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Sexually transmitted bacterial infections among young women in Central Western Brazil

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SUMMARY

Background: Studies on sexually transmitted infections in Brazil are done mainly in large metropolises and screening is available for pregnant women only. We aimed to estimate the prevalence and risk factors for *Chlamydia trachomatis, Neisseria gonorrhoeae*, and *Treponema pallidum* infection among young non-pregnant women in non-clinical settings in middle-sized cities of Central Brazil.
Methods: A cross-sectional community-based sample of 1072 participants was included. Sexually active women (64.9%) provided first-catch urine samples for PCR investigation of chlamydial and gonococcal infection. Syphilis was tested in serum. Univariate analysis investigated risk factors for chlamydial infection. Multivariate logistic regression included associations with a p-value <0.20.
Results: The mean age of participants was 18 years; 73.2% reported unprotected intercourse, 37.6% were married/cohabiting, and 5% reported a previous STI. Prevalence rates of *C. trachomatis*, *N. gonorrhoeae*, and *T. pallidum* were 9.6% (95% confidence interval (CI) 7.4–12.4%), 0.7% (95% CI 0.2–1.9%), and 0.15% (95% CI 0.0–0.7%), respectively. After adjustments, being <20 years old (adjusted odds ratio (aOR) 1.90, 95% CI 1.07–3.37) and having three or more lifetime sexual partners (aOR 2.57, 95% CI 1.46–4.53) were associated with the risk for chlamydial infection.
Conclusions: We observed a high prevalence of chlamydial infection and sexual risk behaviors in this population. These findings are important to guide screening strategies in Brazil.
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1. Introduction

Chlamydia trachomatis, *Neisseria gonorrhoeae*, and *Treponema pallidum* are the most common pathogens responsible for bacterial sexually transmitted infections (STI) worldwide, disproportionately affecting women under 25 years of age.[1] According to the World Health Organization (WHO), approximately 126 million new cases of curable STIs occur each year in the region of the Americas.[1]

C. trachomatis is an obligate intracellular pathogen that infects epithelial cells of the genital tract. The infection resolve spontaneously in most infected women, but persistent infection may occur and lead to the spread of the pathogen to the upper genital tract. *N. gonorrhoeae* is the etiological agent of gonorrhea, an infection on the surface of the urethra, endocervix, and fallopian tubes. Clinically it is indistinguishable from genital chlamydial infection. Asymptomatic infection is frequent among women, increasing the risk of persistent undiagnosed chlamydial and gonococcal infections, which may lead to complications such as pelvic inflammatory disease (PID), infertility, ectopic pregnancy, and chronic abdominal pain.[2] *T. pallidum* is the causative organism of syphilis, a chronic infection that may persist for years, leading to cardiovascular and neurological damage. Syphilis is especially related to adverse outcomes in pregnancy, such as late abortion, prematurity, low birth weight, neonatal death, and congenital infection.[3] Bacterial STI complications are an important prevent-

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able cause of reproductive sequelae, infertility, and adverse pregnancy outcomes in women.[4]

Screening strategies are useful to identify and treat asymptomatic infected people, preventing complications and avoiding dissemination among sexual contacts.[5] *C. trachomatis* and *N. gonorrhoeae* screening has been carried out in countries such as the USA, the UK, and Sweden.[6–9] Chlamydial and gonococcal infections should be investigated in sexually active individuals under the age of 25 years, especially women.[6] Syphilis testing should be performed in all pregnant women and individuals at elevated risk.[10] In Brazil, STI screening is routine only for pregnant women and includes only hepatitis B, HIV, and syphilis testing.[11]

According to previous studies, the prevalence rate of *C. trachomatis* infection in Brazil ranges from 7.3% to 17.6% among young non-pregnant women in non-clinical settings.[12–15] These studies were performed in large cities and there is currently a lack of data from outside metropolises. Brazil is a country of continental proportions. Thus, middle-sized cities may represent an important missing setting for the evaluation of STI prevalence and for the collection of important information for the implementation of screening strategies. The present study aimed to estimate the prevalence of *C. trachomatis, N. gonorrhoeae*, and *T. pallidum* infections and to investigate behavior and social variables potentially associated with STIs in asymptomatic non-pregnant young women in middle-sized cities of Central Western Brazil.

2. Methods

2.1. Study design, setting, and sampling

This study was a community-based, cross-sectional survey performed in three cities (Ceres, Catalão, and Inhumas) in the state of Goiás, Central Western Brazil, between 2007 and 2009. The overall population of these cities was 141 046 inhabitants, with an estimated 4500 women under 25 years of age monitored by a public health program known as the Family Health Program (FHP). The FHP is an assistance public health service covering all Brazilian cities and is responsible for promoting primary health care by implementing interventions according to community risk factors. The FHP consists of health units with multidisciplinary teams responsible for monitoring a defined number of families.[16]

Ceres is a city in the north of Goiás State, with 18 637 inhabitants and six family health units. Catalão is located in the southeast, with 75 623 inhabitants and three family health units. Inhumas is situated in the central region of the state, at a distance of 54 km from the capital Goiânia, and has a population of 46 786 inhabitants with 13 family health units. These cities were randomly selected to represent the urban population outside the capital in Central Western Brazil.

All women aged 15–24 years monitored by the FHP in the selected cities were potentially eligible. Census information was provided by the local health department. The sample size was calculated using Open Source Epidemiologic Statistics for Public Health software (OpenEpi version 2.3.1). The sample size was based on the study design (prevalence study without clustering, i.e., effect design = 1). The number of participants required was 646 sexually active non-pregnant women aged 15–24 years, based on a minimum expected STI prevalence of 2.0%, with a 1.0% precision and a confidence interval of 95%. Syphilis was expected to be the STI with the lowest prevalence. Taking into consideration that 20% of young women would not be living at the recorded address (could not be contacted), that approximately 40% of the invited young women would not be sexually experienced (would not be eligible), that 10% of the sexually experienced would be pregnant or would have used antimicrobials in the previous 15 days (would not be eligible), and anticipating a refusal rate of 25%, the final estimated sample size was 1250 women.

The recruitment process was done in two phases. The sampling process was done in the first phase of recruitment. The local health departments provided a list of households with potential participants (women aged 15–24 years registered at a family health unit). A number was assigned to each potential participant, and a simple sampling was done using a computer-generated list of random numbers (Epi Info software 3.4. version). From a finite population of approximately 4500 young women, 1250 were invited by letter to present to the nearest family health unit. In the second phase, all women who accepted the invitation and met the inclusion criteria were included in the study.

Volunteers who presented to the local family health unit were first informed about the study and then interviewed by a trained nurse or physician using a structured questionnaire to collect information on socio-demographic variables, including level of education, mother's schooling, and family income, which was measured in minimum Brazilian wages (US$ 213 at the time of the study). Participants were questioned on their previous sexual experience and issues related to general health knowledge and health concerns. Women who reported previous sexual activity were then invited for further procedures, including a second interview using a more specific questionnaire containing questions about sexual practices and reproductive life, such as the onset of sexual life, number of partners, condom use, and STI history. Women who referred to antimicrobial use within the previous 15 days or who were pregnant were excluded. All eligible young women were requested to sign a written informed consent form and were referred for first-catch urine and blood sample collection for the diagnosis of *C. trachomatis*, *N. gonorrhoeae*, and *T. pallidum*.

2.2. Laboratory procedures

For *C. trachomatis* and *N. gonorrhoeae* diagnostics, participants were instructed to collect an approximately 20-ml first-catch urine specimen after at least 2 h without urinating. PCR for *C. trachomatis* and *N. gonorrhoeae* was performed using the Amplicor CT/NG Kit (Roche Molecular Systems, Branchburg, NJ, USA) in accordance with the manufacturer's instructions, at the Infectious Diseases Immunology and Molecular Biology Laboratory of the Institute of Tropical Pathology and Public Health of the Federal University of Goiás, Brazil. In brief, a ~208-bp nucleotide sequence of *C. trachomatis* and a ~201-bp nucleotide sequence of *N. gonorrhoeae* DNA were amplified with biotinylated primers. A hybridization chain reaction was then performed to identify target sequences. Internal controls were used in all amplification assays.

For syphilis diagnostics, a 10-ml sample of venous blood was collected from an arm vein, and 5 ml of serum was obtained and stored at −70 °C until analysis. All basic biosafety standards were assured during blood collection. The non-treponemal VDRL (Venereal Disease Research Laboratory) test was performed on all serum samples. Confirmation of positive results was done with an FTA-Abs test (fluorescent treponemal antibody absorption test).

2.3. Data analysis

A descriptive analysis was performed for the socio-demographic and sexual behavior variables. Continuous variables were described as the median with interquartile range (IQR). Categorical variables were presented as the frequency and percentage. The Chi-square test or Fisher's exact test was used to analyze the differences among categorical variables, with a significance level of 5%. The prevalence of *C. trachomatis*, *N. gonorrhoeae*, and *T. pallidum* infections and their respective 95% confidence intervals (95% CI)

were estimated as the number of positive tests divided by the total number of women tested.

Univariate analysis was performed to investigate possible associations between *C. trachomatis*-positive tests and socio-demographic and behavior variables. All associations with a *p*-value <0.20 were then included in a multivariate logistic regression analysis. In the final analysis, a *p*-value of ≤0.05 was considered statistically significant. Risk factors for gonococcal infection and syphilis were not assessed due to a low expected prevalence rate requiring a larger sample size. Data analyses were conducted using Epi Info version 3.5.1 and SPSS version 13.0.

3. Results

A total of 1072 asymptomatic young women (15–24 years) were recruited; 696 (64.9%) of them declared being sexually experienced and not pregnant, and were therefore eligible for the study. No significant differences regarding socio-demographic and behavioral characteristics were observed among volunteers from each city. The median age of the 696 sexually active participants was 20 years (IQR 17–22 years) and 37.8% of them were already married or cohabiting. Most of the eligible participants had completed more than 8 years of schooling (72.8%), had poorly educated mothers (70%), and a low family income (<4 minimum wages/month, 85.1%).

Concerning sexual behavior, more than 20% of participants were younger than 15 years at first intercourse (median age at first intercourse 16 years, IQR 15–17 years). Half of them reported two or more lifetime sexual partners. Nevertheless, more than 80% reported only one sexual partner in the last 3 months. Approximately 70% of subjects reported inconsistent use of condoms and 5% reported a previous STI diagnostic. Regular use of condoms was more frequently reported by single women than by married or cohabiting women ($p < 0.001$). Almost 40% of participants had been pregnant at least once and more than 25% of them were younger than 15 years at first pregnancy.

Table 1
Prevalence of bacterial sexually transmitted infections in asymptomatic sexually active young women in Central Western Brazil

	Positives	Tested	Prevalence % (95% CI)
Chlamydia trachomatis	55	574	9.6 (7.4–12.4)
Ceres	13	106	10.9 (5.9–18.0)
Catalão	12	99	12.1 (6.4–20.2)
Inhumas	30	356	8.4 (5.8–11.9)
Neisseria gonorrhoeae	4	574	0.7 (0.2–1.9)
Treponema pallidum	1	685	0.1 (0.0–0.9)

CI, confidence interval.

Five hundred seventy-four (82.5%) subjects provided a first-catch urine sample for *C. trachomatis* and *N. gonorrhoeae* testing. Those who did not provide a urine sample were in menses or were taking antimicrobial therapy at that time or had taken such therapy in the prior 15 days, and failed to attend for a second visit. No specific information was gathered about non-compliers. Six hundred and eighty-five (98.4%) blood samples were available for syphilis diagnosis. Eleven blood samples (1.6%) were excluded due to hemolysis.

3.1. Prevalence of bacterial STIs

The overall prevalence of *C. trachomatis* infection was 9.6% (95% CI 7.4–12.4%), while the prevalences of *N. gonorrhoeae* and *T. pallidum* infection were 0.7% (95% CI 0.2–1.9%) and 0.15% (95% CI 0.0–0.7%), respectively. None of the participants with a positive test presented a co-infection. There was no significant difference in prevalence of *C. trachomatis* infection among participants from the three cities (Table 1).

3.2. Socio-demographic and sexual behavior variables associated with the risk for STI

Univariate analysis demonstrated that *C. trachomatis* positivity was associated with younger age (15–19 years old), early onset of sexual life (<15 years), and having three or more lifetime sexual

Table 2
Univariate analysis of socio-demographic variables potentially associated with the risk for *Chlamydia trachomatis* infection in sexually active young women in Central Western Brazil

Variables	Number of patients (%) ($n = 696$)	*C. trachomatis*-positive ($n = 574$)	OR (95% CI)	*p*-Value
Origin				
Ceres	145 (62.2)	13	1.01 (0.41–2.53)	0.855
Catalão	123 (65.4)	12	1.50 (0.69–3.20)	0.353
Inhumas	428 (65.7)	30	1.52 (0.72–3.17)	0.315
Age, years				
15–19	346 (49.7)	35	1.90 (1.07–3.37)	0.039[a]
20–24	350 (50.3)	20		
Marital status				
Single	431 (61.9)	37	1.45 (0.78–2.73)	0.213
Married/cohabiting	262 (37.6)	18		
NA	3 (0.4)			
Schooling, years				
≤8	189 (27.2)	15	1.05 (0.53–2.03)	0.883
>8	506 (72.7)	40		
NA	1 (0.1)			
Mother's schooling, years				
≤8	486 (70.0)	35	0.61 (0.33–1.12)	0.147
>8	160 (23.1)	18		
NA	48 (6.9)			
Family income, minimum wage[b]				
≤4	592 (85.1)	45	0.69 (0.31–1.54)	0.496
>4	79 (11.4)	8		
NA	25 (3.6)			

OR, odds ratio; CI, confidence interval; NA, not available.
[a] Statistically significant, *p*-value < 0.05; Fisher's exact test was used for frequencies <5.
[b] Minimum Brazilian wage at the time of study: R$ 380.00 (approximately US$ 213.00).

Table 3
Univariate analysis of behavioral variables potentially associated with *Chlamydia trachomatis* infection in sexually active young women in Central Western Brazil

Variables	Number of patients (%) (n = 696)	*C. trachomatis*-positive (n = 574)	OR (95% CI)	p-Value
Age at first intercourse, years				
<15	165 (23.7)	20	2.14 (1.18–3.86)	0.013[a]
≥15	527 (75.7)	34		
NA	4 (0.6)			
Number of lifetime sexual partners				
≥3	213 (31.1)	27	2.57 (1.46–4.53)	0.001[a]
<3	471 (68.9)	27		
NA	12 (1.7)			
Number of sexual partners in the last 3 months				
≥3	9 (1.3)	1	1.61 (0.19–13.60)	0.503
<3	683 (98.1)	53		
NA	4 (0.6)			
Regular partner				
No	101 (14.5)	9	1.31 (0.61–2.80)	0.622
Yes	585 (84.1)	45		
NA	10 (1.4)			
Condom use				
Never	98 (14.1)	8	1.05 (0.38–2.88)	0.915
Sometimes/rarely	408 (58.6)	33	1.05 (0.50–2.24)	0.935
Always	185 (26.6)	13		
NA	5 (0.7)			
Previous pregnancy				
Yes	271 (38.9)	18	0.76 (0.42–1.38)	0.449
No	416 (59.8)	35		
NA	9 (1.3)			
Age at first pregnancy, years				
<15	33 (12.2)	5	2.90 (0.95–8.85)	0.115
≥15	234 (86.3)	13		
NA	4 (1.5)			
STI history				
Yes	33 (4.7)	2	0.73 (0.17–3.18)	1.000
No	638 (91.7)	50		
NA	25 (3.6)			
Sexual intercourse with a symptomatic partner				
Yes	36 (5.2)	3	1.15 (0.34–3.95)	0.742
No	623 (89.5)	47		
NA	37 (5.3)			

OR, odds ratio; CI, confidence interval; NA, not available; STI, sexually transmitted infection.
[a] Statistically significant, p-value < 0.05; Fisher's exact test was used for frequencies <5.

partners. Participants who reported a previous pregnancy or inconsistent condom use did not present a higher risk for chlamydial infection. Socio-demographic and behavior variables potentially associated with the risk for *C. trachomatis* positivity are shown in Tables 2 and 3.

To further explore these associations, a multivariate analysis was performed. The following exposure variables were included in the logistic regression model: age (<20 vs. ≥20 years), age at first intercourse (<15 vs. ≥15 years), and number of lifetime sexual partners (<3 vs. ≥3). Age ranging from 15 and 19 years (odds ratio (OR) 2.16, 95% CI 1.18–3.97) and having three or more sexual partners in life (OR 2.56, 95% CI 1.38–4.74) remained independently associated with the risk for *C. trachomatis* infection even after adjustments for possible confounding factors (Table 4).

Risk factors for *N. gonorrhoeae* and *T. pallidum* infection were not assessed due to the low prevalence observed. There were four positive cases of *N. gonorrhoeae* infection and one positive case of syphilis. The women with gonococcal infection were between 18 and 22 years old; two of the four reported less than 8 years of schooling, two were single, and three were aged 15 years at first intercourse. These participants reported one to six lifetime sexual partners and only one sexual partner in the last 3 months. Two of them reported a previous pregnancy and only one reported regular use of condoms. The only case positive for syphilis was a 24-year-

Table 4
Multivariate analysis of socio-demographic and sexual behavior associated risk factors for *Chlamydia trachomatis* infection in young women in Central Western Brazil

Variable	*C. trachomatis*-positive (n = 574)	OR (95% CI)	p-Value	aOR (95% CI)	p-Value
Age, years					
15–19	35	1.90 (1.07–3.37)	0.039[a]	2.16 (1.18–3.97)	0.0128[a]
20–24	20				
Age at first intercourse, years[b]					
<15	20	2.14 (1.18–3.86)	0.013[a]	1.40 (0.73–2.66)	0.3069
≥15	34				
Number of lifetime sexual partners[b]					
≥3	27	2.57 (1.45–4.53)	<0.001[a]	2.56 (1.38–4.74)	0.0028[a]
<3	27				

OR, odds ratio; CI, confidence interval; aOR, adjusted odds ratio.
[a] Statistically significant, p-value < 0.05; Fisher's exact test was used for frequencies <5.
[b] Some variables were not available for all participants.

old married woman with less than 8 years of schooling who reported an onset of sexual life at 15 years of age and who had only had one sexual partner in life. She referred to never having used a condom in her life and a previous pregnancy at the age of 18 years.

4. Discussion

The prevalence rate observed for chlamydial infection is in accordance with those found in studies involving sexually active young Brazilian women in the northeast region (6–17.1%) and southeast region of the country (7.4–12.2%).[17–20] A similar prevalence rate (9.8%) was also observed in a national population-based study conducted in parturient women aged 15–24 years.[21] However, previous studies performed in young women recruited in the capital Goiânia, Goiás (Central Western Region) presented higher prevalence rates (14.5–17.6%).[12,13] Our findings are also in accordance with estimated prevalence rates found in other studies performed in asymptomatic young women outside STI clinics in other countries: USA (8%), Norway (7.2%), and Canada (9.3%).[22–24]

Regarding *N. gonorrhoeae* infection, previous studies in asymptomatic Brazilian women recruited in non-clinic settings demonstrated similar prevalence rates to that found in the present study, ranging from 0.8% to 2.1%.[13,17,18,21] Otherwise, a higher prevalence rate of gonococcal infection (3.2%) was observed in a population recruited in 2000 at family health units in three Brazilian slums of Salvador, Bahia.[18] A higher rate of gonococcal prevalence (4.0%) was observed in women at public health services recruited in 2011 in large cities of the southeast of Brazil.[25] The lower prevalence rate found in our study could be explained by the type of specimen used for diagnosis. Although nucleic acid amplification tests are highly appropriate for the detection of genital gonococcal infection,[26] a low sensitivity of PCR has been demonstrated for *N. gonorrhoeae* detection in urine samples.[27]

The syphilis prevalence rate observed in this population was consistent with data from a large urbanized area of Brazil.[28] Meanwhile, a significantly higher rate of syphilis was identified in female public health attendees in the northeast region of Brazil (5.1%, 95% CI 2.4–9.6%).[18] Regardless of the low prevalence observed for *T. pallidum* infection in this study, this is a cause for concern, since this is a population of child-bearing age. Teenage pregnancy represents a higher vulnerability for STIs and their associated complications, including an increased susceptibility to HIV-1 acquisition.[29] In this young population, a previous pregnancy was reported frequently and a high percentage had become pregnant at less than 15 years of age, reflecting a public health issue.

C. trachomatis positivity was significantly associated with being aged less than 20 years and having three or more lifetime sexual partners, corroborating the results of a prior study in a similar population.[21] Inconsistent condom use was not associated with positivity for chlamydial infection. This lack of association may be related to the definition of 'inconsistent use', in which one may answer 'always' for the use of condoms in most sexual encounters or just for the use of condoms with the current partner. As a great proportion of participants reported having a steady partner, the lack of association may also be related to the fact that those women in a regular relationship were more likely to use condoms inconsistently.

The study presents some limitations, a few of them inherent to cross-sectional studies. Survey studies are valuable for estimating the burden of an event and for generating hypotheses, but they do not provide strong evidence for risk factors. It should be pointed out that the low prevalence of gonococcal infection and syphilis found in the present study prevented us from evaluating the risk factors associated with these infections. The investigation of risk factors for chlamydial infection may also have been hampered due to the low prevalence of exposure factors among the participants, such as a history of exchanging sex for money or illegal drug use. The low report of inconsistent condom use or previous STI could be due to constraint issues related to face-to-face interviews, leading to incorrect reporting. The rate of refusal to participate, although within the predetermined limits, may have led to an underestimation of the STI prevalence due to self-exclusion procedures.

This study was a community-based survey of bacterial STI prevalence in young women attending a public health service in Central Western Brazil. Although there is substantial information about STI epidemiology in developed countries, limited data are available in developing settings. In Brazil, most STI prevalence studies have been carried out in large urban centers. We observed a high prevalence of *C. trachomatis* infection in young Brazilian women living in middle-sized cities in the central region of Brazil. Nevertheless, *N. gonorrhoeae* and *T. pallidum* infections exhibited low prevalence rates.

Although most of the volunteers reported an adequate level of education and had free access to condoms in health units, they still engaged in sexual behaviors associated with the risk for STIs. This emphasizes the need for policies directed at sexual education and STI prevention. Furthermore, awareness of the magnitude of these infections outside high risk groups is important to determine the necessity for screening strategies directed at asymptomatic young women.

In conclusion, a high prevalence of *C. trachomatis* infection was observed among sexually active young women in three middle-sized cities of Central Western Brazil. The Family Health Program is a feasible setting for adolescent recruitment, screening, and early treatment of these infections in Brazil. Asymptomatic young women represent a target population for STI control intervention strategies.

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Ethics statement: This study was approved by the Ethics Review Board of the University Hospital, Federal University of Goiás. Potential participants who were not sexually experienced received counseling concerning general health issues or were referred for a medical appointment and/or vaccination when necessary. Tests for chlamydia, syphilis, and gonorrhea infections were offered free-of-charge for those participants who reported being sexually active. After being informed and providing a written agreement, the participants were referred for blood sample collection and urine self-collection. Samples were kept until the end of the study. Biological samples and questionnaires were labeled with code identification numbers in order to preserve participant anonymity. The code identification was held in reserve with the research team. At the end of the study, remaining samples were discarded and questionnaires were stored in a safe place at the Federal University of Goiás. Participants with a positive result for any of the STIs investigated, received counseling and treatment in accordance with the Brazilian Guidelines for Sexually Transmitted Diseases Treatment (Manual de Controle das Doenças Sexualmente Transmissíveis (DST). Brasília, DF: Ministry of Health; 2006). Participants with a positive result were advised to inform their sexual partners and to encourage them to make an appointment at a Family Health Unit for counseling and treatment.

Conflict of interest: The authors declare that there are no conflicts of interest.

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