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Incidence of *Chlamydia trachomatis* detected by PCR in women's endocervical samples in Lages, Santa Catarina, Brazil

Incidência de Chlamydia trachomatis *detectada por PCR em amostras endocervicais de mulheres em Lages, Santa Catarina, Brasil*

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ABSTRACT

Introduction: *Chlamydia trachomatis* is one of the major causative agents of sexually transmitted infections (STI) in men and women, but the greatest impact of infection occurs in the reproductive system of women, causing, among other problems, infertility. **Objective:** To estimate the incidence of *C. trachomatis* detected by the polymerase chain reaction (PCR) technique in endocervical samples from women in Lages, Santa Catarina, Brazil. **Methods:** A descriptive, quantitative, cross-sectional study was conducted from May to November 2017. Endocervical cell samples were collected from 126 women that receive medical assistance at three primary healthcare units and one gynecology clinic in the city. The samples of endocervical material were evaluated for the diagnosis of *C. trachomatis* by the PCR technique. Prior to sample collection, a Cancer Information System (SISCAN) standard questionnaire was answered by the participants. Fisher's exact test and chi-square test were used to evaluate the association between infection and the variables of the questionnaire. **Results:** From the 126 endocervical samples analyzed, 39.7% were positive for *C. trachomatis*. The average age of the participants was 31.7 years old. From the 118 women who did not show changes in the cervix, 43 were positive for *C. trachomatis*, which reinforces the asymptomatic character of the infection. There was no significant association between endocervical infection by *C. trachomatis* and age, use of contraceptive pill, time of last pap test and signs of STI. **Conclusion:** The incidence of *C. trachomatis* infection in the endocervical samples from these women can be considered high, which emphasizes the importance of measures designed to clarify and prevent this important STI.

Key words: *Chlamydia trachomatis*; diagnosis; polymerase chain reaction; sexually transmitted diseases; public health.

RESUMO

Introdução: Chlamydia trachomatis *é um dos principais agentes causadores de infecções sexualmente transmissíveis (IST) em homens e mulheres, porém, o maior impacto da infecção ocorre no sistema reprodutivo das mulheres, ocasionando, entre outros problemas, infertilidade.* ***Objetivo:*** *Estimar a incidência de* C. trachomatis *detectada pela técnica da reação em cadeia da polimerase (PCR) em amostras endocervicais de mulheres em Lages, Santa Catarina, Brasil.* ***Métodos:*** *Estudo transversal descritivo quantitativo, realizado entre maio e novembro de 2017, com coleta de material endocervical de 126 mulheres atendidas em três unidades básicas de saúde e em um consultório médico ginecológico. As amostras de material endocervical foram avaliadas para diagnóstico de* C. trachomatis *pela técnica da PCR. Todas as participantes responderam a um questionário padrão do Sistema de Informação do Câncer (SISCAN) antes da coleta das amostras. Teste exato de Fischer e qui-quadrado foram utilizados para avaliar a associação entre a infecção e as variáveis do questionário.* ***Resultados:*** *Das 126 amostras endocervicais, 39,7% foram positivas para* C. trachomatis*. As participantes tinham idade média de 31,7 anos. Das 118 mulheres que não apresentaram alterações no colo do útero, 43 foram positivas para* C. trachomatis*, o que reforça o caráter assintomático da infecção. Não houve associação significativa entre a infecção endocervical por* C. trachomatis *e a idade, o uso de anticoncepcional, o tempo do último exame preventivo e os*

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sinais de IST. ***Conclusão:*** *A incidência de infecção por* C. trachomatis *no material endocervical dessas mulheres pode ser considerada elevada. Isso mostra a importância de medidas para esclarecimento e prevenção dessa importante IST.*

Unitermos: Chlamydia trachomatis*; diagnóstico; reação em cadeia da polimerase; doenças sexualmente transmissíveis; saúde pública.*

RESUMEN

Introducción: Chlamydia trachomatis *es uno de los principales agentes causantes de infecciones de transmisión sexual (ITS) en hombres y mujeres, pero el mayor impacto de la infección se produce en el aparato reproductor de las mujeres, causando, entre otros problemas, infertilidad.* ***Objetivo:*** *Calcular la incidencia de* C. trachomatis *detectada por la técnica de reacción en cadena de la polimerasa (PCR) en muestras endocervicales de mujeres en Lages, Santa Catarina, Brasil.* ***Métodos:*** *Estudio descriptivo, transversal, cuantitativo, realizado entre mayo y noviembre de 2017, con recolección de material endocervical de 126 mujeres atendidas en tres unidades básicas de salud y en un consultorio ginecológico. Se evaluaron las muestras de material endocervical para diagnóstico de* C. trachomatis *mediante la técnica de PCR. Todas las participantes respondieron a un cuestionario estándar del Sistema de Información del Cáncer (SISCAN) antes de la recolección de las muestras. Las pruebas exacta de Fisher y de ji cuadrado fueron utilizadas para evaluar la asociación entre la infección y las variables del cuestionario.* ***Resultados:*** *De las 126 muestras endocervicales, el 39,7% fueron positivas para* C. trachomatis*. Las participantes tenían edad media de 31,7 años. De las 118 mujeres que no presentaron alteraciones en el cuello uterino, 43 fueron positivas para* C. trachomatis*, lo que refuerza el carácter asintomático de la infección. No hubo asociación significativa entre la infección endocervical por* C. trachomatis *y edad, uso de anticonceptivo, fecha del último Papanicolaou y señales de ITS.* ***Conclusión:*** *La incidencia de infección por* C. trachomatis *en el material endocervical de esas mujeres puede ser considerada alta. Eso muestra la importancia de medidas de aclaración y prevención de esa seria ITS.*

Palabras clave: Chlamydia trachomatis*; diagnóstico; reacción en cadena de la polimerasa; enfermedades de transmisión sexual; salud pública.*

INTRODUCTION

Chlamydia trachomatis is one of the most prevalent bacteria that cause sexually transmitted infections (STI) in men and women[1]. Infection risk factors include being young or adolescent, the number of sexual partners, using contraceptive pill, recordings of sexual infection and low educational and socioeconomic levels[2]. In general, the infection is asymptomatic in women and less commonly observed in men, but the infection may serve as a bacterium transmission reservoir to sexual partners[3].

C. trachomatis can infect various types of cells and tissues in the human body. A considerable infection are found in the urogenital tract, where this agent mainly infects the columnar epithelium cells of the genital mucosa, the cervix is the most commonly infected site in women[4]. This bacterium may cause infectious cervicitis and urethritis in women as well as pelvic inflammatory disease (PID) that, consequently, may cause complications in the woman's reproductive system, such as chronic pelvic pain, ectopic pregnancy, and infertility[5].

Regarding the prevalence of STI by *C. trachomatis*, according to the World Health Organization (WHO), the number of cases reached 105.7 million worldwide in 2008[6]. In Europe and the US, this infection requires mandatory notification, and only in the US, two to five million new cases are reported every year[7]. For this reason, it is considered the main bacterial STI, and is called silent epidemics because it is asymptomatic in more than 80% of the cases[8]. In Europe, 345,000 cases of infections by *C. trachomatis* were diagnosed in 2010[3]. In England, 200,000 people were diagnosed with STI caused by this bacterium in 2016, representing almost half of all STIs recorded in the year, with an incidence more usually found in young people aged 15 to 24 years[9].

In Latin America, infections caused by *C. trachomatis* are not notifiable, and the estimates are based on local studies. As an example, the prevalence of this infection in sexually active women was 26.4% in the northeastern region of Argentina[10], and 5% in female sex workers in Peru[11].

In Brazil, there are no consolidated data of infections caused by *C. trachomatis* because it is not a notifiable disease[12]. The prevalence of this infection in Brazil, according to clinical

studies with women who received medical care in gynecological ambulatories and in gynecologist's offices ranged from 4.3% to 31%[13]. But this prevalence may vary according to the region, method of diagnosis and the participants' characteristics. In a study conducted in Manaus, for example, 52.8% of the women who had infertility problems were infected by *C. trachomatis*[14]. In São Miguel do Oeste, Santa Catarina (SC), Brazil, the *C. trachomatis* infection prevalence in endocervival samples was 11%[15]; another study carried out with women from Santa Catarina e São Paulo showed a prevalence of 56,4%[16]. The tests used for the diagnosis of *C. trachomatis* can be a limiting factor for the determination of its prevalence in certain studies. Conventional methodologies, such as cell culture, require viable microorganisms, invasive collection, and its lack of sensitivity makes the bacterium identification difficult[17]. In recent years, techniques for amplification of nucleic acids based on the polymerase chain reaction (PCR) offered improvements in the sensitivity of *C. trachomatis* infection diagnosis, and when it is possible, screening of the bacterium is recommended[18, 19].

Therefore, due to the lack of studies on genital infection caused by *C. trachomatis* in Santa Catarina, the aim of this study was to estimate the incidence of *C. trachomatis* detected by the PCR technique in endocervical samples from women in Lages-SC, Brazil.

METHODS

Participants and study design

A quantitative, descriptive, cross-sectional study was carried out for the detection of *C. trachomatis* in endocervical cells of women in the period from May to November 2017. This study was approved by the Research Ethics Committee of the Universidade do Planalto Catarinense (Process no. 2.028.254). An Informed Consent Form was signed by all the participants.

Endocervical cells collection tests for *C. trachomatis* were carried out at three Primary Healthcare Units (PHU) located in Tributo, Santa Mônica and Coral neighborhoods, and in a gynecologist's office in the city of Lages-SC. These sites were selected by convenience, due to the researcher's facility to obtain samples and access to the records of the women's monthly visits for routine screening tests.

The study included women aged 18 years and older, non-pregnant, non-postmenopausal, no hysterectomy, no prior report of human immunodeficiency (HIV) seroconversion, no vaginal bleeding, no urinary loss, no use of antibiotics or vaginal cream in the preceding 30 days, abstinence from sexual intercourse in the 72 hours prior to the exam, with pap smear appointments scheduled in the above mentioned healthcare units. Women who did not fit the eligibility criteria or the ones who refused to participate were excluded.

The women who met the eligibility criteria were referred to a nurse who explained the objectives and importance of participating in the research. As a result, 126 women participated in the study and all of them answered a standard questionnaire provided by the Cancer Information System [Sistema de Informação do Câncer (SISCAN)], routinely used at the PHUs and gynecology clinics/offices before the collection of the sample for the pap test. The following information was obtained from the questionnaire: age, the reason for the test, if she had ever done a pap test, date of last pap test, if she had been using an intrauterine device, if she had been using birth control pills and if she had experienced bleeding after intercourse. After the pap test, information about the cervix (normal or abnormal) was collected and if there was a signal of STIs.

Sample collection

After the patient's consent, she was taken to the collection room where the nurse first collected the sample for the appointed pap smear exam, and then performed the endocervical cell collection, for the *C. trachomatis* test. For this second collection the nurse used an endocervical brush (Cytobrush), rotating it 360º in the endocervix to collect squamous cells. Each sample was stored in 400 microliters of TE solution [tris-HCl pH 8.0 10 mM/ ethylenediamine tetraacetic acid (EDTA) pH 8.0 1 M] and kept at -20ºC until the time of analysis.

PCR diagnostic

For the tests, each sample was treated with 400 µl of TPK solution (TE 20%, 10 mg/ml of protein K) followed by incubation at 60ºC during 90 minutes, and then boiled for 10 minutes, and deoxyribonucleic acid (DNA) was extracted by the phenol/ chloroform method.

The plasmid primers[20] 1KL10 (5'TCCGGAGCGAGTTACGAAGA3'), KL20 (3'AATCAATGCCCGGGATTGGT5') were used to amplify a 241-base pairs fragment of plasmid DNA from *C. trachomatis* using PCR. PCR consisted of 5 µl DNA, and 5 µl of 10× PCR buffer, 2 µl of $MgSO_4$ 50 mM, 1 µl of dNTP 10 mM, 5 µl of each primer (5 pmol/µl), and 0.2 µl of 5 U/µl Taq DNA polymerase, and 26.8 µl of water to complete 50 µl of volume.

The amplification was performed on a thermocycler using a 30-cycle amplification program, pre-denaturation at 94ºC for 60 seconds, denaturation at 94ºC for 30 seconds, annealing at 57ºC during 60 seconds and extension/elongation at 72ºC during 2 minutes, followed by a final extension at 72ºC during 8 minutes. Amplicons analysis was carried out by electrophoresis in agarose gel (1.5%) stained with ethidium bromide.

To confirm the amplicon identity, all positive samples for *C. trachomatis* were sequenced. To prepare the samples for the amplicon sequencing, a microcentrifuge tube was used, which was filled with 240 ng of DNA and completed with milli-Q water to a final volume of 6 µl. Then it was dried in a SpeedVac at 35ºC for 30 minutes and sent for sequencing, which was carried out on an ABI-Prism 3500 Genetic Analyzer (Applied Biosystems) by ACTGene Company.

In the analysis, a positive control (PC) and a negative control (NC) for *C. trachomatis* were used, in which DNA was not present where there is an amplification of the 241-bp fragment of *C. trachomatis* DNA in endocervical samples subjected to PCR (**Figure**).

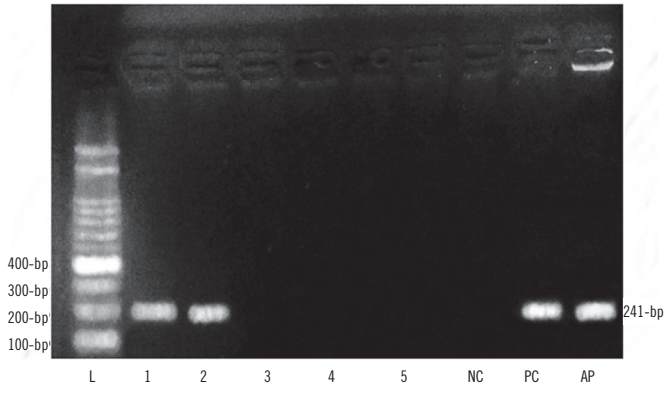

FIGURE – *Amplification of the 241-bp fragment of* C. trachomatis *DNA in 1.5% agarose gel*

Source: Author.

DNA: deoxyribonucleic acid; L: 100-bp molecular size marker (Ludwig); columns 1-2: positive samples; columns 3-5: negative samples; NC: negative control; PC: positive control; AP: sample known for C. trachomatis.

Statistical analyzes

The results of PCR for *C. trachomatis* and the data obtained in the questionnaire were tabulated and subjected to descriptive analysis, and the values were presented in percentage. Fisher's exact test and chi-square test ($p \leq 0.05$) were used to evaluate the association between infection with age, use of birth control pills, time of the last pap test and STI signals (IBM SPSS – Statistical Package for the Social Sciences, version 20).

RESULTS

The average age of the participants was 31.7 years old (18 to 53 years old) and the incidence of *C. trachomatis* was 39.7% ($n = 50$) out of 126 women that participated in the study. Regarding the incidence of *C. trachomatis* at the studied healthcare units, one woman out of three from the Coral PHU was positive for the bacterium; in Santa Mônica PHU, seven out of 18 women were positive; in Tributo PHU, 15 out of 35 were positive, and at the gynecologist's office, 28 out of 70 women were positive. The incidence of *C. trachomatis* in endocervical samples and the information collected from SISCAN questionnaire are presented in **Table**.

TABLE – Information about the participants based on the SISCAN questionnaire and the incidence of *Chlamydia trachomatis* in endocervical samples. There was no significant difference between means ($p > 0.05$)

Variable	Total		*Chlamydia* positive	
	n	%	*n*	%
Reason for doing the test				
Screening	116	92	47	40.5
Follow-up	10	8	4	40
Have you ever done a Pap test?				
Yes	119	94.4	49	41.2
No	7	5.6	7	100
Last Pap test				
One year ago	6	4.8	2	33.3
Two or more years ago	120	95.2	48	40
Use of contraceptive pill				
Yes	46	36.5	22	47.8
No	80	63.5	28	35
Bleeding after intercourse				
Yes	20	15.9	8	40
No/don't known/don't remember	106	84.1	43	40.5
Cervix inspection				
Normal	118	93.6	43	36.4
Abnormal	8	6.3	6	75
Signs of STI				
Yes	16	13	9	56.2
No	110	87	42	38.2

SISCAN: Cancer Information System; STI: sexually transmitted infections.

There was no significant association between endocervical infection by *C. trachomatis* and age, use of birth control pill, time of last pap test (one year ago or two or more years ago) and STI signals ($p > 0.05$).

DISCUSSION

The incidence of 39.7% *C. trachomatis* infection, found in the endocervical cell samples from the women of this study,

is considered high when compared to other regional studies conducted in Brazil, where the incidence of infection with this bacterium, analyzed by PCR, was on average 11%[13, 20, 21]. In recent studies conducted in Santa Catarina, the incidence of this infection varied. In self-collected endocervical samples from university students in Florianópolis, also tested by PCR, the incidence was 3%[22]. In another study conducted in Palhoça-SC with endocervical samples from HIV patients, the incidence for *C. trachomatis* detected by PCR was 1.8%[22]. This research warns about the importance of screening for the incidence of this bacterium in endocervical samples during women's routine visits at the PHUs and gynecologist's offices, even in those patients with no complaints and no signs or symptoms, because it is known that this infection, in most cases, is asymptomatic[8].

According to the Brazilian Ministry of Health, from 2007 to 2017, 8,527 HIV cases were notified in Santa Catarina, especially in 2016, with 1,880 reports; and between 2010 and 2017 16,200 cases of syphilis were notified, and 2016 was the year with the highest number of notifications (5,379 cases), which shows that an increase of STIs over the years[2]. In Lages-SC, according to the Department of Health data, the number of notifiable cases of STIs has also risen in this municipality over the years. The increase in STI transmission occurs as a consequence of non-use of condoms and other preventive measures neglected by part of the population, which is also an alert to non-mandatory STIs such as the *C. trachomatis* infection[13], as demonstrated in the present study.

The sensitivity and specificity of the diagnosis method employed in this study may have contributed to the high incidence of *C. trachomatis* in this study, considering that the technique for amplification of nucleic acids based on the PCR provides higher sensitivity and specificity compared to other methods[18]. Considering that this infection may impair the women's and men's reproductive health, we suggest the use of PCR to screen for this bacterium in preventive health routines of PHUs and gynecologist's offices in Lages-SC.

From 119 women who had already done the pap test, 49 had *C. trachomatis* and from seven women who had never done this test before, all of them were positive for *C. trachomatis*, which underlines the importance of doing this test, not only for diagnosis of cervical cancer but to assist in the diagnosis of STIs. Thus, the screening test for *C. trachomatis* detection is important, mainly during the routine visits to the gynecologist, because this bacterium may cause female infertility as well as premature birth in pregnant women, low birth weight, and may be transmitted to the child at birth causing pulmonary and eye diseases[15].

From all the women who participated in the research, 46 used contraceptive pills, and 22 out of that number were positive for *C. trachomatis*. From 80 women who did not take contraceptive pills, 28 were positive for this bacterium. The use of this pill was not a predisposing factor in this research for the presence of *C. trachomatis* infection, but it can be inferred that women who take birth control pills usually do not use condoms, which may increase the risk for STI[23].

When the healthcare provider collects samples for the pap test, he also examines the cervix and checks for STI signs such as discharge, hyperemia, wounds, and odor. In this study, from 118 women who presented normal cervix, 43 were positive for *C. trachomatis*, which reinforces the fact that this infection is mostly asymptomatic. But, on the other hand, six out of eight women who showed cervix alterations had *C. trachomatis*, which is also a characteristic of this infection. Because it is an intracellular bacterium, it infects particularly the columnar epithelial cells of the genital mucosa, and endocervix is the site most commonly infected in women[4].

The number of women who exhibited signs of STI was 16, nine of them were infected by *C. trachomatis*, and 42 out of 110 women who did not have STI signs were positive for the bacterium. This underlines the importance of the pap smear test, which provides important clinical data for the diagnosis of STIs and also for the need to investigate the incidence of *C. trachomatis* because many women are asymptomatic for this infection.

Regarding that, preventive actions should be adopted, since most people are unaware of the infection caused by *C. trachomatis* and its consequences, even though it is considered one of the bacterial STIs that can be easily treated and cured. Unfortunately, in Brazil, *C. trachomatis* is not investigated and notified such as other STIs (HIV, syphilis, hepatitis), and its diagnosis may have a positive impact on the quality of life of women, considering that it may reduce the risk for cervicitis and urethritis, and the risk for developing pelvic inflammatory disease (PID), which may cause complications on the female reproductive system such as chronic pelvic pain, ectopic pregnancy, and infertility[5]. Moreover, screening of sexually active individuals can decrease the burden of disease, leads to treatment, and prevents severe health implications to women and men[24].

This was the first study about *C. trachomatis* in Lages-SC and the results emphasize the importance of developing health measures for awareness and prevention of this critical STI in this city. The women who participated in this research benefited from the result of the tests, which were delivered to the PHUs and the gynecologist's office for further treatment and clarifications about the consequences of *C. trachomatis* infection.

This study has some limitations. First, because our findings came from four different places of collection in Lages-SC and it

is unclear how they can be extended to other areas of the city, although the proportion of infection detection was similar between the sites. Secondly was the impossibility of assessing risk factors associated with *C. trachomatis* infection in Lages-SC, because we could only use the standard SISCAN questionnaire used in the PHU routine services and in the doctor's offices, where data were collected. Thus, more studies are necessary for a better understanding of its epidemiology and associated factors to *C. trachomatis* infection in this municipality.

CONCLUSION

The incidence of infection by *C. trachomatis* in endocervical samples was 39.7% that is considered high when compared to other regional studies conducted in Brazil. This was the first study carried out on the incidence of *C. trachomatis* infection in Lages-SC and since accurate diagnosis and timely treatment are critical to the control of STIs, we suggest the use of PCR methodology in screening tests for this bacterium.

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