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Frequency of microorganisms in vaginal discharges of high-risk pregnant women from a hospital in Caruaru, Pernambuco, Brazil

Prevalência de microrganismos em secreção vaginal de gestantes de alto risco de uma maternidade em Caruaru, Pernambuco, Brasil

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ABSTRACT

Introduction: During pregnancy, estrogen and progesterone levels may favor vaginal colonization by pathogenic microorganisms that can be associated with obstetric complications and neonatal infections. These pathogens include *Candida* spp., *Trichomonas vaginalis*, *Gardnerella vaginalis* and *Streptococcus agalactiae*. **Objective:** To determine the frequency of such microorganisms amongst high-risk pregnant women receiving prenatal care at a hospital in Caruaru, Pernambuco. **Material and method:** Analytical study in which samples of vaginal discharge were collected from women experiencing high-risk pregnancies, without restriction of age or gestational period. The samples were submitted to Gram-stained direct smear, as well as to fresh wet-mount examination and to cultures in Sabouraud and blood agar. **Results:** From May to December 2018, 92 patients were selected for sampling. The frequency of *Candida* spp. was found to be 31.52%. Concerning *Gardnerella vaginalis*, the frequency was 1.25%. The rate of colonization by *Streptococcus agalactiae* was 3.23%. No cases of *Trichomonas vaginalis* were found in this study. **Discussion:** The frequency of *Candida* that was found corroborates the literature, as it is a common infection during pregnancy due to hormonal increase. However, the frequencies of colonization by *G. vaginalis*, *T. vaginalis* and *S. agalactiae* were lower than those found in other studies. **Conclusion:** Due to the risks that these microorganisms can bring to a pregnant woman and a fetus, health professionals should be alert to signs and symptoms, requesting the screening of these pathogens, as well as treating gestating women when necessary.

Key words: pregnancy; *Candida*; *Streptococcus agalactiae*.

RESUMO

Introdução: *Durante a gestação, os níveis de estrogênio e progesterona podem favorecer a colonização vaginal por microrganismos, incluindo* Candida *spp.,* Trichomonas vaginalis*,* Gardnerella vaginalis *e* Streptococcus agalactiae*, associados ou não a complicações obstétricas e infecções neonatais.* ***Objetivo:*** *Determinar a prevalência desses microrganismos em gestantes de alto risco de uma maternidade na cidade de Caruaru, Pernambuco, Brasil.* ***Material e métodos:*** *Estudo analítico em que amostras de secreção vaginal foram coletadas de gestantes de alto risco, sem restrição de idade ou período gestacional. As amostras foram destinadas à realização de esfregaço corado por Gram, exame a fresco e culturas em ágar Sabouraud e sangue.* ***Resultados:*** *No período de maio a dezembro de 2018, 92 gestantes foram selecionadas. A prevalência encontrada de* Candida *foi de 31,52%. Quanto à* Gardnerella vaginalis*, a prevalência foi de 1,25%. A taxa de colonização por* Streptococcus agalactiae *foi de 3,23%. Nenhum caso de* Trichomonas vaginalis *foi encontrado neste estudo.* ***Discussão:*** *A prevalência de* Candida *encontrada está de acordo com a literatura, visto que é uma infecção comum durante a gestação em razão do aumento hormonal. Entretanto, as prevalências de* G. vaginalis*,* T. vaginalis e S. agalactiae *foram baixas em comparação com outros estudos.* ***Conclusão:*** *Devido ao risco que esses microrganismos podem trazer à gestante e ao feto, os profissionais devem estar atentos aos sinais e sintomas, solicitando pesquisa desses patógenos, além de iniciar o tratamento da paciente, quando necessário.*

Unitermos: *gravidez;* Candida*;* Streptococcus agalactiae.

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RESUMEN

Introducción: *Durante el embarazo, los niveles de estrógeno y progesterona pueden favorecer la colonización vaginal por microorganismos, incluyendo* Candida *spp.,* Trichomonas vaginalis*,* Gardnerella vaginalis *y* Streptococcus agalactiae*, asociados o no a complicaciones obstétricas e infecciones neonatales.* ***Objetivo:*** *Estimar la prevalencia de eses microorganismos en mujeres embarazadas de alto riesgo de una maternidad en la ciudad de Caruaru, Pernambuco, Brasil.* ***Material y métodos:*** *Estudio analítico en el que se tomaron muestras de flujo vaginal de mujeres embarazadas de alto riesgo, sin restricción de edad o edad gestacional. Las muestras iban destinadas a la realización del frotis con tinción de Gram, examen de montaje en fresco y cultivos en agar Sabouraud y sangre.* ***Resultados:*** *En el periodo de mayo a diciembre de 2018, se eligieron 92 mujeres embarazadas. Se encontró una prevalencia de* Candida *del 31,52%. La prevalencia de* Gardnerella vaginalis *fue del 1,25%. La tasa de colonización por* Streptococcus agalactiae *fue del 3,23%. No se encontró ningún caso de* Trichomonas vaginalis *en este estudio.* ***Discusión:*** *La prevalencia de* Candida *está de acuerdo con la literatura, puesto que es una infección común durante el embarazo debido al aumento hormonal. Sin embargo, las prevalencias de* G. vaginalis*,* T. vaginalis *y* S. agalactiae *fueron bajas en comparación con otros estudios.* ***Conclusión:*** *Debido al riesgo que estos microorganismos representan para la mujer embarazada y el feto, los profesionales deben vigilar los signos y síntomas, solicitando investigación sobre esos patógenos, además de comenzar el tratamiento de la paciente, cuando necesario.*

Palabras clave: *embarazo;* Candida*;* Streptococcus agalactiae*.*

INTRODUCTION

During pregnancy, estrogen and progesterone hormone levels induce changes in the genital tract of women. Those changes favor vaginal colonization by pathogenic microorganisms. Vaginitis is common during pregnancy, being associated with adverse perinatal outcomes, such as premature rupture of membranes and preterm delivery[1]. It is normally detected by the presence of odorless or abnormal smelling vaginal discharge[2].

Vulvovaginal candidiasis (VVC) is an infection that affects more than 75% of women at least once in a lifetime, causing symptoms such as pruritus, dyspareunia and whitish curdy discharge. The *Candida albicans* species is responsible for around 80%-90% of the cases and proliferates in acidic environments, as in decreased vaginal pH[2, 3]. Pregnancy is considered a risk factor for VVC, because the high estrogen and progesterone levels facilitate adherence and multiplication of the yeast and predispose pregnant women to primary candidiasis and recurrences[4]. Other risk factors are diabetes, immunosuppression, and the use of antibiotics[5]. In the latest years, studies verified that colonization by *Candida* spp. in early pregnancy is associated with increased risk of preterm delivery and low birth weight[6, 7]. For treatment of VVC in pregnant women, topical nystatin is recommended. The use of systemic antifungal agents is contraindicated, especially in the first trimester of pregnancy[2].

Other causes of vaginitis also associated with adverse perinatal outcomes are trichomoniasis and bacterial vaginosis (BV). Trichomoniasis, caused by the protozoa *Trichomonas vaginalis*, appears as an asymptomatic infection in 10%-50% of the cases; common symptoms are yellow or green foul-smelling vaginal discharge, pruritus, dysuria, and abdominal pain. There is evidence of association between infection by this microorganism during pregnancy and premature delivery and low birth weight[8, 9]. Infection by *T. vaginalis* was also associated with postpartum maternal sepsis[10]. Trichomoniasis during pregnancy can be treated with a single 2-g dose of oral metronidazole[2].

BV is characterized by increase in vaginal pH, reduction in normal microbiota, and overgrowth of anaerobic bacteria, particularly *Gardnerella vaginalis*. The main feature of this infection is the presence of a discharge with foul odor similar to ''rotten fish'', of variable color. This infection has been associated with preterm delivery, premature rupture of membranes, and postpartum endometritis[2, 11]. Larsson *et al*. (2016)[12] demonstrated that, even after treatment with clindamycin, pregnant women with prior diagnosis of BV had higher risk of spontaneous preterm delivery than pregnant women with normal microbiota[12].

Group B streptococcus (GBS), or *Streptococcus agalactiae*, is a major causing agent of gestational complications and neonatal infections. The gastrointestinal tract is a natural reservoir for GBS, which can colonize the vagina asymptomatically. Neonates born to colonized mothers can develop sepsis, pneumonia, and meningitis[13]. After the onset of labor and rupture of membranes, GBS can invade the amniotic fluid, although the microorganism also goes through intact membranes. Thus, fetal aspiration of GBS can lead to bacteremia. The bacterium can also be acquired during passage through the birth canal[14].

According to the guidelines for prevention of perinatal GBS disease, issued by the Centers for Disease Control and Prevention (CDC), universal screening is recommended for pregnant women between the 35th and the 37th gestational weeks. GBS-colonized women must be given antibiotics during labor, and penicillin G crystalline is the drug of choice[13-15]. In Brazil, no consensus exists about prophylactic measures to reduce the incidence of neonatal GBS infection, so that GBS investigation is not part of the protocol of antenatal care proposed by the Ministry of Health[16].

OBJECTIVES

Determine the prevalence of microorganisms in the vaginal discharge of high-risk pregnant women receiving prenatal care at a reference hospital in the municipality of Caruaru, Pernambuco, Brazil.

MATERIAL AND METHOD

Study design and population

Analytical cross-sectional study, in which all women seen at the study period were included, following inclusion and exclusion criteria. Collections were carried out from May to December 2018. Inclusion criterion: being a high-risk pregnant woman under prenatal care at a hospital in the city of Caruaru, with no restriction of age or gestational period. Exclusion criteria: pregnant women suffering any clinical condition that impaired collection, such as miscarriage or bleeding risk, or that did not accept to have samples collected.

Data collection

The patients answered a questionnaire, prior to collection, in which it was possible to evaluate the following variables: age, marital status, level of schooling, income, occupation, race, origin (urban or rural area), presence of diseases, occurrence of miscarriage or preterm delivery in previous pregnancies, presence of discharge, pruritus, and dyspareunia.

Collection of vaginal and rectal specimens

Samples of vaginal and rectal discharge were collected using sterile swabs. Firstly, four vaginal swabs were collected from the distal third of the vagina and, later, a rectal swab. The first vaginal swab sample was used for smear preparation over a microscope slide for later staining with the Gram method. The second swab was placed into a tube containing 1-ml normal saline for fresh wet-mount examination. The third and fourth swabs were seeded onto Petri dishes containing Sabouraud dextrose agar 4% (Merck, Darmstadt, Germany) and sheep blood agar 5%, respectively. The rectal swab was also seeded onto a blood agar plate. After collection, the samples were transported up to the microbiology laboratory of Centro Universitário Tabosa de Almeida (Asces-Unita) to be analyzed.

Sample processing

The heat-fixed Gram-stained slides were microscopically visualized for detection of clue cells, characteristic of the infection by *Gardnerella vaginalis*. For the investigation of *Trichomonas vaginalis* and yeasts, a drop of the saline containing the discharge was deposited on the slide and looked under on a microscope. The plates containing Sabouraud agar and blood agar were kept in an incubator at 37°C for 24 hours.

In the plates where there was bacterial growth, colony morphology was observed. With the colonies suggestive of *Candida* spp. (white/beige, presenting creamy texture), Gram-stained smears were prepared to confirm the presence of oval yeast-like structures of purple color.

With the colonies suggestive of *Streptococcus agalactiae* (small, grayish and beta-hemolytic), streaking was done over a new blood agar plate, to obtain pure culture. For final identification of the possible bacterium, the Christie, Atkins and Munch-Petersen (CAMP) test was conducted. For each GBS isolated found, the profile of susceptibility to antimicrobials was determined as recommended by the manual of the Clinical and Laboratory Standards Institute (CLSI) 2018[17]. The test was carried out on blood agar; the following antibiotic discs were used: ampicillin (10 µg), cefepime (30 µg), vancomycin (30 µg), erythromycin (15 µg), clindamycin (2 µg), chloramphenicol (30 µg), tetracycline (30 µg) and azithromycin (15 µg).

Data analysis

Data, obtained from the questionnaire answers and laboratory results, were entered into an Excel spreadsheet (version 1810), to analyze prevalence and profile of pregnant women. In order to identify associations between clinical variables and laboratory results, Fisher exact test was used, with significance level of 0.05.

Ethics

This study was approved by the Research Ethics Committee of Asces-Unita, under no. 2.432.879. The pregnant women were

duly informed about the procedures and objectives of the research. Those who accepted taking part in the study signed the Free Informed Consent, according to Resolution no. 466/12 of the National Health Council.

RESULTS

Profile of pregnant women

During the cited period, 92 samples from high-risk pregnant women were obtained. Patients' ages ranged from 13 to 43 years, with an average of 29.3 years. The predominant age group was that of 25-29 years (33.7%). A large group of patients had not completed primary school (38.04%). Common-law marriage was the most prevalent marital status (44.57%), and most women worked just at home (68.48%). The white race was the most frequent (45.65%), as well as the patients from the urban areas (69.57%) (**Table 1**).

The found prevalence of *Candida* spp. and *Gardnerella vaginalis* was, respectively, 31.52% and 1.25%. The rate of colonization by GBS was 3.23%. No case of *Trichomonas vaginalis* was found in this study.

Forty-seven patients were in the second trimester of pregnancy (51.09%). The largest number of cases colonized by *Candida* spp. (13) occurred in the gestational period. Among the 29 patients with positive culture for *Candida* spp., three (10.35%) were diabetics, one (3.45%) reported preterm delivery in previous pregnancies, and eight (27.59%) had miscarriage. About the presence of symptoms suggestive of VVC, 18 women (62.07%) reported at least one of the symptoms. Vaginal discharge was reported by 13 (44.83%); genital pruritus, by eight (27.59%); dyspareunia, by seven (24.14%). None of those variables had statistically significant association with the presence of *Candida* spp. in the culture, because they presented value of $p > 0.05$.

The patient who had a result compatible with *Gardnerella vaginalis* was in the second trimester of gestation and reported a case of miscarriage; however, she did not present symptoms suggestive of BV.

Regarding the detected cases of GBS colonization, patients presented positivity just in vaginal culture. About the gestational period, a pregnant woman was in the 18th week and another, in the 29th week. One of the GBS isolates presented susceptibility just to vancomycin and chloramphenicol, being resistant to all the other tested antibiotics, including ampicillin. The other GBS isolate showed resistance to clindamycin, cefepime and tetracycline (**Table 2**).

TABLE 1 – Sociodemographic profile of pregnant women seen at a maternity hospital in the municipality of Caruaru (n = 92)

Variable		*n*	%
Age group (in years)	< 20	4	4.35
	20-24	17	18.48
	25-29	31	33.7
	30-34	19	20.65
	35 or older	21	22.83
Schooling	Complete elementary school	8	8.7
	Incomplete elementary school	35	38.04
	Complete high school	32	34.78
	Incomplete high school	8	8.7
	Complete undergraduate course	8	8.7
	Incomplete undergraduate course	1	1.09
Marital status	Married	36	39.13
	Divorced	1	1.09
	Single	14	15.22
	Common-law marriage	41	44.57
Occupation	Self-employed	1	1.09
	Housewife	63	68.48
	Civil servant	16	17.39
	Private employee	12	13.04
Race	White	42	45.65
	Black	13	14.13
	Brown	37	40.22
Origin	Rural	28	30.43
	Urban	64	69.57
Total		92	100

TABLE 2 – Antimicrobial susceptibility profile of GBS isolates

Antibiotic	Strain I	Strain II
Ampicillin	Resistant	Susceptible
Cefepime	Resistant	Resistant
Vancomycin	Susceptible	Susceptible
Erythromycin	Resistant	Susceptible
Clindamycin	Resistant	Resistant
Chloramphenicol	Susceptible	Susceptible
Tetracycline	Resistant	Resistant
Azithromycin	Resistant	Susceptible

GBS: Group B streptococcus.

DISCUSSION

Candida spp.

In the present study, the most prevalent microorganism in vaginal discharge samples of pregnant women was *Candida* spp., with a prevalence rate of 31.52%.

The VVC epidemiology varies a lot from region to region, depending on the population profile. Climate also interferes with the prevalence of this microorganism, as candidiasis is the most

common type of vulvovaginitis in tropical countries[18, 19]. The results found in this study are close to those obtained by Bonfanti and Gonçalves (2010)[20], who discovered a prevalence of 33.75% during analysis of cytopathological reports of pregnant women from Rio Grande do Sul, Brazil. Lower rates were found in Argentina (28%)[21], United Kingdom (12.5%)[22], Malaysia (17.2%)[23], Nigeria (25%)[24] and India (4,13%)[1].

Pregnancy is considered a risk factor for VVC, given the hormone alterations and the increased deposit of glycogen and other substrates in the vagina during that period[25, 26]. In many cases, the infection is asymptomatic, being called colonization[27]. In the present study, 62.07% of the women with positive culture for *Candida* spp. presented at least one of the symptoms suggestive of VVC, while 37.93% were asymptomatic. Another important risk factor for candidiasis is a situation of hyperglycemia, when excessive glycogen facilitates the establishment of the microorganism[25]. In this study, seven patients were diabetics and among them, three presented positive cultures for *Candida* spp.

Some authors, such as Olowe *et al*. (2014)[5] and Sangaré *et al*. (2017)[27], stated that the incidence of candidiasis increases with gestational age. There are divergences in the literature, however. In the study conducted by Masri *et al*. (2015)[23], pregnant women in the first and second trimesters of gestation had higher risk of acquiring candidiasis than those in the third trimester. Parveen *et al*. (2008)[28] and Brandão (2017)[3], in their turn, did not find association between gestational period and VVC. In this study, the highest prevalence of *Candida* spp. colonization occurred in the second trimester of pregnancy. Kanagal *et al*. (2014)[29] also found higher prevalence of *Candida* spp. in the second trimester.

Gardnerella vaginalis

A case of BV was found in this study, what corresponds to a rate of 1.25% – low, compared to the literature. Akinbiyi *et al*. (2008)[22] also found a low prevalence (3.54%) among asymptomatic pregnant women in the United Kingdom. Rao and Chandini (2017)[11] found a rate of 19.2% among pregnant women of a hospital in India, without statistically significant association with gestational period. In Nigeria, Olowe *et al*. (2014)[5] found a prevalence of 38%, associated with recent use of antibiotics. In the study by Monteiro *et al*. (2017)[30], the found prevalence of BV was 19% among pregnant women treated at a maternity hospital in the city of Natal, Rio Grande do Norte, Brazil[30].

GBS

It is estimated that 10%-30% of pregnant women globally are colonized asymptomatically by GBS[14]. This prevalence varies a lot among studies due to several factors, such as characteristics of the studied population, collection method and laboratory tests.

In this study, the prevalence of GBS colonization was 3.23%, a percentage close to the work conducted by Shirazi *et al*. (2014), which verified prevalence of 4.8%[31]. A lower prevalence was found by Sharmila *et al*. (2014)[32] – 2.3% among Indian pregnant women who were in the 35th-37th weeks of gestation. A factor that might have contributed to this result is the high number of pregnant women that underwent recent treatment for urinary tract infections, what led to decolonization upon collection time[13].

In Brazil, the prevalence of GBS colonization among pregnant women varies widely according to the region. Dias (2014)[33] found a 13.95% rate in pregnant women in Cuiabá, Mato Grosso. In Niterói, Rio de Janeiro, a 6.1% prevalence was found[34]. Nunes *et al*. (2015)[35] analyzed 1,425 records of pregnant women treated in Florianópolis who underwent GBS investigation; 16.5% were colonized. In the study by Senger *et al*. (2016)[36], 22.5% of the pregnant women were colonized, in the municipality of Santo Ângelo, Rio Grande do Sul[36]. Another study, also in Rio Grande do Sul, demonstrated a rate of 8.8%[37].

Penicillin is the drug of choice for intrapartum antibiotic prophylaxis of neonatal infection. Ampicillin can be used as an alternative. Allergic patients are suggested to undergo susceptibility tests to clindamycin and erythromycin, antibiotics that can also be used. In case of resistance, vancomycin is recommended[13-15].

CONCLUSION

Candida spp. was the most prevalent microorganism in the samples from vaginal discharge of high-risk pregnant women. As several studies point the association of this microorganism (although common during pregnancy) with preterm deliveries and low birth weight, it is important to confirm the agent and provide the treatment, because the picture may be asymptomatic. Low prevalence of *Gardnerella vaginalis*, *Trichomonas vaginalis* and *Streptococcus agalactiae* was found. All these microorganisms deserve attention by health professionals because of the possible risk they pose to pregnant women and fetuses.

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