachomatis*. *Mycoplasma genitalium*.

### Resumo

**Fundamentos/Objetivos:** Determinar a prevalência das infeções urogenitais por *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), *Trichomonas vaginalis* (TV) e *Mycoplasma genitalium* (MG) nos utentes duma clínica de infeções sexualmente transmissíveis (IST), aberta e gratuita, localizada em Lisboa, no Centro de Saúde da Lapa, durante 1 ano. **Métodos:** A pesquisa de CT, NG, MG e TV foi realizada por teste de amplificação génica em 1062 espécimes urogenitais (1 amostra por pessoa). Foi efetuado um estudo descritivo, transversal e observacional das pessoas infetadas. Foi realizada análise estatística. **Resultados:** Foram detetadas 237 infeções em 214 utentes. CT foi a IST mais prevalente (11,6%), com

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taxa de infecção similar em homens e mulheres. Seguidamente NG (7,3%), MG (2,9%) e TV (0,5%). Foram encontradas associações estatisticamente significativas: 1) entre idade e prevalência de CT e NG, sendo a idade < 25 anos um fator de risco acrescido; 2) entre prevalência de CT e NG e orientação sexual, mediante a qual os heterossexuais apresentaram risco aumentado para CT e os homens que têm sexo com homens um risco aumentado para NG; e, 3) entre 'sintomas' e infecção gonocócica. **Conclusões:** Este estudo evidencia uma subida da prevalência das infeções por CT e NG em contraste com uma baixa taxa de infecção por MG e um escasso número de casos de TV.

**Palavras-chave:** Sexually transmitted infections. *Trichomonas vaginalis*. *Mycoplasma genitalium*. *Neisseria gonorrhoeae*. *Chlamydia trachomatis*.

## Introduction

The World Health Organization estimates that > 1 million STIs are acquired everyday worldwide, leading to 376 million cases of *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), *Treponema pallidum* (TP), and TV every year<sup>1</sup>. Population-based studies estimate 1-3% the rate of *Mycoplasma genitalium* (MG) infection in sexually active men and women<sup>2</sup>, and that coinfection with CT and/or NG is not uncommon<sup>3</sup>. MSM, intravenous drug users, people who have multiple sexual partners, adolescents, and young adults aged between 15 and 24 years old account for the majority of the STI cases, although TV prevalence rates peak at 40-50 years old<sup>2</sup>.

The most predominant CT and NG symptoms are related to urogenital infections, namely urethritis and epididymitis in men and cervicitis and pelvic inflammatory disease in women. However, because most STIs are frequently asymptomatic, people do not realize they are infected and, accordingly, fail to seek treatment, which may lead to severe complications, namely tubal infertility in women<sup>3</sup>.

In Portugal, there is a lack of data regarding the prevalence of CT, NG, MG, and TV. Therefore, the present work aims to determine the frequency of these four curable urogenital STIs among attendees of an open and free STI clinic located in Lisbon and to evaluate their characteristics.

## Methods

We conducted a descriptive, cross-sectional, observational study, reviewing medical charts from all individuals attending the open and freely available STI clinic (no limitations related to residence area, nationality, or legal status) located in Lisbon, Portugal ("Consulta de DST Lapa", ARSLVT, IP). The "Consulta de DST Lapa" has been operating since 1987, being the only STI clinic in a primary healthcare facility in the country. All attendees (with or without genital symptoms, either due to a new problem or through patient referral) are systematically

screened for human immunodeficiency virus (HIV) and TP through serological testing and for CT and NG in urine and/or urethral and endocervical samples. Additional molecular screening for CT and NG at the oropharynx and rectum, and for MG, TV and herpes simplex virus, are regularly performed according to the patient's clinical presentation. Genital warts are diagnosed through clinical observation.

For the purposes of the present study, during a 1-year study period, between September 2016 and November 2017, demographic and clinical information such as age, gender, sexual orientation, number of sexual partners in the previous 6 months, symptoms, concurrent and previous STI, and HIV status were collected and introduced in an anonymized database for further analysis. A total of 1,062 urogenital samples (97 endocervical or urethral swabs and 965 first-void urines—in general, urethral and endocervical swabs in symptomatic and urines in asymptomatic) were collected from all attendees (one-sample per person) and sent to the National Reference Laboratory for STI at the National Institute of Health (Instituto Nacional de Saude Doutor Ricardo Jorge, INSA, IP) for routine diagnosis by Cobas® 4800 CT/NG (Roche Diagnostics). This test was performed according to the manufacturer's instructions. All eluates from Cobas® 4800 CT/NG testing were further systematically tested by the S-DiaMGTV™ kit (Diagenode S.A.) for MG/TV, according to the manufacturer's instructions.

From the collected samples, 746 (70.2%) were from men and 316 (29.8%) from women. For analysis purposes, we created two groups according to age: "< 25 years old" (n = 234, 22.0%) and "≥ 25 years old" (n = 828, 78.0%). Regarding sexual orientation, four groups were defined—"heterosexual men" (n = 391, 36.8%), "heterosexual women" (n = 301, 28.3%), "MSM" (n = 355, 33.4%), and "women who have sex with women (WSW)" (n = 15, 1.4%).

Attendees were further grouped into "no symptoms" (607, 57.1%), "symptoms", which only included "discharge and/or dysuria" (n = 186, 17.5%), and "other symptoms"

**Table 1.** Sociodemographic characteristics and sexual behavior of the STI clinic attendees

	Men (746/1,062)	Women (316/1,062)	Total study population
Age			
< 25 (n,%)	138 (18.5)	96 (30.1)	234 (22.0)
≥ 25 (n,%)	608 (81.5)	220 (69.6)	828 (78.0)
Sexual orientation			
Heterosexual (n,%)	391 (52.4)	301 (95.3)	692 (65.2)
MSM/WSW (n,%)	355 (47.6)	15 (4.7)	370 (34.8)
Number of sexual partners			
0 (n, %)	23 (3.1)	15 (4.7)	38 (3.6)
1 (n, %)	193 (25.9)	161 (50.9)	354 (33.3)
2-4 (n, %)	385 (51.6)	122 (38.6)	507 (47.7)
5+ (n, %)	144 (19.3)	18 (5.7)	162 (15.3)
Symptoms			
No symptoms (n, %)	403 (54.0)	204 (64.6)	607 (57.1)
Discharge and/or dysuria (n, %)	130 (17.4)	56 (17.7)	186 (17.5)
Other* (n, %)	218 (29.2)	57 (18.0)	275 (25.9)
Previous STI			
CT (n, %)	67 (9.0)	27 (8.5)	94 (8.9)
NG (n, %)	92 (12.3)	13 (4.1)	105 (9.9)
MG (n, %)	1 (0.1)	0 (0.0)	1 (0.1)
TV (n, %)	0 (0.0)	3 (0.9)	3 (0.3)
HIV			
Positive (n, %)	70 (9.4)	4 (1.3)	74 (7.0)
Negative (n, %)	676 (90.6)	312 (98.7)	988 (93.0)

\*Other includes ulcers/erosions, genital warts, or non-STI symptoms with the genital expression.

(n = 275, 25.9%). For analysis purposes, only “discharge and/or dysuria” was considered as these were the ones directly associated with CT, NG, MG, and TV.

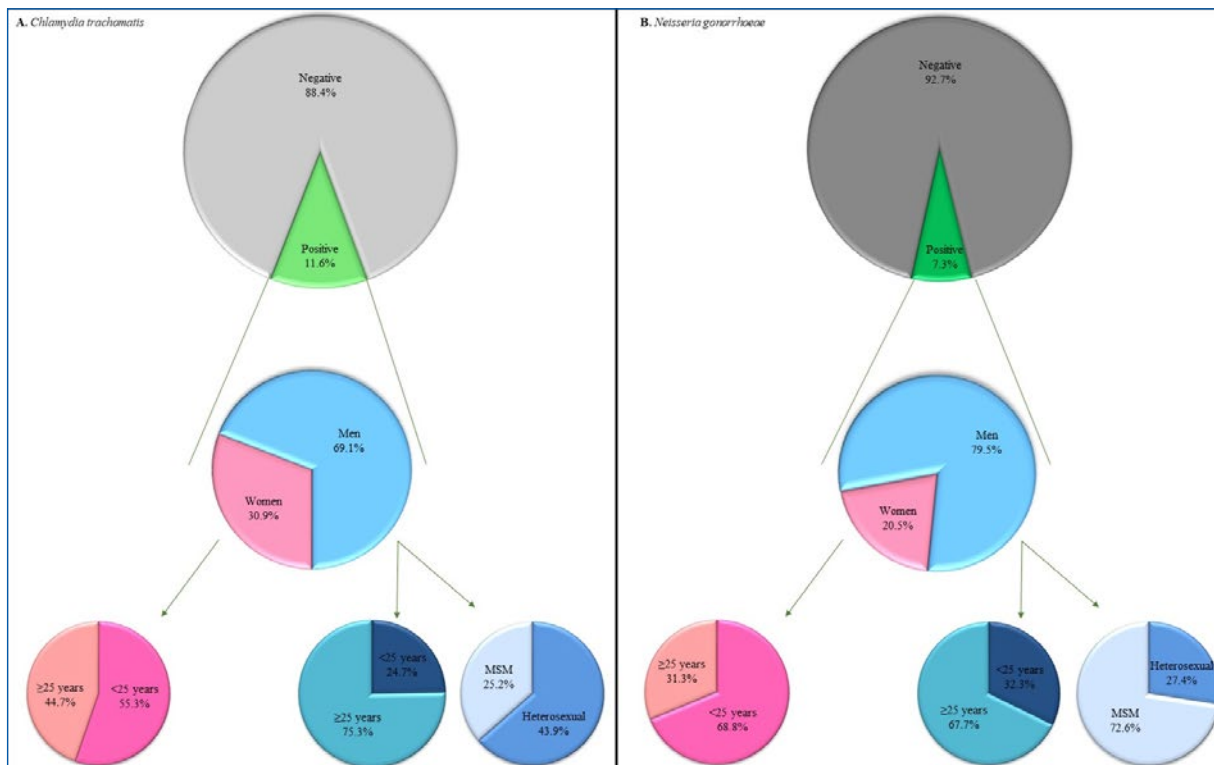
Table 1 summarizes the characteristics of the study population.

The association between the categorical variables concerning potential risk factors and the outcomes of interest was assessed through the Chi-squared test of independence. The odds ratio (OR) and the correspondent 95% confidence intervals (CI) were calculated using a simple logistic regression model (LRM). In these cases, the choice of the reference category of the risk factor (independent variable in the LRM) was made such that the OR would quantify the eventual excess (or shortage) of risk associated with the category a priori thought of as the most interesting, based on the literature (the reference category would be one of the lower reported risks in the literature)<sup>3,4</sup>. Whenever the value “one” does not belong to the 95% CI, it means that the hypothesis OR = 1 is rejected at a 5%

significance level, and hence a significant association is found between the exposure to the risk factor and the occurrence of the disease (outcome). Differences with a p-value of < 0.05 were considered statistically significant. Statistical analysis was performed using software R (4.0.2) and Rstudio (version 1.3.1093). Whenever the number of cases was low, only descriptive statistics were performed.

## Results

Around 237 urogenital STIs were detected in 214 patients, while the remaining 848 attendees tested negative for the four STIs under evaluation. CT was the most prevalent STI, with 11.6% (123/1,062) and a similar infection rate between men (85/746, 11.4%) and women (38/316, 12.0%). NG was the second most frequently detected STI, with 7.3% (78/1,062), followed by MG and TV, with 2.9% (31/1,062) and 0.5% (5/1,062), respectively (Fig. 1). Probably due to the low number



**Figure 1. A:** *Chlamydia trachomatis*; **B:** *Neisseria gonorrhoeae* infections in “Consulta de DST Lapa”: % of positives in the total population, in men (heterosexual and MSM) and women, and in < 25 and ≥ 25 years old.

of cases, no statistically significant association was found between TV infection and any of the variables.

Table 2 describes positive cases for any of the four STIs under study regarding gender, age, sexual orientation, number of sexual partners, symptoms, and HIV status.

Regarding gender, for every infection except NG, the odds of infection for women were slightly higher than for men (OR estimates consistently higher than one). For NG, the opposite was observed (Table 2).

We have detected that there was a statistically significant association between age and STI, except for MG; accordingly, being “< 25 years old” old constituted an increased risk factor for both CT and NG infections. A statistically significant association could also be established between STI and sexual orientation, namely with “heterosexual men” and “heterosexual women” presenting an increased risk for CT infections and “MSM” for NG infections (p-value of < 0.01 and 0.01, respectively). In both cases (age and sexual orientation), the odds of infection were more than duplicated when compared to the reference group (OR = 2.07 and 2.87 for age; OR = 2.22 and 2.31 for sexual orientation) (Table 2).

The number of sexual partners was statistically associated with having any of the four STIs (p-value = 0.03), but there was no specific association with CT, NG, or MG. However, having more than one sexual partner during the last 6 months constituted an increased risk factor for presenting any of the four STIs (Table 2).

The prevalence of coinfections among the STI clinic attendees was 2.0% (21/1,062): 14 CT/NG, 3 CT/MG, 1 CT/TV, 1 NG/MG, 1 CT/NG/TV, and 1 CT/NG/MG. A statistically significant association was observed between being simultaneously CT positive and NG positive (p-value = 0.01), and patients who are NG positive have increased odds for infection by CT (OR = 2.43). From 237 patients diagnosed with any of the four STIs, 51 previously had CT (n = 33) and/or NG (n = 36), being there a statistically significant association and increased odds of infection between having any of the four STI and having had previously one of the same four STI (p-value of < 0.01, OR = 2.07). Also, being NG positive and having previously had any of the four STIs were associated (p-value of < 0.01) with increased odds of gonococcal infection when patients had previously had one of those four STIs in their clinical history (OR = 2.08).

**Table 2.** STI cases by gender, age group, sexual orientation, number of sexual partners, symptoms, and HIV status

	Any of the four STI (237/1,062)	<i>Chlamydia trachomatis</i> (123/1,062)	<i>Neisseria gonorrhoeae</i> (78/1,062)	<i>Mycoplasma genitalium</i> (31/1,062)	<i>Trichomonas vaginalis</i> (5/1,062)
<b>Gender</b>					
Men (n + /n, %)	156/746 (19.6)*	85/746 (11.4)*	62/746 (8.3)	21/746 (2.8)*	1/746 (0.1)
Women (n + /n, %)	58/316 (18.4)	38/316 (12.0)	16/316 (5.1)*	10/316 (3.2)	4/316 (1.3)
$\chi^2$	0.03	0.70	0.23	0.05	a
p-value	0.87	0.40	0.63	0.82	a
OR (95% CI)	1.04 (0.74, 1.46)	1.22 (0.80, 1.84)	1.20 (0.68, 2.23)	1.19 (0.53, 2.51)	a
<b>Age</b>					
< 25 (n + /n, %)	78/234 (33.3)	42/234 (17.9)	31/234 (13.2)	10/234 (4.3)	1/234 (0.4)
≥ 25 (n + /n, %)	136/828 (16.4)*	81/828 (9.8)*	47/828 (5.7)*	21/828 (2.5)*	4/828 (0.5)
$\chi^2$	27.44	11.10	15.13	0.18	a
p-value	<< 0.01	<< 0.01	<< 0.01	0.67	a
OR (95% CI)	2.49 (1.76, 3.49)	2.07 (1.35, 3.13)	2.87 (1.68, 4.86)	1.31 (0.54, 2.88)	a
<b>Sexual orientation</b>					
Heterosexual (n + /n, %)	76/391 (19.4)*	54/391 (13.8)	17/391 (4.3)*	9/391 (2.3)	1/391 (0.3)
MSM (n + /n, %)	80/355 (22.5)	31/355 (8.7)*	45/355 (12.7)	12/355 (3.4)*	0/355 (0.0)
$\chi^2$	0.42	8.90	6.16	0.19	a
p-value	0.51	< 0.01	0.01	0.65	a
OR (95% CI)	0.87 (0.59, 1.26)	2.22 (1.34, 3.77)	2.31 (1.24, 4.48)	0.74 (0.29, 1.81)	a
<b>Number of sexual partners</b>					
0-1 (n + /n, %)	61/392 (15.6)*	37/392 (9.4)*	19/392 (4.8)*	9/392 (2.3)*	1/392 (0.3)
2+ (n + /n, %)	153/669 (22.9)	86/669 (12.9)	59/669 (8.8)	22/669 (3.3)	4/669 (0.6)
$\chi^2$	4.62	1.74	1.27	1.01	a
p-value	0.03	0.19	0.26	0.31	a
OR (95% CI)	1.47 (1.05, 2.07)	1.36 (0.90, 2.08)	1.44 (0.8, 2.59)	1.64 (0.75, 3.96)	a
<b>Symptoms</b>					
No symptoms (n + /n, %)	93/607 (15.3)	64/607 (10.5)	19/607 (3.1)*	13/607 (2.1)	5/607 (0.8)
Symptoms (discharge and/or dysuria) (n + /n, %)	99/186 (53.2)*	44/186 (23.7)*	57/186 (30.6)	13/186 (7.0)*	0/186 (0.0)
$\chi^2$	116.31	20.25	141.25	12.97	a
p-value	<< 0.01	<< 0.01	<< 0.01	< 0.01	a
OR (95% CI)	0.16 (0.11, 0.23)	0.37 (0.24, 0.58)	17.66 (9.90, 33.02)	0.26 (0.12, 0.55)	a

\*Reference group: a, no statistics were performed due to a low number of cases. Each cell of Table 2 should be read as the number of positive cases in each category, regarding the total number of cases for that same variable in the category, and the respective percentage. E.g., the information concerning variable gender regarding CT infection should be read as 85 out of the total 746 men are positive for CT; which means that 11.4% of men are infected with CT; 38 out of the 316 women are positive for CT; meaning that 12.0% of women are infected with CT. Chi-squared test statistic for independence between gender and infection by CT is equal to 0.7, to which a p-value of 0.4 is associated; OR of infection by CT is of 1.22, with a 95% CI equal to (0.80, 1.84), meaning that the odds of infection by CT is estimated to be 1.22 higher among women than in among men (reference category); hypothesis that OR = 1 is not rejected at a 0.05 significance level, as one belongs to the 95% CI.

Concerning the existence of “symptoms”, there was a very high-risk of infection associated (OR = 1 7.70) and a strong statistically significant association between the presence of “discharge and/or dysuria” and gonococcal infection (p-value of << 0.01) (Table 2).

Having any of the four STIs under study and HIV status and syphilis provided no statistically significant association (p-value = 0.70 and 0.13, respectively).

## Discussion

A fifth of the STI clinic attendees (n = 214, 20.2%) were diagnosed with at least one of the four curable urogenital STIs (CT, NG, MG, and TV), and the most frequent was CT (11.6%) (Fig. 1) and (Table 2), as expected, according to the literature<sup>5-8</sup>.

In 2014, a national system for the epidemiological surveillance of obligatory reporting diseases, including

CT, was implemented in Portugal. In the early years, it was based on clinical notification, and since 2017, it includes both clinical and laboratory notification (<https://sinave.min-saude.pt/>). Thus, the Portuguese history of CT infection was short by the start of our study. However, prior data from this same STI clinic evidenced lower prevalence rates, namely 8.4% between 2000 and 2007<sup>9</sup>, and 6.0% among HIV-infected patients during the 2009-2013 period<sup>10</sup>; thus, CT seems to be on the rise. This putative growth is particularly striking when our study was mostly based on urine samples (only 97 endocervical or urethral swabs from symptomatic patients), which are not considered ideal for CT diagnosis in asymptomatic women because of its lower sensitivity compared to endocervical (or vaginal) swabs<sup>2</sup>; as such, some chlamydial (and even gonococcal) infections might have remained undiagnosed in women.

*Neisseria gonorrhoeae* (NG) has been under surveillance in our country since the 1950s, but its large under-reporting is common knowledge. It was the second most frequent STI in the present study with a prevalence of 7.3% (Fig. 1 and Table 2), a rise from the 3.1% determined during the 1998 and 2006 period (data not shown), and contrasting to the 1.3% positivity rate determined by a Netherlander study<sup>6</sup> held in a similar clinical setting. The reasons underlying this rise are hard to determine. Increased risky behaviors related to preexposure prophylaxis (PrEP) for HIV could be implicated, as proposed by other studies reporting higher rates in those patients<sup>11</sup>; however, this should not be the case in our study, as PrEP was only approved by Portuguese health authorities later in 2018. Our findings reinforce the need for continuous surveillance of gonorrhea dissemination because of the risk of antimicrobial-resistant strains, which have been described in our country<sup>12</sup>, and the putative risk of treatment failure.

Regarding MG, an STI that is not under surveillance in Portugal nor in other European countries, its prevalence was 2.9%, which is lower than the described for similar clinics in other countries. In fact, several European studies<sup>6,13-15</sup>, evidenced percentages varying from 3.0 to 9.8%, and it surely contrasts with the obtained in similar clinical settings in the United States (16.1% for women and 17.2% for men) and Canada (7.2% for women and 5.3% for men)<sup>16,17</sup>. In fact, the observed 2.9% MG rate is similar to the described for low-risk populations (2.0%)<sup>6</sup>; considering that the demographic and behavioral risk factors for this infection are shared with CT and NG<sup>18</sup>, it would be expected that the high percentages detected for the later infections would be reflected for MG too. The reasons underlying this low rate are hard to determine but are

consistent with previous data from the “Consulta de DST Lapa” (data not shown), in which this microorganism was rarely detected; thus, specificities of the Portuguese MG epidemiological scenario, putatively evidenced by our study, require disclosure.

*Trichomonas vaginalis* (TV) is not under surveillance in Portugal, similar to most countries. The “Consulta de DST Lapa” has been observing a decline in TV since the 90s, and the present prevalence of 0.5%, the lowest of the four curable STIs under evaluation, was not a complete surprise. In addition, it is in accordance with a prevalence ranging between 0.6 and 1.5% described for Netherlander and French STI clinics<sup>6,13</sup>. It is of note that, in our study, men represented about two-thirds of the population, and men are usually considered less prone to TV infection. Nonetheless, a study involving Portuguese women of childbearing age somehow corroborates our findings, as a 1.0% prevalence rate was determined<sup>19</sup>. On the contrary, in Iran, a study performed in STI clinics only involving women reported that the overall TV prevalence was 8.3%<sup>8</sup>, and in North America, incarcerated people prevalence rates varied between 2 and 47%<sup>20</sup>. Thus, the TV prevalence outside Europe may not parallel the European situation. The impact of a putative indiscriminate use of vaginal antiseptics or vaginosis therapeutics could be contributing to the apparent disappearance of the protozoan; further evaluation of this phenomenon is needed.

Previous studies showed that some variables such as age, gender, concurrent STI, and/or number of sexual partners constitute risk factors for acquiring a new STI<sup>3</sup> and, in general, women were considered more prone to getting an STI<sup>21</sup>. However, in our study, the most obvious association was NG infection with men as described by others<sup>4</sup>.

Sexually transmitted infections (STI) were expected to be associated with young age<sup>21</sup>; in fact, ectocervical everted columnar epithelium that characterizes women of young age should contribute to a high-risk of acquiring an STI<sup>22</sup>. As such, in our study, being “< 25 years old” constituted an increased risk factor for both CT (p-value of << 0.01, OR = 2.07) and NG (p-value of << 0.01, OR = 2.87). This is of great concern, as nontreated infections can ascend to the upper genital tract and cause pelvic inflammatory disease and related sequelae<sup>23</sup>. For MG, and in line with the described by others<sup>13,14</sup>, no such association was observed; in our study, older people presented an increased risk for MG infection (OR = 1.31).

Concerning the association between urogenital STI and sexual orientation, being “heterosexual” constituted an increased risk for CT infection (p-value of < 0.01), while being “MSM” constituted an increased risk for NG

infection (p-value = 0.01), which is in accordance with other studies<sup>21</sup>. Although no association could be established between MG and sexual orientation, the odds in MSM were slightly higher (Table 2), corroborating the results of a Spanish study<sup>24</sup>.

Having multiple sexual partners has been often associated with an increased risk of acquiring and transmitting STIs<sup>18,21</sup>. Accordingly, more than half of our patients (n = 669 and 63.0%) reported having more than one sexual partner during the last 6 months.

Considering that the only symptom that was recorded, for analysis purposes, was “discharge and/or dysuria”, 82.5% of attendees were considered asymptomatic, a feature that was statistically significantly associated, p-value << 0.01 and < 0.01, with CT and MG, respectively. On the contrary, “having symptoms” and NG were statistically significantly associated, evidencing very high odds of infection when compared to “not having symptoms” (OR = 17.66). However, if only patients who had symptoms were tested, many NG cases would have been missed; therefore, a generalized screening accompanied by appropriate treatment should surely contribute to reducing the NG reservoir in high-prevalence communities.

Regarding having had at least one of the four STIs previously, despite the development of some immunity upon suffering several STI episodes, it does not provide enough protection against the acquisition of a new STI<sup>25</sup>. As such, almost one-fifth of our study population (n = 182, 17.1%) had at least one of the four STIs in the past, and 28.6% (n = 52) of the people from this group had also an STI in the present. Constant reinfections evidence lack of use of preventive methods and repeated high-risk behaviors, which should also facilitate acquisition and transmission of HIV<sup>26</sup>; nonetheless, no statistically significant association was observed between having any of the four curable STIs under study and being HIV positive.

This study presents some limitations regarding the anatomical sites of infection-as it only included urogenital samples. Also, regarding symptoms, only “discharge and/or dysuria” were systematically recorded, missing potential curable STI-related symptoms such as itching and pelvic pain. Nonetheless, it contributes to fulfilling the lack of data on the prevalence of CT, NG, MG, and TV in Portugal, evidencing that among STI clinic attendees in Lisbon, Portugal, TV reveals rare, MG occasional, and NG and CT on the rise. NG was associated with being MSM, being young, and having discharge. CT was the most prevalent STI and was associated with being “heterosexual men” and being “< 25 years old”.

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## Conflicts of interest

None.

## Ethical disclosures

**Protection of human and animal subjects.** The authors declare that no experiments were performed on humans or animals for this study.

**Confidentiality of data.** The authors declare that no patient data appear in this article.

**Right to privacy and informed consent.** The authors have obtained the written informed consent of the patients or subjects mentioned in the article. The corresponding author is in possession of this document.

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