BJGH Brazilian Journal of Global Health
Revista Brasileira de Saúde Global

Chlamydia trachomatis molecular screening in women of poor region of São Paulo: challenges and relevance in low-income regions

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ABSTRACT

OBJECTIVE

Chlamydia trachomatis is a gram-negative bacterium. Its infection often presents asymptomatically in women but can lead to severe reproductive complications if left untreated. Among the most frequent sexually transmitted infections (STIs), Chlamydia accounts for around 130 million out of the 374 million new cases globally in 2020 and poor socioeconomic conditions may contribute to this situation, yet C. trachomatis infection is often under-investigated in population in these conditions. Therefore this study evaluates the frequency of C. trachomatis in women living in impoverished areas of São Paulo city.

METHODS

Samples were collected from patients at a gynecological outpatient clinic in the far southern region of São Paulo city. The participants were women aged 18 to 78 years. Cervical scraping samples were collected and screened for C. trachomatis using PCR. Additionally, sociodemographic information was gathered through a questionnaire, and clinical data were retrieved from medical records.

RESULTS

Out of 102 samples analyzed through Real-Time PCR, 17 were positive, indicating a prevalence of 16.67% (95% CI) for chlamydial infection in the studied population. There was also a significant association between the frequency of chlamydial infection and the age of the first sexual intercourse ($x^2 = 34.05$, $p < 0.0001$).

CONCLUSIONS

This study reveals a high frequency of chlamydial infection among women attending an outpatient clinic. Our findings emphasize the importance of regular screening to enable early treatment and prevent further reproductive complications.

DESCRIPTORS

Chlamydia trachomatis; Sexually transmitted infections; PCR.

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DOI: https://doi.org/10.56242/globalhealth;2024;4;14;24-27

INTRODUCTION

Sexually transmitted infections (STIs) are a major reproductive health issue of public health concern worldwide. WHO has estimated that there was around 374 million new STIs cases in 2020, and among them, Chlamydia represents nearly 130 million cases[1]. Still, data reported by the Pesquisa Nacional da Saúde (PNS) and Instituto Brasileiro de Geografia e Estatística (IBGE) have estimated that around 0.6% of the population have acquired STIs in Brazil[2].

Chlamydia trachomatis is an ubiquitous gram-negative bacteria that replicates intracellular obligately [3]. Interestingly, infections by *C. trachomatis* in women are usually asymptomatic, but they can result in reproductive complications if left untreated. These complications may vary from chronic pelvic pain and tubal infertility to ectopic pregnancy and Pelvic Inflammatory Disease [4–6]. Unfortunately, there is no vaccine for *C. trachomatis* prevention and in the lack of vaccination, screening for *C. trachomatis* infection relies as a possible tool for preventive measure and early diagnosis for treatment. In fact, previous findings supports that screening may impact on the frequency of infection [4,7].

Importantly, poor socioeconomic conditions may interfere with the frequency of Chlamydia infection, and also deprived individuals are at higher risk for IST infections, including Chlamydia [8]. In this sense, São Paulo is the biggest city of the country and the most densely populated, with around 11milion inhabitants. The South Region of São Paulo is home to around 2.5 million individuals, mainly composed by young people [9]. Although the wealth condition of the city is good, the South Region is the poorest and some neighborhoods have concerning socioeconomic conditions, where per capita income is around 0.4 of the Brazilian minimum wage. [10]. This finding highlights the possibility that this region may be at higher risk of ISTs infection and in fact, our group have revealed high frequency of infection do high risk HPV and coinfection by multiple HPV types [11]and also high frequency of *N. gonorrhoeae* frequency and antimicrobial resistance [12] in poor regions of Sao Paulo. For this reason, it is feasible to suppose that these individuals may be at higher risk for *C. trachomatis* infection. Therefore, the aim of this study is to determine the frequency of *C. trachomatis* infection in women who live in poor areas of São Paulo city, Brazil.

METHODS

Study population and ethics

The following study is retrospective transversal study. Sampling was performed by convenience, patients who attended to the gynecology out care patient service of Hospital Escola Wladimir Arruda located in the South Zone of São Paulo, a poor region of São Paulo city, were invited to participate on the study. A total of 102 women were enrolled in the study, the participants were aged between 18-78 years-old. The sample collection was obtained by scraping cervical-vaginal material from the uterine cervix, specifically from the ectocervix, squamo-columnar junction (SCJ), and endocervical canal using a plastic bristle brush rotated 360º and then placed in a liquid-based cytology medium ThinPrep®. Sociodemographic, gynecological, sexual and health data were collected through a questionnaire, their symptoms and clinical manifestation were obtained by the medical records. This study was approved by the ethical committee of University Santo Amaro under protocol #4.237.904. Participants were invited to read and sign an informed consent form prior to participating in the study, and the sampling occurred during February to November 2021.

Sample preparation, DNA extraction and quality control

Around 5ml ThinPrep media of each sample were placed in falcon tubes and were centrifuged 1500 x g for 3 minutes, the cell pellets were then washed with sterile PBS and centrifuged again and the supernatant was discarded. The samples were then resuspended in the 1mL of PBS. An aliquot of 200µL was used to perform DNA extraction with PureLink TMGenomic DNA Mini Kit (Germany), following the manufacturer's instructions. Additionally, in order to assure the quality of the extracted DNA, a PCR was conducted with primers complementary to GAPDH gene, as previously described protocol[13].

Chlamydia trachomatis detection

A Sybr Green Real-Time PCR assay was used to perform the test in order to detect Chlamydial infection. For each samples 3.2mM of each primer were added, and the sequence of each primer are as follows: Forward 5'-GGATTGACTCCGACAACGTATTC-3' Reverse: 5'- ATCATTGCCATTAGAAAGGGCATT -3' these primers were previously described by [14] and amplifies the cryptic plasmid of *C. trachomatis*. The cycling conditions were as follows: 95ºC for 10 minutes, 40 cycles of 95ºC for 15 seconds, 60ºC for 1 minute a melting curve stage was used to confirm the amplification curves. *A C. trachomatis* strain was used as a positive control, also negative controls were added in every reaction, and all samples were tested in duplicates.

Statistical Analysis

All statistical data from the study were analyzed by *GraphPad Prism 8*. Frequencies were shown as descriptive data, also data was analyzed by Chi-Square test was used for comparing different qualitative variables and averages between two groups. Kappa test was used in order to determine possible associations between the frequency of infection and other risk factors. In all steps a level of 5% (0.05) was considered statistically significant.

RESULTS

Study population

A total of 102 women were enrolled in this study, and their sociodemographic, gynecological and sexual behavior and health characteristics are described in table 1. Most of them were married or with stable union (50.99%), with age from 21 – 60 years old, with one partner in the last 6 months and with first sexual intercourse at 11 – 20 years old (80.40%).

Table 1 - Demographic data and sexual behavior of the women who were enrolled in the study.

Variables	N	%
Marital status		
Married/stable union	52	50.99
Single	33	32.35
Divorced	11	10.78
Widow	4	3.92
Uninformed	2	1.96
Age (years-old)		
18-20	4	3.92
21-30	17	16.67
31-40	20	19.61
41-50	24	23.53
51-60	25	24.51
61-70	10	9.80
71-80	2	1.96
First Sexual Intercourse		
11 to 20	82	80.40
21 to 30	17	16.60
31 to 40	3	3
Number of sexual partners (last 6 months)		
0	25	24.51
1	67	65.69
2	8	7.84
>3	2	1.96
Total	102	100%

Source: Authors.

Interestingly, most of the patients, 84 (80.40%) have reached out to seek the health services without any clinical manifestation. On the other hand, 18 (17.64%) patients have sought gynecological medical assistance due to a clinical symptom/manifestation, and among them, the most frequent clinical manifestation was urinary incontinence and abdominal lower pain (dysmenorrhea). All sam-

ples were positive for GAPDH detection, and therefore, all of them were tested for Chlamydia. Of the total participants, 17 patients were positive for *C. trachomatis*, which corresponds to a general frequency of a chlamydial infection in the studied population of 16.67% (CI = 95 %). The majority of the infected patients were asymptomatic, but only 3 (2.94%) of them presented dysmenorrhea.

Next, we sought to determine if the frequency of infection could be associated with some known risk factors. According to table 2 there was no association of the frequency of infection according to the number of sexual partners in the last 6 months.

Table 2 - Association of Chlamydia infection status and the number of sexual partners in the last six months. $x^2 = 0.52$ p=0.7699.

Number of sexual partners (last 6 months)	Positive for Chlamydia	Negative for Chlamydia	% of positive samples
0	5	20	20
1	11	56	16
>2	1	9	10
Total	17	85	16,67

Source: Authors.

Additionally, as presented in Table 3, there is a significant association between the age at FSI and the frequency of Chlamydia infection. The data indicates that patients who had their FSI at a younger age exhibit a higher frequency of infection ($x^2 = 34.05$, $p < 0.0001$). Notably, all patients in this group reported having had fewer than four sexual partners over their lifetime. Additionally, they predominantly used contraceptive methods other than condoms.

Table 3 - Association of Chlamydia infection status and the age of the first sexual intercourse. $x^2 = 34.05$ *=p<0.0001.

Age of the First Sexual Intercourse	Positive for Chlamydia	Negative for Chlamydia	% of positive samples
15-20	14	11	56.00*
21-30	3	65	4.41
31-40	0	3	0,00
	17	85	56.00

Source: Authors.

Additionally, we have observed no association of *C. trachomatis* infection with previous spontaneous abortion, only one out of 17 patients have reported previous abortion and we have not observed any association with marital status, education or age.

DISCUSSION

We have detected a prevalence of 16.67% of *C. trachomatis* in our study population. This frequency is high according to a previous global prevalence estimate. By using a Bayesian meta-analysis approach, the authors claimed that the global prevalence is around 3.6% of the population [15]. Interestingly, the number of sexual partners in the last 6 months was considerably low, even though this was collected by a self-reported questionnaire, it is not possible to precisely determine the sexual life behavior of the patients. And, in this sense, our findings reinforce the need for constant surveillance to screen *C. trachomatis*. Our findings may reveal a concerning issue, regarding the asymptomatic profile of our patients, which may be a considerable risk for further reproductive complications, also considering the high prevalence found in our population compared to other regions of the country. In the amazon region, previous report by Santos et al., revealed an overall prevalence of 4.6% [16], our finding is also higher than previous study performed in São Paulo (2.2%), which did not considered poor individuals [17], in Minas Gerais state an overall prevalence of 6,3% [18] and women who lives with HIV (2,1%) [19]. An interesting study performed in Pelotas city, Rio Grande do Sul state investigated pregnant women attended in public hospitals and revealed similar prevalence of 12,3% [20].

Therefore, our findings bring a concerning issue regarding the dynamics of *C. trachomatis* infection in the general population. Our population is located in a poor region of São Paulo, Brazil, which depends most exclusively on the governmental health services (SUS)[21]. It is predominantly composed of poor people who live with less than 2 Brazilian minimum wages (around 400U$) as a total income and also by low education grade. Importantly, the lack of compulsory notification and also the lack of frequent screening may be considered a major public health issue, considering the consequences of clinical manifestations and impact in the fertility of the affected women. And may favor, as previously observed the increase of bacteria transmission throughout the years [17]. And in fact, this seems not to be observed exclusively to the C. trachomatis infection, the profile of STI has been changing during the last decades and it is estimated a significant increase of STI occurrence cases among women population [22].

Importantly, our study was conducted during the SARS-CoV2 pandemic. Unfortunately, during this time there was an increase on the access and use of antibiotics, which could impact the self-medication and probably the resistance acquirement for *Chlamydia trachomatis*. Specially in countries with poor antibiotics control actions and restricted access to the health settings. And in fact, a previous study reported that around 70% patients who had been infected by SARS-CoV2, and 33% of them reported self-medication prior hospital admission [23]. In addition, C. trachomatis infection may alter the cervical environment and may also leads to higher oxidative stress [24] and inhibiting DNA repair pathways[25], and therefore may predispose the development of cervical squamous carcinoma by co-infection with HPV[24].

It is also important to consider the antimicrobial resistance of *C. trachomatis* that is commonly reported among the affected patients. This is crucial to precisely determine the treatment and reduce the risk for complications. In this sense, our study presents some limitations, mostly due to low sampling, lack of resistance investigation and quantification of the bacterial in each sample. However, our findings are still concerning and reinforces the need for constant screening studies to be rolled out, especially in those socially and economically vulnerable regions, in order to prevent chlamydia infection to occur and prevent infertility complications, other clinical manifestations and worse scenarios with coinfections with other microbiological agents that could certainly impact the quality of life of the affected women and treatment scenarios.

Conflict of interest

The authors declare to not have any conflict of interest that might interfere in the study.

Funding Source

This study was funded by Universidade Santo Amaro.

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