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Characterization of *Chlamydia trachomatis* and *Neisseria gonorhoeae* Infections in Populations Volunteering for Screening in Lisbon, Portugal

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Abstract

Background: Sexually Transmitted Infections (STI) are a public health issue, which should be promptly diagnosed and treated.

Objectives: The objectives of this study were to characterized *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections, namely co-infections between the two and with other STI, and associated demographic factors in population groups volunteering for screening.

Methods: *C. trachomatis* and *N. gonorrhoeae* infections were diagnosed through a multiplex Real-Time PCR. The presence of *N. gonorrhoeae* DNA was confirmed by RFLP technique. The presence of antibodies against other STI and of antigens was performed with recommended techniques.

Results: The overall prevalence of *C. trachomatis* and *N. gonorrhoeae* infection was 3, 4%, and 1, 0%, mostly being asymptomatic in 90% and 83, 3%, respectively. Syphilis, hepatitis C, hepatitis B and HIV were diagnosed respectively in 2, 7%, 5, 1%, 4% and 2%. The majority of infected participants were <25 years old and those with *N. gonorrhoeae* males, from whom 50% had a co-infection with *C. trachomatis*. *N. gonorrhoeae* infection was associated with a diagnosis of gonorrhoea in the past. Most of the people infected had more than one sexual partner in the previous 6 months (73, 7%) and an inconstant condom use (80, 7%). Furthermore, most individuals are not aware of their high risk sexual behaviour.

Conclusion: This study suggests that STI sexual health education, decreasing barriers to health care and non-invasive STI screening are essential in this population and in similar ones.

Introduction

Sexually Transmitted Infections (STIs) are a growing public health social and economic worldwide problem, mostly affecting adolescent women and young adults [1,2]. According to the World Health Organization (WHO), over 1 million people acquire an STI every day [3]. Early diagnosis, timely and correct treatment is as important in interrupting the chain of transmission, as in the prevention of complications and serious sequelae [1].

STI are among the most common infectious diseases that increase morbidity rate, with a high impact on sexual and reproductive health throughout the world [1,3].

Chlamydia trachomatis and *Neisseria gonorrhoeae* urogenital infections are the most prevalent bacterial STI worldwide and have been increasingly reported in Europe [2,4].

The major challenge in the diagnosis and consequently in the control of these infections is the high proportion of asymptomatic individuals, which allows its uncontrolled spreading [5]. Moreover, these infections are an important co-factor in HIV infection, increasing susceptibility to this virus [1].

The STI screening aims to control and stop transmission, to decrease its incidence and thus preventing consequences of these infections and reducing associated economic, physical and psychological costs [6,7].

Screening for urogenital infection caused by *C. trachomatis* and *N. gonorrhoeae* is simple, non-invasive and painless [5,8]. Nucleic Acid Amplification Tests (NAATs) are the recommended

methodology and biological samples (urine in men and vaginal swabs in women) may be self-collected [5,6,8].

Actually, screening for *C. trachomatis* and *N. gonorrhoeae* infections is recommended annually for all sexually active females aged <25 years, those with risk factors independently of age and in all sexually active men who have sex with men (MSM). All pregnant women should also be tested for all STI [8,9-11].

However, the prevalence of *N. gonorrhoeae* infections varies among population groups (screening might be considered for individuals in high-prevalence settings), and therefore screening generalization must always be based on cost-effectiveness.8,12 The implementation of preventive measures and of an effective screening program needs assessment of target groups attitudes, as well an estimate of infection prevalence in those groups [2,7].

Taking that into account, the present study intends to characterize *C. trachomatis* and *N. gonorrhoeae* infections with respect to their prevalence, co-infections and associated sociodemographic factors in populations volunteering for screening.

Materials and Methods

The study population consisted in individuals who volunteered for STI screening at a mobile unit from Portuguese League Against AIDS (Liga Portuguesa Contra a SIDA - LPCS) and Oblata Sisters and Orientation Center for Women (Centro de Acolhimento e Orientação à Mulher das Irmãs Oblatas - CAOMIO), in the period between September 2013 and June 2015 in Lisbon. Each volunteer was given a questionnaire including questions about sociodemographic characteristics, sexual behaviour and current STI symptoms, after informed consent was obtained.

Vaginal swabs were obtained from females and first catch urine and urethral swabs in males, the last one whenever symptoms were present. Blood was drawn from every individual who agreed to do so.

C. trachomatis and *N. gonorrhoeae* DNA was identified through a Multiplex real-time polymerase chain reaction (PCR) in accordance with Jalal et al.[13] and Whiley et al.,[14] using the Rotor-Gene 3000 (Corbett Robotics, Australia). The presence of *N. gonorrhoeae* DNA was confirmed by restriction fragment length polymorphism (RFLP) using a technique described by Ho et al. [15]. Positive controls were *N. gonorrhoeae* DNA extracted from a culture of N gonorrhoeae ATCC 49226 and *C. trachomatis* DNA extracted from a cell culture *C. trachomatis* of strain L2. All primers and probes were from Nzytech, Lda. Antibodies against other STI were detected with a nontreponemal test (Rapid Plasma Reagin – Macro-Veu™ RPR Card Test) and a treponemal test (*T. pallidum* passive particle agglutination TPHA- assay). An immunochromatographic assay was used for the detection of antibodies against HCV and HIV-1/ HIV-2 (Hepatitis C Virus – Abon and Determine HIV 1/2 - Alere, respectively) and HBs antigen (Hepatitis B surface antigen – Abon), containing serum controls. All patients found to be positive for any of these tests were sent for confirmation tests.

Statistical analysis was performed with SPP-IBM version 18.0. Pearson's chi-squared and Fisher's exact tests were used when applicable: 95% confidence intervals were calculated for each variable and *C. trachomatis* and *N. gonorrhoeae* infections using a multivariate logistic regression analysis. p-values of <0.05 were considered statistical significant.

Results

The study included 596 participants, from which 340 (57%) were males and 256 (43%) females, with a median age of 40, 5 years (range: 17-87 years).

Reasons for participants to volunteer for screening were opportunity/curiosity in 480/596 (80,5%), engaging in risk behaviours for 103/596 (17,3%) and the presence of symptoms in 13/596 (2,2%).

With respect to nationality, 46,5% (275/591) of the participants were Portuguese, 34,5% (204/591) from the African Portuguese-speaking countries (PALOPS), 9,1% (54/591) South Americans, 3,2% (19/591) Western Africans, 3% (18/591) Eastern Europeans, 1,5% (9/591) Asians, 1,4% (8/591) Western Europeans and 0,7% (4/591) Central Americans.

Not every individual answered to all questions of the questionnaire. In relation to civil status, 58, 2% (334/573) of the participants were not married/not cohabiting. Primary school was completed by 80, 4% (439/549), while 4% (22/549) attended high school and 16% (88/549) graduated from university. Unemployment affected 65, 2% (356/546) of these participants.

In this population, 92, 6% (528/570) were heterosexual. One sexual partner during the last six months was reported by 48, 2 % (271 /562) and two or more partners by 32, 6% (183 /562) of them. When asked about condom use, 19, 9% referred "always to use it", 53, 2% "sometimes" and 27% "never"; 12,1% "always", 24,8% "sometimes" and 63,1% "never"; 26% "always", 36,4% "sometimes" and 37,7% "never" for vaginal, oral and anal sex, respectively Table 1.

Table 1: Condom use in relation with type sex

Type of sex	Always	Sometimes	Never
Vaginal	19,9% (123/619)	53,2% (298/619)	27% (167/619)
Oral	12,1% (34/282)	24,8% (70/282)	63,1% (178/282)
Anal	26% (42/162)	36,4% (59/162)	37,7% (61/162)

The overall prevalence of *C. trachomatis* and *N. gonorrhoeae* infections was 3, 4% (20/596) and 1,0% (6/596), respectively. The rate of asymptomatic infection was 90% (18/20) for *C. trachomatis* and 83, 3% (5/6) for *N. gonorrhoeae.* Symptoms were shown to be independent of infections in the multivariate logistic regression analysis.

Table 2, describes sociodemographic and behavioural characteristics and clinical data of individuals with *C. trachomatis* and *N. gonorrhoeae* infections. The rate of *C. trachomatis* infection was 3, 5% (12/340) for males and 3,1% (8/256) for females, being 1,5% (5/340) in males and 0,4% (1/256) in females for *N. gonorrhoeae.* Differences between genders were not statistically significant. *C. trachomatis*-infected participants were statistically significantly younger (median age: 25,5 years; Mann-Whitney U-test, p=0.001), the majority being ≤ 25 years of age, while in those with *N. gonorrhoeae* infection there was no association with age, with five in six infected individuals (83,3%) having more than > 25 years of age.

Table 2: Socio-demographic and behavioural characteristics and clinical data of *C. trachomatis* and *N. gonorrhoeae* infected participants

	Selected variables	*C. trachomatis* positive	*p*-value	*N. gonorrhoeae* positive	*p*-value
Gender (n= 596)	Male (n=340)	12 (3,5%)	0,786*	5 (1,5%)	0,244**
	Female (n=256)	8 (3,1%)		1 (0,4%)	
Age (n=588)	< 25 years (n=77)	9 (11,7%)	**0,000****	1 (1,3%)	0,571**
	≥ 25 years (n=511)	11 (2,2%)		5 (1,0%)	
Nationality (n=591)	Portuguese (n=275)	8 (2,9%)	0,286*	4 (1,5%)	0,587**
	Portuguese-speaking countries (n=240)	10 (4,9%)		2 (1,0%)	
	Not Portuguese (n=112)	2 (1,8%)		-	
Marital status (n=573)	Married/cohabiting (n=239)	4 (1,7%)	**0,045***	3 (1,3%)	0,698**
	Not married/not Cohabiting (n=334)	16 (4,8%)		3 (0,9%)	
Professional occupation[a] (n=546)	Yes (n=190)	6 (3,2%)	0,764*	2 (1,1%)	1,000**
	No (n=356)	13 (3,7%)		4 (1,1%)	
Education level (n=549)	Primary school (n=439)	15 (3,4%)	0,571**	3 (0,7%)	0,098**
	High school/ University (n=110)	5 (4,5%)		3 (2,7%)	
Sexual behaviour (n=570)	Heterosexual (n=528)	16 (3,0%)	0,059**	6 (1,1%)	1,000**
	Homosexual (n=25)	2 (8,0%)[b]		-	
	Bisexual (n=17)	2 (11,8%)[c]		-	
Partners in previous 6 months (n=562)	1 (n=271)	6 (2,2%)	**0,008***	1 (0,4%)	**0,029****
	2-10 (n=135)	12 (8,9%)		5 (3,7%)	
	>10 (n=48)	2 (4,2%)		-	
Use of condom (n=569)	Always (n=110)	4 (0,7%)	**0,049****	1 (0,2%)	0,424**
	Sometimes/never (n=459)	16 (2,8%)		5 (0,9%)	
Past IST (n=541)	Yes (n=154)	4 (2,6%)	0,961*	3 (1,9%)	
	No (n=396)	16 (4,0%)		3 (0,8%)	

[a]A *C. trachomatis* infected individual did not respond to this category; [b]Female participants; [c]Male participants; * Pearson's chi-squared test; ** Fisher's exact test. Values in bold are statistically significant

There was no significant statistically association between each of these infections and nationality, professional occupation, education level and sexual behaviour. For *N. gonorrhoeae* infection, there was also no association for marital status and use condom. On the contrary, there was a statistically significant difference in the association between *C. trachomatis* infection and marital status (p=0,045), number of partners (p=0,008) and use of condom (p=0,049), while for *N. gonorrhoeae* infection this is only true for the number of partners (p=0,029).

When individuals with any one of these infections were asked about past STIs, from those with gonorrhoea 3/6 (50, 0%) have had it before, while 4/20 (20%) in which *C. trachomatis* was detected, one remembered to have had hepatitis B, one was infected with HIV and the remaining individuals did not know the name. The only statistically significant association was a present infection with *N. gonorrhoeae* a previous gonorrhoea diagnosis (Fisher's exact test, p = 0.047).

Co-infection with both microbial agents (*C. trachomatis* and *N. gonorrhoeae*) was observed in 3 patients. This association was not statistically significant (Fisher's exact test, p=0.001).

Other STI were diagnosed in 2, 7% (16/594), 5, 1/ (30/594), 4% (24/594) and in 2% (2/594) respectively syphilis, hepatitis C, hepatitis B and HIV.

A relationship between *C. trachomatis* or *N. gonorrhoeae* and other STI was also looked for. Ten percent (2/20) of the participants in whom *C. trachomatis* was detected were concurrently infected with HIV and hepatitis B virus. However, no other co-infections were observed. When *N. gonorrhoeae* was present no other infections were detected. Regarding co-infections there were no statistically significant differences.

Discussion

Recently, the incidence of STI is increasing with the total number of chlamydia, gonorrhoeae and syphilis cases reaching the highest number ever reported [16]. This has been mainly attributed to high risk sexual behaviour in conjunction with increased sensitivity and easy to perform diagnostic tests [2,5].

STI numbers will only decrease if screening and treatment are part of STI prevention and control programs. Otherwise, many STI will not be diagnosed and treated. Screening programs also need to be cost effective and for that it is necessary to know the prevalence of STI in specific populations, since we know that the prevalence of those infections depends on heterogeneity of the populations studied [2,7].

The population of the present study was similarly of Portuguese origin and from PALOPS and other African countries, mostly heterosexuals, unemployed and of primary school level.

The prevalence of *C. trachomatis* infection is variable (0, 1 to 30%) depending on the population studied [17-23]. We found that 3.4% of individuals in this study were infected with these microorganisms, similar to Pedrosa et al. [24] findings, who reported a prevalence of *C. trachomatis* infection of 4.0%. In another publication by Santo et al. [25] in which participants were also mostly heterosexuals (89.4%), but younger (mean age: 29 years) and attending a STI clinic, the overall prevalence was 8.4%.

In the present study and when relating *C. trachomatis* infection with gender, this was similar (3.5% in males and 3.1% in females) and statistically associated with < 25 years of age for both genders. This was also found in a meta-analysis study, which concluded that although *C. trachomatis* prevalence is highly variable between different countries of the world, it is similar between genders and in other Portuguese studies [17,24,26-27]. Most STI guidelines, however, recommend annual screening of all sexually active women under 25 years of age, based in the fact that infection is frequently in this age group; it is a frequent cause of infertility and also on the basis of being frequently asymptomatic. More studies should be performed on this subject to confirm these results and to establish screening cost effectiveness also in men of that age, independently of being MSM.

The overall prevalence of *N. gonorrhoeae* infection was 1.0%, slightly higher than in a Portuguese study performed by Guedes et al. [28], in which it was 0.8%. This population was also predominantly male and heterosexual. Many studies have approached this subject with prevalences varying from 0.4% to 14% [12,19-22,29]. We found *N. gonorrhoeae* infection occurring mainly in older males. However, statistically significant differences between genders were not observed, as also described by Guedes et al [28]. A high prevalence of *N. gonorrhoeae* infection in males was also noted by Borges da Costa et al [26]. According to the data reported in Europe and the United States of America (USA), most *N. gonorrhoeae* cases are in males between the ages of 20-24 years [2,30]. In this study, all infected male were above 25 years of age. This is consistent with a previous study presented by Bayette et al. (2014) [31] in France, where the prevalence of *N. gonorrhoeae* infection was higher in males aged between 25-30 years.

Only 10% of *C. trachomatis*-infected participants reported symptoms, an expected finding, since it has been described that the majority of these patients do not report any symptomatology [17,24,26,28,32]. Differently from publications where *N. gonorrhoeae* infection is said to be frequently symptomatic, especially in males we found that only 16.7% of patients with gonorrhoea presented symptoms [5,8,10,26,28].

As expected, the analysis of the sociodemographic and behavioural characteristics of our participants showed that having two or more sexual partners during the previous six months was a risk factor for *C. trachomatis* and *N. gonorrhoeae* infections.

Non-consistency in the use of condoms was identified as a risk factor in the acquisition of *C. trachomatis* and *N. gonorrhoeae* infection, although non-significant for the latter, in the multivariate analysis.

According to several studies, STI increase HIV transmission, which is considered a useful marker for risk sexual behaviours. The CDC then recommends screening other STI in people

diagnosed with HIV and vice-versa8. This study found no evidence of links between HIV infection and the presence of *C. trachomatis* and *N. gonorrhoeae,* what is probably due to the small number of participants. However, a previous episode of gonorrhoeae was statically associated with a new *N. gonorrhoeae* infection, with quite a number of individuals infected with *C. trachomatis* (20%) having a past STI, 50% of individuals with gonorrhoea also having clamidiosis, while 15% with *C. trachomatis* had *N. gonorrhoeae* infection.

Limitations of this study were the relatively small sample size of infected patients which may have influenced the analysis of some individual characteristics.

There is also a risk, as in all studies based on questionnaires that some participants may have answered according to what is more accepted socially.

In conclusion, it should be noticed that although the majority of participants volunteered for screening out of curiosity and not because they were aware of their risk sexual behaviour, unprotected intercourse and multiplicity of sexual partners in a short period of time was very frequent. Therefor it seems that most individuals are not aware that they are engaging in high risk sexual behaviour.

It should also be highlighted that most infected participants did not show symptoms, which emphasizes the importance of screening, as asymptomatic individuals constitute a reservoir of infection.

In view of the lack of awareness about engaging in risk factors that promote the acquisition of STI, as well as the absence of symptoms in most infected individuals that prevents them from seeking health services or adjust their risk practices, sexual health education, decreasing barriers to health care and non-invasive STI screening are essential in this population and in similar ones.

References

1. World Health Organization. Global Strategy for the Prevention and Control of Sexually Transmitted Infections: 2006-2015: breaking the chain of transmission. 2007. Available at: http://www.who.int/reproductivehealth/publications/rtis/9789241563475/en/
2. European Centre for Disease Prevention and Control. Annual epidemiological report 2014 - sexually transmitted infections, including HIV and blood-borne viruses. 2015. Available at: https://ecdc.europa.eu/sites/portal/files/media/en/publications/Publications/sexually-transmited-infections-HIV-AIDS-blood-borne-annual-epi-report-2014.pdf
3. World Health Organization. Sexually transmitted infections Fact Sheet. 2016. Available at: http://who.int/mediacentre/factsheets/fs110/en
4. World Health Organization. Global Incidence and Prevalence of Selected Curable Sexually Transmitted Infections - 2008. 2012. Available at: http://apps.who.int/iris/bitstream/10665/75181/1/9789241503839_eng.pdf
5. World Health Organization. Laboratory diagnosis of sexually transmitted infections, including human immunodeficiency virus. 2013. Available at: http://apps.who.int/iris/bitstream/10665/85343/1/9789241505840_eng.pdf?ua=1
6. Papp JR, Schachter J, Gaydos CA, Van Der Pol B. Recommendations for the laboratory-based detection of Chlamydia trachomatis and Neisseria gonorrhoeae--2014. MMWR Recomm Rep. 2014;63:1-19.
7. European Centre for Disease Prevention and Control. Chlamydia control in Europe: literature review. 2014. Available at: https://ecdc.europa.eu/sites/portal/files/media/en/publications/Publications/chlamydia-control-europe.pdf
8. Workowski KA, Bolan GA. Sexually transmitted diseases treatment guidelines, 2015. MMWR Recomm Rep. 2015;64:1-137.
9. Chan PA, Janvier M, Alexander NE, Kojic EM, Chapin K. Recommendations For the Diagnosis of Neisseria gonorrhoeae and Chlamydia trachomatis, Including Extra-Genital Sites. Med Heal R I. 2012;95:252-254.
10. Bignell C, Unemo M. 2012 European guideline on the diagnosis and treatment of gonorrhoea in adults. Int J STD AIDS. 2013;24:85-92. doi: 10.1177/0956462412472837
11. LeFevre ML. Screening for chlamydia and gonorrhea: U.S. Preventive Services Task Force recommendation statement. Ann Intern Med. 2014;161:902-910. doi: 10.7326/M14-1981
12. Cunha CB, Friedman RK, de Boni RB, Gaydos C, Guimarães MRC, Siqueira BH, et al. Chlamydia trachomatis, Neisseria gonorrhoeae and syphilis among men who have sex with men in Brazil. BMC Public Health. 2015;15:686.
13. Jalal H, Stephen H, Curran MD, Burton J, Bradley M, Carne C. Development and Validation of a Rotor-Gene Real-Time PCR Assay for Detection, Identification, and Quantification of Chlamydia trachomatis in a Single Reaction. J Clin Microbiol. 2006;44:206-213.
14. Whiley DM, Buda PJ, Bayliss J, Cover L, Bates J, Sloots TP. A new confirmatory Neisseria gonorrhoeae real-time PCR assay targeting the porA pseudogene. Eur J Clin Microbiol Infect Dis. 2004;23:705-710. DOI: 10.1007/s10096-004-1170-0
15. Ho BS, Feng WG, Wong BK, Egglestone SI. Polymerase chain reaction for the detection of Neisseria gonorrhoeae in clinical samples. J Clin Pathol. 1992;45:439-442.

16. Centers for Disease Control and Prevention. Sexually Transmitted Disease Surveillance 2015. 2016. Available at: https://www.cdc.gov/std/stats15/std-surveillance-2015-print.pdf

17. Dielissen PW, Teunissen DA, Lagro-Janssen AL. Chlamydia prevalence in the general population: is there a sex difference? a systematic review. BMC Infect Dis. 2013;13:534. doi: 10.1186/1471-2334-13-534

18. Belozorov A, Fedets O, Chastii T, Milutina E, Sokol O, Grigorova R, et al. Chlamydia trachomatis infection positivity rates determined by nucleic acid amplification test in patients of hospitals in the northeastern region of Ukraine. Int J STD AIDS. 2017;28(14)1405-1409. doi: 10.1177/0956462417709831

19. Del Prete R, Ronga L, Lestingi M, Addati G, Angelotti UF, Di Carlo D, et al. Simultaneous detection and identification of STI pathogens by multiplex Real-Time PCR in genital tract specimens in a selected area of Apulia, a region of Southern Italy. Infection. 2017;45(4):469-477. doi: 10.1007/s15010-017-1002-7

20. Ginindza TG, Stefan CD, Tsoka-Gwegweni JM, Dlamini X, Jolly PE, Weiderpass E, et al. Prevalence and risk factors associated with sexually transmitted infections (STIs) among women of reproductive age in Swaziland. Infect Agent Cancer. 2017;12:29. doi: 10.1186/s13027-017-0140-y

21. Kojima N, Park H, Konda KA, Joseph Davey DL, Bristow CC, Brown B, et al. The PICASSO Cohort: baseline characteristics of a cohort of men who have sex with men and male-to-female transgender women at high risk for syphilis infection in Lima, Peru. BMC Infect Dis. 2017;17:255. doi: 10.1186/s12879-017-2332-x

22. Miranda AE, Silveira MF, Travassos AG, Tenório T, Val ICCD, Lannoy L et al. Prevalence of Chlamydia trachomatis and Neisseria gonorrhea and associated factors among women living with Human Immunodeficiency Virus in Brazil: a multicenter study. Brazilian J Infect Dis 2017. doi: 10.1016/j.bjid.2017.03.014

23. Thomas P, Spaargaren J, Kant R, Lawrence R, Dayal A, Lal JA, et al. Burden of Chlamydia trachomatis in India: a systematic literature review. Pathog Dis 2017;31:75(5). doi: 10.1093/femspd/ftx055

24. Pedrosa AF, Azevedo F, Lisboa C. Screening for Chlamydia infection in a sexually transmitted infection clinic: a missed opportunity?. Int J Dermatol 2015;54:405-409. doi: 10.1111/ijd.12338

25. Santo I, Azevedo J, Nunes B, Gomes JP, Borrego MJ. Partner notification for Chlamydia trachomatis urogenital infections: eight years of patient referral experience in the major Portuguese sexually transmitted infections clinic, 2000-07. Int J STD AIDS. 2011;22(10):548-551. doi: 10.1258/ijsa.2011.010268

26. Borges Da Costa J, Azevedo J, Santo I. Sexually transmitted infections and related sociodemographic factors in Lisbon's major Venereology Clinic: A descriptive study of the first 4 months of 2007. J Eur Acad Dermatology Venereol. 2010;24(7):811-814. doi: 10.1111/j.1468-3083.2009.03530.x

27. Dinis M, Cordeiro D, Santo I, Azevedo J, Gomes JP, Borrego MJ. Diagnóstico laboratorial da infeção por Chlamydia trachomatis , 1991-2014. Boletim epidemiologico, INSA. 2015;16-18.

28. Guedes R, Simões JS, Azevedo F, Lisboa C. Infecção por Chlamydia trachomatis e Neisseria gonorrhoeae em Utentes de uma Consulta de Doenças de Transmissão Sexual - Análise de Dez Anos. Soc Port Dermatologia e Venerol. 2012;70(1):91-97.

29. Clarivet B, Picot E, Marchandin H, Tribout V, Rachedi N, Schwartzentruber E, et al. Prevalence of Chlamydia trachomatis, Neisseria gonorrhoeae and Mycoplasma genitalium in asymptomatic patients under 30 years of age screened in a French sexually transmitted infections clinic. Eur J Dermatology 2014;24(5):611-616. doi: 10.1684/ejd.2014.2413

30. Centers for Disease Control and Prevention. Reported STDs in the United States 2013 National Data for Chlamydia, Gonorrhea, and Syphilis. 2014. Available at: https://www.cdc.gov/std/stats13/std-trends-508.pdf

31. Bayette J, Jreige R, Marchandin H, Laurens C, Joullié F, Clarivet B, et al. Prevalence of Chlamydia trachomatis, Neisseria gonorrhoeae and Mycoplasma genitalium infections in the emergency department. Pathol Bio. 2013;61:245-249. doi: 10.1016/j.patbio.2012.04.001

32. Corbeto EL, Lugo R, Martró E et al. Prevalencia de la infección por *C. trachomatis* y N. gonhorroeae y determinantes para su adquisición en jóvenes y adultos-jóvenes en Cataluña. Enferm Infecc Microbiol Clin. 2011;29(2):96-101. DOI: 10.1016/j.eimc.2010.08.011