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ARTIGO ORIGINAL

RASTREAMENTO DE *CHLAMYDIA TRACHOMATIS, NEISSERIA GONORRHOEAE, MYCOPLASMA GENITALIUM E TRICHOMONAS VAGINALIS* EM GESTANTES ATENDIDAS NA REDE PÚBLICA DE SAÚDE: ESTUDO TRANSVERSAL NO SUL DO BRASIL

CHLAMYDIA TRACHOMATIS, NEISSERIA GONORRHOEAE, MYCOPLASMA GENITALIUM, AND TRICHOMONAS VAGINALIS SCREENING IN PREGNANT WOMEN ATTENDING PUBLIC HEALTH SERVICES: A CROSS-SECTIONAL STUDY IN SOUTHERN BRAZIL

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RESUMO

Contexto: As infecções sexualmente transmissíveis (IST) representam um problema relevante de saúde pública, especialmente em países em desenvolvimento, e não são rastreadas rotineiramente em gestantes brasileiras. Objetivos: Determinar a prevalência de *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium* e *Trichomonas vaginalis* em gestantes assintomáticas e analisar associações com características sociodemográficas. Métodos: Estudo transversal que incluiu 148 gestantes selecionadas aleatoriamente em serviços de pré-natal da rede pública no sul do Brasil, entre novembro de 2021 e março de 2022. Foram excluídas mulheres que haviam recebido antibioticoterapia nos três meses anteriores, com histórico de excisão da zona de transformação cervical ou com perdas gestacionais de repetição. *Swabs* vaginais foram analisados por amplificação de ácidos nucleicos (NAAT) baseada em transcrição mediada (TMA). As análises estatísticas foram realizadas no software R versão 4.1.1. Resultados: *C. trachomatis*, *M. genitalium* e *T. vaginalis* foram detectadas em 11 (7,4%),

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13 (8,8%) e 3 (2,0%) participantes, respectivamente. *N. gonorrhoeae* não foi identificada em nenhuma amostra. No total, 16,2% (n=24) das participantes testaram positivo para pelo menos um patógeno. A infecção por *M. genitalium* associou-se significativamente ao estado civil solteira, enquanto *T. vaginalis* esteve relacionada a menor idade na primeira gestação. Nenhum patógeno apresentou associação com faixa etária inferior a 30 anos. Conclusão: Este estudo, entre os primeiros a descrever a prevalência de *M. genitalium* em gestantes brasileiras, demonstra taxas elevadas desse patógeno emergente. A prevalência de *C. trachomatis* foi consistente com dados nacionais anteriores. Os achados reforçam a necessidade de ampliar o rastreamento de IST no pré-natal no Brasil.

Descritores: Cuidado pré-natal; *Mycoplasma genitalium*; Programas de rastreamento; *Chlamydia trachomatis*; Infecções sexualmente transmissíveis.

ABSTRACT

Background: Sexually transmitted infections (STIs) are prevalent in developing countries, yet are not routinely screened for in pregnant Brazilian women. **Objectives:** This study aimed to determine the prevalence of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, and *Trichomonas vaginalis* among asymptomatic pregnant women and to examine associations with sociodemographic characteristics. **Methods:** This cross-sectional study enrolled 148 randomly selected pregnant women attending prenatal care in southern Brazil between November 2021 and March 2022. Women were excluded if they had received antibiotic therapy in the preceding three months, had a history of cervical transformation zone excision, or had experienced recurrent pregnancy loss. Vaginal swabs were tested by polymerase chain reaction (PCR)-based nucleic acid amplification. Statistical analyses were performed using R version 4.1.1. **Results:** *C. trachomatis*, *M. genitalium*, and *T. vaginalis* were detected in 11 (7.4%), 13 (8.8%), and 3 (2.0%) participants, respectively. *N. gonorrhoeae* was not identified. Overall, 16.2% (n=24) tested positive for at least one pathogen. *M. genitalium* infection was significantly associated with single marital status, whereas *T. vaginalis* was linked to a younger age at first pregnancy. No association with younger age (under 30 years) was observed for any pathogen. **Conclusion:** This study—among the first to describe *M. genitalium* prevalence in pregnant Brazilian women—demonstrates a notably high rate of this emerging pathogen. The prevalence of *C. trachomatis* was consistent with prior Brazilian data, and these findings support broadening prenatal STI screening in Brazil.

Keywords: Prenatal care; *Mycoplasma genitalium*; Diagnostic screening programs; *Chlamydia trachomatis*; Sexually transmitted infections.

INTRODUCTION

Sexually transmitted infections (STIs) constitute a significant and growing burden on global public health.[1] Among these, *Mycoplasma genitalium* has emerged as an important pathogen in women, frequently causing subclinical infection and appearing in co-infections with organisms such as *Chlamydia trachomatis*.[2] Nevertheless, available evidence from female populations remains insufficient to define optimal screening strategies for this pathogen.[3,4]

C. trachomatis, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis* are associated with preterm birth, premature rupture of membranes, fetal death, intrauterine growth restriction, and puerperal endometritis.[5,6,7] Evidence also links *M. genitalium* to adverse obstetric outcomes, including an elevated risk of preterm labour that appears independent of co-infection with other STIs.[2,3,4,8,9,10] Cervicitis caused

by these pathogens is frequently underdiagnosed owing to the high proportion of asymptomatic presentations, non-specific clinical signs, and limited access to diagnostic testing, rendering syndromic management unreliable.[11] Nucleic acid amplification tests (NAATs) represent the diagnostic gold standard, offering superior sensitivity and specificity for *C. trachomatis*, *N. gonorrhoeae*, *M. genitalium*, and *T. vaginalis*, including on vaginal swab specimens.[12,13,14]

Prenatal screening for *C. trachomatis* appears justifiable given its impact on perinatal outcomes;[15,16] however, randomised evidence to support systematic screening for non-viral STIs in developing countries remains scarce.[17] In Brazil, diseases caused by *C. trachomatis*, *N. gonorrhoeae*, *M. genitalium*, and *T. vaginalis* are not included in the national list of compulsory notifiable conditions.[18] Current Brazilian guidelines recommend *C. trachomatis* screening only for pregnant women under 30 years of age, individuals with other STIs or living with human immunodeficiency virus (HIV), victims of sexual violence, and those receiving HIV pre-exposure prophylaxis.[6] Because a substantial proportion of infected women are asymptomatic, these criteria likely underestimate the true burden of infection, and many women who could benefit from treatment remain undetected[11].

This study aimed to determine the prevalence of *C. trachomatis*, *N. gonorrhoeae*, *M. genitalium*, and *T. vaginalis* among pregnant women attending public health services in southern Brazil, and to examine associations between pathogen detection and sociodemographic and reproductive health characteristics. To our knowledge, this is among the first studies to report the prevalence of *M. genitalium* in pregnant women in Brazil.

METHODS

This observational, cross-sectional study was conducted between November 2021 and March 2022 in a city in southern Brazil. The study was approved by the Ethics Research Board (ERB) of the Health Sciences Sector at the Clinics Hospital, Federal University of Paraná (UFPR), and was conducted in accordance with the principles of the Declaration of Helsinki. All participants provided written informed consent.

Pregnant women aged 18–49 years attending scheduled outpatient prenatal consultations at Basic Health Units and the regional maternity hospital—facilities collectively serving approximately 600 pregnant women per month—were eligible for inclusion. Participants were randomly selected from the clinic appointment lists. Eligibility required willingness to provide a vaginal swab for laboratory analysis, regardless of gestational age.

Women were excluded if they had received systemic antibiotic therapy in the three months preceding enrolment, had a history of excision of the cervical transformation zone, had experienced recurrent pregnancy loss, or presented with threatened miscarriage at the time of enrolment. Of 167 women initially screened, 19 were excluded: one due to recent antibiotic use, six due to technical sample

failures, and 12 who declined vaginal swab collection. The final analytic sample comprised 148 participants.

Because no prior data on the prevalence of *M. genitalium* in pregnant Brazilian women were available to support a formal power calculation, the target sample size of approximately 150 participants was defined pragmatically by the Ministry of Health, using population-proportional criteria based on the study city's share of the national territory. This approach, while operationally justified given the absence of baseline prevalence estimates, did not derive from a statistical power calculation for the primary outcome.

All enrolled participants were interviewed privately by a member of the study team. Sociodemographic and clinical data were extracted from prenatal records and supplemented by structured interview, including date of birth, gestational age, obstetric history (number of pregnancies, live births, miscarriages, and stillbirths), serology for HIV, syphilis, and hepatitis B, educational attainment, occupation, household income, STI symptoms during the current pregnancy, history of STI in the preceding 12 months, age at first sexual intercourse, age at first pregnancy, number of current and recent sexual partners, and partner behaviour.

Vaginal swabs were obtained by a clinician during gynaecological examination and immediately placed in the Aptima Multitest Swab Specimen Collection Kit (Hologic, Massachusetts, USA). Samples were stored at −20°C for up to 90 days before being transported to the Molecular Biology, Microbiology, and Serology Laboratory at the Federal University of Santa Catarina for analysis. Testing was performed on the Panther automated platform (Hologic) using the Aptima® Combo 2 Assay (for *C. trachomatis* and *N. gonorrhoeae*), the Aptima® *Trichomonas vaginalis* Assay, and the Aptima® *Mycoplasma genitalium* Assay, all based on transcription-mediated amplification (TMA). Participants with a positive result were managed according to the Brazilian Protocol for Sexually Transmitted Infections.[4]

Qualitative variables were summarised as frequencies and proportions. Continuous variables that did not follow a normal distribution (assessed by the Shapiro-Wilk test) were expressed as medians with interquartile ranges (IQR)[19]. Associations between categorical variables were evaluated using Fisher's exact test with Bonferroni post hoc correction applied for multiple comparisons[20,21]. The Mann-Whitney U test was used to compare continuous variables between groups. A two-sided significance level of 5% was adopted throughout. All analyses were performed using R, version 4.1.1 (R Core Team, Vienna, Austria).[22]

RESULTS

One hundred and forty-eight pregnant women were included. The population studied was predominantly composed of white, married women with a household income of approximately USD 4,800–9,600 per year, as shown in Table 1. The median participant age was 29 years (IQR 25–34), the median level of formal education was 11 years, and the median gestational age at enrolment was 28

weeks. The median age at first sexual intercourse was 16 years and at first pregnancy was 21 years. One participant had received a diagnosis of syphilis in the preceding 12 months; two were living with HIV.

C. trachomatis was detected in 11 participants (7.4%; 95% CI 3.2–11.6%), *M. genitalium* in 13 (8.8%; 95% CI 4.2–13.4%), and *T. vaginalis* in three (2.0%; 95% CI 0–4.3%). *N. gonorrhoeae* was not detected in any sample. Three participants had co-infections: two with *C. trachomatis* and *M. genitalium*, and one with *M. genitalium* and *T. vaginalis*. Overall, 24 of 148 women (16.2%) tested positive for at least one pathogen. No statistically significant difference in *C. trachomatis* prevalence was observed when participants were stratified by age (under vs. over 30 years), consistent with previously published data[8,12].

All participants with positive results for *C. trachomatis* or *M. genitalium* were asymptomatic. Among the three participants with *T. vaginalis*, one reported vaginal discharge. *M. genitalium* infection was significantly more prevalent among single women (n=3; p=0.014; Supplementary Table 2). *T. vaginalis* positivity was associated with a younger age at first pregnancy (median 17 years; p=0.045) and with having had a greater number of sexual partners in the preceding year (p=0.042; Supplementary Table 3).

When comparing the 24 women who tested positive for any pathogen with the 124 who tested negative, no statistically significant differences were identified with respect to age, marital status, parity, number of sexual partners (lifetime or in the preceding year), or age at first pregnancy. Analyses restricted to participants with *C. trachomatis* or *M. genitalium* yielded similar findings. The sociodemographic profile of *C. trachomatis*-positive and *M. genitalium*-positive participants did not differ significantly from one another (Table 1).

DISCUSSION

This cross-sectional study identified a high prevalence of *M. genitalium* and *C. trachomatis* among pregnant women attending public prenatal services in southern Brazil. To our knowledge, this is among the first studies to characterize the prevalence of *M. genitalium* specifically in pregnant Brazilian women. Single marital status was independently associated with *M. genitalium* infection, and *T. vaginalis* was linked to a younger age at first pregnancy partners.Notably, neither younger age nor low socioeconomic status—conventional risk factors for STIs[1]—was associated with pathogen detection in this study.

The *C. trachomatis* prevalence of 7.4% in this study falls within the reported range for pregnant Brazilian women (6.1–16.7%),[4] and is lower than estimates for Latin America as a whole (11.2%),[23] Haiti (14.7%),[3] and Papua New Guinea (22.9%).[11] Among European pregnant women aged under 25 years, *C. trachomatis* was detected in 7.6% in France and 18.4% in Spain,[12,24] suggesting that our estimate is broadly comparable with lower-prevalence settings. The absence of *N. gonorrhoeae* in this population (0/148) is consistent with the reported prevalence of 1.0% in Brazilian pregnant women[4] and

1.2% across Latin America,[23] and likely reflects the low endemic prevalence in this region. *T. vaginalis* was detected in 2.0% of participants, a rate lower than the regional Latin American estimate of 3.9%.[23]

Our prevalence of *M. genitalium* (8.8%) substantially exceeds rates reported from high-income countries: 0.7% in England,[25] and 0.8% in France.[12] It is also higher than the 5.2% reported from Argentina,[18] although comparable with estimates from the Solomon Islands (11.9%) and Papua New Guinea (12.5%).[26] A systematic review reported *M. genitalium* prevalence in pregnant women ranging from 0.7% to 8.5%, rising to 2.4–10% in high-risk subgroups such as young women, those with risky sexual behaviors, and those with low socioeconomic status.[7,8] Among pregnant women under 30 years of age, prevalence was 1.3% in Spain[8] and 2.4% in France;[12] the rate in our study exceeds both.

In our study, the finding that *M. genitalium* was more prevalent among single women—although it showed no association with age group—may have potential implications for targeting screening efforts (reescrito – não temos referência porque esse é um achado do nosso estudo e foi significativamente relevante nos resultados). Importantly, all *M. genitalium*-positive participants were asymptomatic, underscoring the limitations of symptom-based case detection and the need for laboratory-based screening during antenatal care.

The co-infection rate of of 2.0% (3/148) observed in this study is comparable to the 1.0% reported among Argentine pregnant women,[18]though lower than the approximately 10% described in Australian women undergoing termination of pregnancy [27,28] and the 13.5% rate reported in an African population. [26]

Although the sample size of this study limits generalizability, the co-occurrence of these pathogens supports the value of multiplex NAAT-based screening panels capable of simultaneously detecting multiple pathogens [5,14].

Several limitations should be considered. As with any cross-sectional design, this study cannot establish temporal or causal relationships between the sociodemographic and behavioural characteristics examined and pathogen detection; prospective, longitudinal designs would be required to determine whether these characteristics precede or result from infection. The study was conducted at a single site and over a relatively short recruitment window, which may limit the representativeness of findings. The sample size, even predetermined by the Ministry of Health, was modest and not derived from a formal power calculation, which may have limited the statistical power to detect associations in smaller subgroups. Furthermore, the uniformity of gestational age at enrollment prevents examination of trimester-specific prevalence differences.g.

CONCLUSION

This study, demonstrates that *M. genitalium* and *C. trachomatis* are prevalent among pregnant women in southern Brazil, with detectable co-infection among a subset of participants. All positive cases of *C. trachomatis* and *M. genitalium* were clinically silent. Single marital status and a younger age at first pregnancy were associated with *M. genitalium* and *T. vaginalis* detection, respectively, but these

associations should be interpreted with caution given the small number of positive cases in each subgroup. Notably, no association was observed between any pathogen and age under 30 years, the threshold currently used to define screening eligibility under national guidelines. These findings suggest that age-based criteria alone may not adequately identify infected pregnant women in this population. Given the exploratory nature and limited sample size of this study, larger, prospective, multicentric investigations are needed to confirm these associations and inform potential revisions to prenatal STI screening policy in Brazil.

LIST OF ABBREVIATIONS

ERB Ethics Research Board
HIV Human immunodeficiency virus
IQR Interquartile range
NAAT Nucleic acid amplification test
PCR Polymerase chain reaction
STI Sexually transmitted infection
TMA Transcription-mediated amplification
UFPR Federal University of Paraná
USA United States of America

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TABLES

Table 1. Description of demographic and health data of the sample population of 148 pregnant women, aged 18–49 years, in Joinville/SC, from November 2021 to March 2022.

Variable (n assessed)	Median [IQR]
Age – years (148)	29 [25 – 34]
Education – years (119*)	11 [8 – 11]
Gestational age (completed weeks) (148)	28 [22 – 34]
Age at first sexual intercourse – years (142*)	16 [15 – 17]
Age at first pregnancy – years (148)	21 [18 – 24.5]
Number of pregnancies (148)	2 [1 – 3]
Number of children (148)	1 [0 – 2]
Number of miscarriages (148)	0 [0 – 1]
Number of stillbirths (140*)	0 [0 – 0]
Number of lifetime sexual partners (124*)	2 [1 – 4]
Number of partners in the last year (148)	1 [1 – 1]

Note: SD: standard deviation; IQR: Interquartile range (1st and 3rd quartiles).
*n assessed lower than the total number of patients, as some patients did not report this information during the interview

Table 2. Comparison of age, marital status and human immunodeficiency virus (HIV) infection among 22 pregnant women with isolated or associated *Mycoplasma genitalium* and *Chlamydia trachomatis*.

Variable	Class	MG (%, CI95%)	P value	CT (%, CI95%)	P value
Patient's age (years)	<=30 (84)	9 (10.71, 4.10 -	0.511	7 (8.33, 2.42 -	0.871

		17.32)	14.24)
	>30 (64)	4 (6.25, 0.32 - 12.18)	4 (6.25, 0.32 - 12.18)
Marital status	Married (127)	7 (5.51, 1.54 - 9.48) 0.335	8 (6.30, 2.07 - 10.52) 0.999
	Single (8*)	3 (37.50, 3.95 - 71.05)	1 (12.5, 0.00 - 35.42)
HIV infection	No (145*)	13 (8.96, 4.31 - - 13.61)	11 (7.58, 3.28 - - 11.85)
	Yes (2*)	0 (0, 0 - 0)	0 (0, 0 - 0)

Note: Fisher's exact test (5% significance level); P value: 2 sample test for equality of proportion. CT: *Chlamydia trachomatis* ; MG: *Mycoplasma genitalium.* *n assessed lower than the total number of patients, as some patients did not report this information during the interview

Supplementary table 3. Comparison between the group of patients with detected and undetected *M. genitalium*, considering marital status, symptoms of sexually transmitted infections (STIs), complications during pregnancy, number of stillbirths, number of partners in the last year, with a significance level of 5%.

Variable	**Class**	**MG**		**P-value**
		Detected (%)	**Not Detected (%)**	
Marital status	MARRIED	7 (70)	120 (96)	0,014
	SINGLE	3 (30)	5 (4)	
Symptoms of STIs in the current pregnancy	NO	13 (100)	123 (91,11)	0,601
	YES	0 (0)	12 (8,89)	

Any complications in the current pregnancy	NO	13 (100)	123 (91,11)	0,601
	YES	0 (0)	12 (8,89)	
Number of stillbirths*	0	12 (100)	119 (97,54)	0,999
	1	0 (0)	3 (2,46)	
Number of partners in the last year*	0	1 (7,69)	0 (0)	0,189
	1	12 (92,31)	129 (96,99)	
	2	0 (0)	3 (2,26)	
	5	0 (0)	1 (0,75)	

Note: Fisher's exact test (5% significance level); P value: 2 sample test for equality of proportion. MG: *Mycoplasma genitalium*. STIs sexually transmitted infection. *n assessed lower than the total number of patients, as some patients did not report this information during the interview

Supplementary Table 4. Comparison between the group of patients with detected and undetected *T. vaginalis*, considering marital status, symptoms of sexually transmitted infections (STIs), complications during pregnancy, number of stillbirths, number of partners in the last year, with a significance level of 5%.

Variable	**Class**	**TV**		**P-value**
		Detected (%)	**No Detected (%)**	
Marital status*	Married	1 (50)	126 (94,74)	0,115
	Single	1 (50)	7 (5,26)	
Symptoms of STI in the current pregnancy*	No	3 (100)	142 (98,61)	0,999
	Yes	0 (0)	2 (1,39)	
Any complications in the current pregnancy*	No	3 (100)	142 (98,61)	0,999
	Yes	0 (0)	2 (1,39)	
Number of stillbirths*	0	3 (100)	128 (97,71)	0,999
	1	0 (0)	3 (2,29)	

Number of partners in the last year*	0	1 (33,33)	0 (0)	0,042
	1	2 (66,67)	139 (97,2)	
	2	0 (0)	3 (2,1)	
	5	0 (0)	1 (0,7)	

Note: Fisher's exact test (5% significance level); P value: 2 sample test for equality of proportion. TV: *Trichomonas vaginalis*. STIs sexually transmitted infection. *n assessed lower than the total number of patients, as some patients did not report this information during the interview