Original Article

Sexually transmitted infections with *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, and *Trichomonas vaginalis* in pregnant women as detected by molecular testing

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Abstract

Context: During pregnancy, sexually transmitted infections can be transmitted vertically to the fetus, leading to an increase in morbidity and mortality for both mother and child. **Aims:** This study aimed to determine the profile of cervical and vaginal infections in pregnant women receiving prenatal care in a single institute. **Settings and Design:** The study was conducted in a tertiary hospital. Molecular testing was used to detect *Chlamydia trachomatis*, *Neisseria gonorrhoeae, Mycoplasma genitalium,* and *Trichomonas vaginalis*. **Materials and Methods:** Samples of vaginal secretions were collected from pregnant women using the Aptima® Multitest Swab Specimen Collection kit to test for the pathogens. The inclusion criteria consisted of pregnant women of 15–45 years of age receiving prenatal care at the institute, irrespective of gestational age, who agreed to provide vaginal swab. The exclusion criterion was the use of antibiotics in the preceding 3 months. **Statistical Analysis:** Frequencies and percentages were calculated for the pathogens detected in the samples evaluated. **Results:** Overall, 200 samples were tested. Of the pathogens detected, there was a predominance of *T. vaginalis* (15.5% of the samples) and *C. trachomatis* (14.5%), followed by *M. genitalium* (10.0%) and *N. gonorrhoeae* (0.5%). **Conclusion:** Identifying the microorganisms present in the microbiota of pregnant women is of the utmost importance in assuring the appropriate treatment for each pathogen, thus avoiding complications both for the woman and for her fetus. These results should serve to stimulate the debate on implementing these tests as routine during prenatal care.

Key words: Coinfection, infections, microorganisms, transmission

Introduction

Sexually transmitted infections (STIs) are known to originate from a variety of microorganisms transmitted principally through sexual contact or by vertical transmission. Changes that occur during pregnancy, including relative immunosuppression and anatomical and hormonal changes, can facilitate transmission of different pathogens and may alter the course of STIs.[1]

Although most STIs remain asymptomatic for prolonged periods of time, they can develop into severe clinical conditions. *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium,* and *Trichomonas vaginalis*, the pathogens investigated in the present study, can result in chronic pelvic pain, sexual dysfunction, and infertility in women. Furthermore, during pregnancy, they are associated with an increased risk of prematurity, premature rupture of membranes, fetal loss, intrauterine growth restriction, and even neonatal sepsis.[2]

C. trachomatis infection can progress to pelvic inflammatory disease, with the consequent sequelae resulting from that condition. In addition, this bacterial infection is an important risk factor for the development

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of cervical cancer.[3] Deaths resulting from *C. trachomatis* infection are rare and in general are the result of complications caused by progression of the infectious disease. During pregnancy, the possibility of vertical transmission involves a high risk of neonatal complications as the infection may affect the throat and eyes of the new-born infant, as well as other organs.[4]

N. gonorrhoeae infection, when symptomatic, presents as pain at urination, pain in the lower abdomen, a yellow or light-colored discharge, and pain or bleeding during sexual intercourse. This infection can be associated with severe complications during pregnancy and repercussions for the new-born infant, including neonatal conjunctivitis, pneumonia, endocarditis, and meningitis.[5]

When symptomatic, infections by the *M. genitalium* bacterium are associated with cervicitis, dysuria, dyspareunia, and burning or irritation of the genital organs. Complications seen in women include sexually acquired reactive arthritis, pelvic inflammatory disease, spontaneous miscarriage, and a possibility of tubal factor infertility.[6]

T. vaginalis infects the urethra and vagina. The infection caused by this pathogen is symptomatic in approximately 50% of cases, with signs and symptoms including macular colpitis and vaginal discharge that is malodorous, frothy yellow or greenish yellow, as well as itching, vulvar irritation, and urinary symptoms such as dysuria and frequent urination.[7] In pregnant women, infection by this pathogen has been associated with premature rupture of membranes, low birthweight, and perinatal death.[8]

According to data from the Ministry of Health, STIs are associated with a possible 18-fold increase in the likelihood of human immunodeficiency virus (HIV) transmission as the pathogens involved may lead to lesions on genital organs, facilitating contact with the secretions and blood of HIV-positive individuals.[9]

All pregnant women and their sexual partners should be investigated for STIs and be provided with information on the risk of perinatal infection. To be effective, the screening for STIs in a population depends on various factors including access to a health-care service, testing coverage, and appropriate treatment.[10] Factors that must be taken into consideration when diagnosing and treating STIs during pregnancy include identifying the pathogen that causes the disease, its incubation period, and the clinical presentation of the disease.[2]

Given the importance of diagnosing and treating STIs in pregnant women in a timely fashion to avoid severe maternal and neonatal complications, the objective of the present study was to determine the profile of the vaginal microbiota of pregnant women receiving prenatal care at a public health-care facility, with specific molecular testing being used to detect *C. trachomatis*, *N. gonorrhoeae*, *M. genitalium,* and *T. vaginalis*.

Materials and Methods

This study forms part of the procedures involved in implanting pilot tests within the public health-care service, in which a total of 3654 pregnant women receiving prenatal care in all the geographical regions of Brazil (north, northeast, mid-west, southeast, and south) were evaluated. The total sample size was calculated by estimating the prevalence rate of infection by *N. gonorrhoeae* in pregnant women of 15–45 years of age, with a 95% two-sided confidence interval of 1.5%. Calculation was based on a prevalence rate of 0.6%.[11]

This cross-sectional study was conducted with pregnant women receiving care in a tertiary hospital in the southeast of the country between February 2022 and March 2022. Vaginal swab were collected from 200 pregnant women attending the prenatal clinic, using the Aptima® Multitest Swab Specimen Collection kit. These samples were stored at −20°C for 20 days and then sent in dry ice to the Molecular Biology, Microbiology and Serology Laboratory for testing and analysis. At the laboratory, the samples were stored at −80°C until the assays were performed. After the samples were thawed, they were checked against a spreadsheet used to anonymize the samples, labeled with the barcode corresponding to the code for each individual sample, and tested using a Hologic Panther instrument. The method applied to detect RNA targets was isothermal transcription-mediated amplification using reverse transcriptase and DNA-dependent RNA polymerase, based on the ability of specific hybridization of complementary DNA oligonucleotides to determine the presence or absence of targets.

The inclusion criteria were pregnant women of 15–45 years of age receiving prenatal care at the institute, irrespective of gestational age, who agreed to provide vaginal swab to test for the microorganisms evaluated in this study. The exclusion criterion was having used antibiotics in the preceding 3 months and advanced stage of pregnancy because specimens cannot be collected.

The institutional review board approved the study protocol under reference number 63837722.3.0000.5065. All the participants gave their written informed consent and their anonymity and privacy were guaranteed.

The statistics procedures were based on the descriptive statistics tools and as the variables were categorical, data collection of frequencies and calculation of proportions ware carried out with 95% confidence interval with graphic design.

The statistics procedures were made using SPSS 26.0 program for statistical analysis by IBM® from Brazil.

Results

Analysis of the 200 samples collected from the pregnant women who participated in the study allowed the percentage of each one of the pathogens tested here to be calculated. The most common infections found in this sample of pregnant women were *T. vaginalis,* found in 31 samples (15%) and *C. trachomatis,* detected in 29 samples (14.5%). *M. genitalium* was detected in 20 samples (10%) and *N. gonorrhoeae*, the least common infection, was detected in 1 sample (0.5%) [Table 1].

In 11 (5.5%) of the 200 samples, two different pathogens were found simultaneously in the vaginal flora: *C. trachomatis* and *N. gonorrhoeae* were present concomitantly in one of the samples (0.5%); *C. trachomatis* and *M. genitalium* were found together in three samples (1.5%); *C. trachomatis* and *T. vaginalis* were found in four samples (2.0%); and *M. genitalium* and *T. vaginalis* were found in another three samples (1.5%) [Figure 1].

Discussion

Overall, the results of this study are in agreement with the most recent data for Brazil as a whole, as discussed hereafter.

The prevalence of *C. trachomatis* found in the present study was 14.9%, a figure that is similar to rates reported

Table 1: Results of the analysis of vaginal swab from pregnant women using molecular testing

Pathogen	Detected, *n* (%)	Not detected, *n* (%)	Invalid results, *n* (%)	95% CI
Chlamydia trachomatis	29 (14.5)	171 (85.5)	0	14.5 (9.6-19.4)
Neisseria gonorrhoeae	1 (0.5)	199 (99.5)	0	0.5 (0.0-1.5)
Mycoplasma genitalium	20 (10.0)	180 (90.0)	0	10 (5.8-14.2)
Trichomonas vaginalis	31 (15.5)	169 (84.5)	0	15.5 (10.5-20.5)
Total	200 (100.0)		0	

CI: Confidence interval

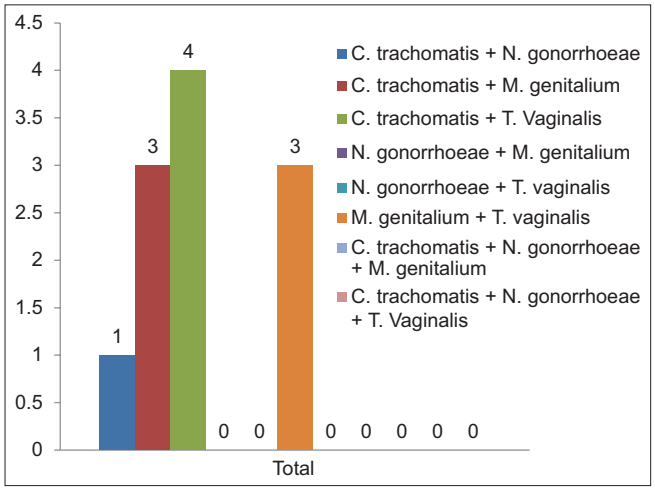

Figure 1: Number of samples testing positive for more than one pathogen. *C. trachomatis: Chlamydia trachomatis, N. gonorrhoeae: Neisseria gonorrhoeae, M. genitalium*: *Mycoplasma genitalium, T. vaginalis: Trichomonas vaginalis*

in other studies. A study on maternal *C. trachomatis* infections and preterm births, conducted in a University Hospital in Vitória, Brazil, investigated 323 pregnant women and reported a prevalence of *C. trachomatis* of 13.9% (45 cases).[12] In another Brazilian study on the prevalence of *C. trachomatis* and risk behaviors, samples were collected from 562 pregnant women aged 15–24 years, with a prevalence of *C. trachomatis* infection of 12.3% being reported.[13]

The prevalence of chlamydia and gonococcus infection was investigated in 3303 pregnant women with a mean age of 23.8 years who were receiving prenatal care within the public healthcare network in the following Brazilian cities: Manaus, Fortaleza, Goiânia, Rio de Janeiro, São Paulo, and Porto Alegre. Vaginal swab were collected using a specific swab for hybrid capture. Results showed a prevalence of chlamydia infection of 9.4% and a prevalence of gonococcus infection of 1.5%. Furthermore, of the 273 women who tested positive for chlamydia infection in that study, 27 (10%) also tested positive for gonococcus.[14]

A study on *M. genitalium* infection and premature birth evaluated vaginal swab from 1349 pregnant women with a gestational age of 20–25 weeks, with results showing a prevalence of *M. genitalium* of 18%.[15]

A study on the prevalence and validation of tests for the diagnosis of *T. vaginalis* in pregnant, nonpregnant, and HIV-positive women evaluated 100 pregnant women receiving care in Goiânia, Brazil. Four diagnostic techniques were compared for detection of the parasite: wet mount examination, culture, Papanicolaou stained smears, and polymerase chain reaction (PCR). For the PCR test, a positive control for *T. vaginalis* was prepared from culture isolates followed by DNA extraction using the QIAamp DNA mini kit (brand QIAGEN®). The frequency of *T. vaginalis* in the pregnant women analyzed was 19%.[16]

The composition of the vaginal microbiota is affected by various factors, including hormonal factors, sexual behaviors, infections, the use of antibiotics, hygiene, and even the woman's life cycle (infancy, menace, or post menopause).[17] Progesterone and estrogen levels during pregnancy affect vaginal pH and can play a role in the colonization of the vagina by different microorganisms. Identifying these microorganisms is crucial in determining potential complicating factors during pregnancy and in avoiding vertical transmission to newborns.[17]

However, controlling STIs requires the engagement of healthcare professionals in applying the resources available within the public health-care network to organize health-care services and provide satisfactory care for individuals with STIs. Appropriate care and treatment are crucial steps in breaking the chain of transmission of STIs and HIV.[10]

According to the Ministry of Health guidelines, in suspected cases of STIs, vaginal swab should be collected for laboratory examination at primary health-care level; however, easy access to laboratory facilities to carry out molecular testing, as used in the present study, is crucial in guaranteeing early detection and adequate treatment in pregnant women and their sexual partners.[10]

The clinical protocol and treatment guidelines for the comprehensive health care of individuals with STIs[9] define the treatment to be given for each one of the agents investigated here and recommend treating the sexual partners of pregnant women. The recommendations on the appropriate drugs and doses are summarized in Table 2.

When access to treatment is poor, the duration and transmissibility of infections tend to be greater. Combined actions of prevention and appropriate care can contribute toward achieving the expected results in the control of STIs. The Ministry of Health recommends the combined prevention strategy that includes individual and collective prevention, offering laboratory diagnosis and the treatment of asymptomatic STIs, and monitoring symptomatic STIs through protocol-based management. One of the principal focuses is on compliance with treatment and treating sexual partners.[10]

Providing care for individuals with an STI should not be based exclusively on curing the infection identified, but also on preventing coinfection by other STIs and avoiding the complications resulting from the infection. Applying health-promoting actions through individual and collective education is a strategy that should be adopted when the patients are receiving care at the health-care units.[10]

In the case of the sexual partners of pregnant women with STIs, diagnosis should be presumed based on the agent identified in the woman, and the same treatment should be prescribed. If the partner does not attend the scheduled appointment, some strategies can be adopted according to the availability at each healthcare unit, as long as

Table 2: The treatment recommended according to the etiological agent, as defined in the clinical protocol and treatment guidelines for the comprehensive healthcare of individuals with sexually transmitted infections[9]

Etiological agent	Recommended treatment
Neisseria gonorrhoeae	Ceftriaxone 500 mg, intramuscular, single dose + azithromycin 500 mg, 2 tablets, orally, single dose
Chlamydia trachomatis	Azithromycin 500 mg, 2 tablets, orally, single dose
Mycoplasma genitalium	Azithromycin 500 mg, 2 tablets, orally, single dose
Trichomonas vaginalis	Metronidazole 400 mg, 5 tablets, orally, single dose (total dose for treatment 2 grams) or metronidazole, 250 mg, 2 tablets, orally, twice a day for 7 days

Source: The clinical protocol and treatment guidelines for the comprehensive healthcare of individuals with sexually transmitted infections[9]

confidentiality is respected, there is no coercion and the individual is protected against discrimination.[10]

The safety of drugs during pregnancy is an essential choice for treatment. Drugs should be used during pregnancy only if the expected benefits to the mother outweigh the potential risks to the fetus, and all drugs should be avoided, if possible, during the first trimester because of the risk of a teratogenic effect.

An important discussion to be held if the results of the present study are to be used to implement public policies concerns the ideal moment in pregnancy at which to collect vaginal swab and begin treatment according to the potential for complications during pregnancy and the possibility of vertical transmission.

Conclusion

The present study found a predominance of *T. vaginalis* and *C. trachomatis*, followed by *M. genitalium* and *N. gonorrhoeae*. Evaluating the vaginal microbiota of pregnant women is of the utmost importance in implementing the appropriate treatment for each pathogen, consequently avoiding complications, both for the woman and for her fetus.

Although the use of molecular testing for the detection of *C. trachomatis*, *N. gonorrhoeae*, *M. genitalium,* and *T. vaginalis* in pregnant women has yet to be included in the public policies of the Brazilian national health service, the results of the present study may encourage reflection and stimulate debate regarding the future inclusion of these tests as routine in prenatal care.

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Conflicts of interest

There are no conflicts of interest.

References

1. Costa MC, Bornhausen Demarch E, Azulay DR, Périssé AR, Dias MF, Nery JA. Sexually transmitted diseases during pregnancy: A synthesis of particularities. An Bras Dermatol 2010;85:767-82.
2. Silva FC, Medeiros HA, Anjos TF, Viana TC. Sexually transmitted infections in pregnant women: An integrative review. Braz J Surg Clin Res 2020;33:98-103.
3. Yang X, Siddique A, Khan AA, Wang Q, Malik A, Jan AT, *et al. Chlamydia trachomatis* infection: Their potential implication in the etiology of cervical cancer. J Cancer 2021;12:4891-900.
4. He W, Jin Y, Zhu H, Zheng Y, Qian J. Effect of *Chlamydia trachomatis* on adverse pregnancy outcomes: A meta-analysis. Arch Gynecol Obstet 2020;302:553-67.
5. Brazil. Ministry of Health. Department of Health Surveillance on Chronic Conditions and Sexually Transmitted Infections – Gonorrhoea and Chlamydia; 2022. Available from: https://www.gov.br/aids/pt-br/assuntos/ist/gonorreia-e-clamidia. [Last accessed on 2022 Oct 11].
6. De Carvalho NS, Palú G, Witkin SS. *Mycoplasma genitalium*, a stealth female reproductive tract. Eur J Clin Microbiol Infect Dis 2020;39:229-34.
7. Brígido HA. Municipal Health Department. Sexually Transmitted Diseases in Pregnant Women. Coordination: Department of Health Actions – Municipal STD/AIDS Coordination. Belém; 1999. Available from: https://bvsms.saude.gov.br/bvs/publicacoes/cartilha_dst.pdf. [Last accessed on 2022 Oct 11].
8. Silva CP, Ambrozim F, Silveira LF, Costa NL, Carvalho NLF, Pinto GVS, Trichomoniasis and Risk Factors in Pregnant Women. Available from: http://www.cic.fio.edu.br/anaisCIC/anais2019/pdf/05.06.pdf. [Last accessed on 2022 Oct 11].
9. Ministry of Health. Department of Health Surveillance on Chronic Conditions, STD, AIDS and Viral Hepatitis. Clinical Protocol and Therapeutic Guidelines on Comprehensive Care for People with Sexually Transmitted Infections. Brasília: Ministério da Saúde; 2020. Available from: https://portaldeboaspraticas.iff.fiocruz.br/wp-content/uploads/2020/08/pcdt_ist_final_revisado_020420.pdf. [Last accessed on 2022 Oct 11].
10. Ministry of Health. Department of Health Surveillance on STD, AIDS and Viral Hepatitis. Clinical Protocol and Therapeutic Guidelines on Comprehensive Care for People with Sexually Transmitted Infections. Brasília: Ministério da Saúde; 2015. Available from: https://bvsms.saude.gov.br/bvs/publicacoes/protocolo_clinico_diretrizes_terapeutica_atencao_integral_pessoas_infeccoes_sexualmente_transmissiveis.pdf. [Last accessed on 2022 Oct 11].
11. Brazil. Federal University of Santa Catarina. Laboratory of Molecular Biology, Microbiology and Serology. Technical Report on the analysis of vaginal secretion samples from Vitória - ES regarding the strategy: "Pilot implementation of service network for access to molecular biology tests for the detection of Chlamydia trachomatis, Neisseria gonorrhoeae, Mycoplasma genitalium and Trichomonas vaginalis in pregnant women receiving prenatal care within the scope of the Unified Health System. Florianópolis, 2022.
12. Schmidt R, Muniz RR, Cola E, Stauffert D, Silveira MF, Miranda AE. Maternal *Chlamydia trachomatis* infections and preterm births in a university hospital in Vitoria, Brazil. PLoS One 2015;10:e0141367.
13. Pinto VM, Szwarcwald CL, Baroni C, Stringari LL, Inocêncio LA, Miranda AE. *Chlamydia trachomatis* prevalence and risk behaviors in parturient women aged 15 to 24 in Brazil. Sex Transm Dis 2011;38:957-61.
14. Jalil EM, Pinto VM, Benzaken AS, Ribeiro D, Oliveira EC, Garcia EG, *et al*. Prevalence of chlamydia and *Neisseria gonorrhoeae* infections in pregnant women in six Brazilian cities. Rev Bras Ginecol Obstet 2008;30:614-9.
15. Cunha GK. Genital Mycoplasma Infection and Preterm Birth Outcome: BRISA Cohort 2021. (MS dissertation in Obstetrics and Gynecology) Ribeirão Preto: Universidade de São Paulo; 2021. Available from: https://www.teses.usp.br/teses/disponiveis/17/17145/tde-06122021-143746/pt-br.php. [Last accessed on 2022 Oct 11].
16. Lemos PA. Prevalence and Validity of Techniques for the Diagnosis of *Trichomonas vaginalis* in Pregnant, Non-Pregnant and HIV Positive Women (Doctoral thesis). Goiânia: Universidade Federal de Goiás; 2017. Available from: https://repositorio.bc.ufg.br/tede/handle/tede/6834. [Last accessed on 2022 Oct 11].
17. Espinheiro RF, Monteiro MC, Batista RH, Gomes MP, Pantoja RE, Araujo SA. Aspects of vaginal microbiota and the relationship with candidiasis in pregnant women: A literature review. Res Soc Dev 2022;11:e2911124704.