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Streptococcus agalactiae: colonization of high-risk pregnant women in a regional hospital in the Brazilian Amazon and antimicrobial sensitivity profile

Streptococcus agalactiae: colonização de gestantes de alto risco em um hospital regional da Amazônia brasileira e perfil de sensibilidade aos antimicrobianos

Edlainny Araujo Ribeiro[1], Georgia Miranda Tomich[1], Brena de Almeida Costa[1], Rodrigo Alves de Oliveira[1], Lorrany Karen Batista de Jesus[1]

[1] *Faculdade de Ensino Superior da Amazônia Reunida, Redenção, Pará, Brasil*

ABSTRACT

INTRODUCTION: Group B streptococcus (GBS) or *Streptococcus agalactiae* in immunosuppressed individuals, such as neonates, can result in a series of complications and diseases, which can even lead to death. OBJECTIVES: To characterize the clinical-epidemiological profile of pregnant women colonized by *S. agalactiae* and determine the isolates' sensitivity profile in a hospital in the Amazon. MATERIALS AND METHODS: Clinical specimens were collected from March 15 to April 15, 2019, following the Centers for Disease Control and Prevention guidelines. The phenotypic identification was performed according to the Brazilian Health Regulatory Agency (Anvisa), and for the antimicrobial sensitivity testing, the Clinical and Laboratory Standards Institute specifications were followed. RESULTS: Colonization by GBS was found in 34.0% of the pregnant women; the most frequent chronic diseases were hypertension (26.0%) and diabetes (10.0%). The antimicrobials linezolid, vancomycin, and meropenem were the most effective against the bacteria. There was a high resistance rate for ciprofloxacin (82.4%) and chloramphenicol (70.6%); 88.2% of the strains analyzed were multidrug-resistant. CONCLUSION: The presence of GBS among high-risk pregnant women and the detection of multidrug-resistant strains, including those with resistance to penicillins and cephalosporins, bring up the importance of screening for the detection of this bacteria during pregnancy and the beginning of antibiotic prophylaxis, emphasizing the need to adapt the practice of local prenatal care to the current recommendations.

Keywords: *Streptococcus agalactiae*; Pregnant Women; Microbial Drug Resistance.

RESUMO

INTRODUÇÃO: Estreptococo do grupo B (EGB) ou *Streptococcus agalactiae* em indivíduos imunossuprimidos, como os neonatos, pode resultar em uma série de complicações e doenças, podendo levar até à morte. OBJETIVOS: Caracterizar o perfil clínico-epidemiológico de gestantes colonizadas por *S. agalactiae* e determinar o perfil de sensibilidade antimicrobiana dos isolados em um hospital na Amazônia. MATERIAIS E MÉTODOS: As coletas dos espécimes clínicos foram realizadas no período de 15 de março a 15 de abril de 2019 considerando as diretrizes do Centers for Disease Control and Prevention. A identificação fenotípica foi realizada de acordo com as recomendações da Agência Nacional de Vigilância Sanitária, e para o teste de sensibilidade antimicrobiana, foram seguidas as especificações do Clinical and Laboratory Standards Institute. RESULTADOS: A colonização por EGB foi detectada em 34,0% das gestantes; as doenças crônicas mais frequentes foram hipertensão (26,0%) e diabetes (10,0%). Os antimicrobianos linezolida, vancomicina e meropeném foram os mais eficazes contra as bactérias. Verificou-se alta taxa de resistência para ciprofloxacina (82,4%) e cloranfenicol (70,6%). Das cepas analisadas, 88,2% eram multirresistentes. CONCLUSÃO: A presença de EGB entre as gestantes de alto risco e a detecção de cepas multirresistentes, inclusive com resistência a penicilinas e cefalosporinas, traz à tona a importância da triagem para a detecção dessa bactéria durante a gestação e o início da antibioticoprofilaxia, ressaltando a necessidade de adequar a prática de acompanhamento pré-natal local às recomendações vigentes.

Palavras-chave: *Streptococcus agalactiae*; Gestantes; Resistência Microbiana a Medicamentos.

Correspondence / Correspondência:
Edlainny Araujo Ribeiro
Faculdade de Ensino Superior da Amazônia Reunida, Curso Biomedicina
Av. Brasil, 1435. Bairro: Alto Paraná. CEP: 68550-0325 – Redenção, Pará, Brasil – Tel.: +55 (94) 99178-0799
E-mail: dyy_araujo77@hotmail.com

INTRODUCTION

Streptococcus agalactiae or Lancefield group B Streptococcus (GBS) shows cocci chain morphology and gram-positive dye reaction. It can colonize women's gastrointestinal and genitourinary tracts asymptomatically and cause severe infections in newborns[1,2]. When the bacterium is transferred to the newborn at the time of delivery, it can cause sepsis and meningitis, increasing the risk of mortality[3]. The Centers for Disease Control and Prevention (CDC) treats infection/colonization by this bacterium as one of the leading infectious causes of neonatal morbidity and mortality in the United States of America[4]. A study carried out in Mozambique with a total of 183 stillborns detected GBS in 2.1% of them through umbilical blood analysis[5].

Complications during pregnancy, childbirth, and puerperium can be prevented through prenatal care, making it possible to identify risk situations for the mother and fetus[6]. However, a study conducted with 100 pregnant women to detect colonization by GBS demonstrated a positivity of 14%[7]. Another study carried out in Rio de Janeiro State revealed that among the 3,647 pregnant women between the 35th and 37th gestational week, 26.2% were colonized by this bacterium[8].

Thus, to reduce the associated perinatal damage, Toyofuku et al.[9] demonstrated that intrapartum prophylaxis in colonized pregnant women reduced neonatal death by 32.5% in a region with high prevalence rates of this bacterium. The CDC[4] recommends screening for GBS colonization in women between the 35th and 37th gestational week and starting antibiotic prophylaxis as soon as the presence of GBS is identified. The Ministry of Health recommends the investigation of these bacteria only in high-risk pregnant women who present signs and symptoms, such as bleeding. However, the pregnant woman may be colonized and have no symptoms and may transmit this microorganism to the fetus, which will be susceptible to infection that can lead to sepsis[10].

A high-risk pregnancy is characterized by greater susceptibility to damage to the health of the mother and fetus, such as complications in labor (premature delivery), maternal clinical diseases, and fetal changes that can trigger complications during this period[11]. Additionally, sociodemographic factors, such as low levels of income, education level, high pregnancy rates, and poor obstetric care favor the persistence of mortality indicators among pregnant women[12,13,14].

Besides, bacteria of the genus *Streptococcus* may present resistance to a prophylactic antimicrobial drug due to chemical changes in the antimicrobial binding sites in the cell wall and transfer of resistance coding genes[15]. They can also form communities that allow their survival in hostile environments for long periods, called biofilms[15]. This bacterium is a significant cause of infection in pregnant women and their newborns, and despite this, it has been little studied in Latin America[16].

Knowledge about the colonization and sensitivity profile of isolated GBS in pregnant women is of great value, considering the possibility of vertical transmission, its pathogenicity, inherent risks to the health of the mother and fetus, and the possibility of antimicrobial resistance. In addition to these factors, during pregnancy, the administration of antimicrobials is complicated, especially in the case of high-risk pregnant women, and, in Pará State, Brazil, there is a paucity of epidemiological data on this subject.

Given the above, this research aimed to characterize the clinical-epidemiological profile of pregnant women colonized by *S. agalactiae* and determine the sensitivity profile of these isolates in a hospital that provides medium and high-complexity services in the Amazon.

MATERIALS AND METHODS

ETHICAL ASPECTS

The research was approved by the Comitê de Ética em Pesquisa do Sul do Pará, belonging to the Faculdade de Ensino Superior da Amazônia Reunida, on October 29, 2018, under the number CAAE: 99503018.2.0000.8104; and, in compliance with Resolution No. 466/12 of the National Health Council, the collections were only started in March 2019, after approval by the Committee.

TYPE AND LOCATION OF STUDY

The study was descriptive, prospective, and cross-sectional, with a quantitative approach, carried out in a public hospital, a reference in high-risk pregnancy, which treats an average of 100 pregnant women per month and provides medium and high complexity services to patients from 15 municipalities in Pará State southeast. It belongs to the 12th Regional Health Center, which encompasses a population of 541,347 inhabitants. The hospital is located in Redenção, at a distance of 1,018 km from the capital Belém[17,18].

STUDY PERIOD AND POPULATION

The sample collections were performed from March 15 to April 15, 2019, totaling 32 consecutive days. The following inclusion criteria were considered for the study population: women undergoing prenatal care at the hospital with a high-risk pregnancy. Although the CDC[4] recommends performing the exam from the 35th gestational week onwards, pregnant women from the 22nd onward were also included in this research. In compliance with the resolution of the National Health Council No. 466/12[19], all pregnant women who agreed to participate in the study signed the Free and Informed Consent Form (FICF). Pregnant women who could not sign the FICF were excluded, that is, those who needed the signature of their guardian or curator, as well as those who took antibiotics in the last 10 days.

Thus, 50 pregnant women participated in the study. Two sterile alginate *swabs* were collected from each of them, one rectal and one vaginal, which were

immediately sent for analysis in the hospital's laboratory, following recommendations described later.

Sociodemographic and clinical-obstetric data were analyzed considering the variables: race, education attainment, housing (rural/urban), city of origin, age group, chronic diseases (baseline), sexually transmitted infections (STI), number of pregnancies, and gestational age at the time of *swabs* collection.

PROCEDURES FOR SAMPLE COLLECTION, PHENOTYPIC DETECTION, AND SENSITIVITY PROFILE

Collections of clinical specimens were performed following CDC recommendations[4]. Samples were obtained from the vaginal introitus and rectum using sterile alginate *swabs*. The material was immediately inoculated into Todd Hewitt selective enrichment broth (Probac®) supplemented with antibiotics, hermetically sealed, and taken to the hospital's laboratory.

In the microbiology sector, inoculated samples were incubated at 35–37 °C for 24 h; later, the streaking technique was performed on Todd Hewitt Blood (Probac®), first adding a Hemolysinabac tape (Probac®) in the center of the plate. Streaks perpendicular to the tape were made with a sterile and disposable bacteriological loop, and the plates were reincubated at 35–37 °C for 24 h. The interpretation of the test with detection of β-hemolysis with arrow formation was performed using a positive control for the CAMP test after 24 h. Following the recommendations of the Brazilian Health Regulatory Agency for the phenotypic identification of *S. agalactiae*[20], the strains were submitted to the catalase and PYR (Probac®) tests to determine the pyrrolidonyl arylamidase enzyme activity, in which negativity was observed for all strains included in this research.

The antimicrobial susceptibility test was performed from recent cultures, with sample suspensions in sterile saline solution, with turbidity corresponding to 0.5 of the MacFarland standards in blood agar, by the disk diffusion method described by Clinical and Laboratory Standards Institute (CLSI)[21]. As a control, a strain of *Streptococcus pneumoniae* (ATCC® 49619™) was used. After 24 h of incubation at 35–37 °C, the diameter of the growth inhibition zone was recorded in millimeters, and the isolates were classified as sensitive, intermediate, and resistant, according to CLSI criteria.[21]. To verify the induced resistance to clindamycin, the D-test was performed, in which the clindamycin disk (Polisensidisc®) has been added to a different site for better viewing.

The antimicrobials tested were penicillin (10 U), ampicillin (10 μg), cefotaxime (30 μg), erythromycin (15 μg), clindamycin (2 μg), chloramphenicol (30 μg), oxacillin (1 μg), azithromycin (15 μg), ciprofloxacin (5 μg), tetracycline (30 μg), linezolid (30 μg), vancomycin (30 μg), and meropenem (10 μg)[22]. The resistance to three or more classes of antimicrobials tested was defined as resistance to multiple drugs[22].

DATA ANALYSIS

The data were organized in Microsoft Excel (2013) spreadsheets and consolidated according to the appropriate coding for each studied variable. Descriptive data analysis was performed using absolute, relative (percentage), and medium frequencies and standard deviation.

RESULTS

Samples of vaginal and rectal secretions from 50 high-risk pregnant women attended at a regional hospital in the southeast of Pará State were screened for the presence of *S. agalactiae*. The bacteria was found in 34.0% (17/50) of the pregnant women, of which 35.3% (6/17) in vaginal secretions, 23.5% (4/17) in the anal region, and 41.2% (7/17) in both. The average age of participants was 26.4 years (standard deviation ± 7.2). The highest frequency of positive tests was observed in full-term pregnant women, with ≤ eight years of schooling, non-white skin color, living in rural areas, and who had been pregnant more than once (Table 1).

Among the factors that justify the monitoring of these pregnant women in medium and high-complexity care, the most frequently observed were hypertension (26.0%; 13/50), diabetes (10.0%; 5/50), twin pregnancy (10.0%; 5/50), kidney disease (8.0%; 4/50), and maternal age (8.0%; 4/50). Another associated risk was the STIs, such as syphilis, viral hepatitis B, and HIV infection, which was observed in 6.0% (3/50) of pregnant women.

Regarding the sensitivity profile of the isolated microorganisms, 100.0% (17/17) were sensitive to the antimicrobials linezolid, vancomycin, and meropenem (Figure 1). However, high rates of resistance were detected for ciprofloxacin (82.4%; 14/17), followed by chloramphenicol (70.6%; 12/17), azithromycin (58.8%; 10/17), and tetracycline (58.8%; 10/17). Of the strains analyzed, 88.2% (15/17) were multiresistant, showing resistance to erythromycin, ampicillin, penicillin, ciprofloxacin, and tetracycline (Table 2). The classes of antimicrobials with the highest resistance rates (*in vitro*) were the quinolones, followed by β-lactams, amphenicols, macrolides, lincosamides, and tetracyclines.

DISCUSSION

In this study, colonization by GBS was found in high-risk pregnant women. This fact can be associated with anatomical and physiological changes, such as hypertrophy of the vaginal walls, increased blood flow, changes in pH, temperature, and vaginal acidity, in addition to some personal hygiene habits such as, for example, the use of showers and intimate soaps[23,24].

Although sampling is a limiting factor in this research, studies aimed at analyzing sociodemographic factors can infer the existence of groups more susceptible to colonization by GBS[25,26]. This fact was demonstrated in a study conducted in Saudi Arabia with 1,328 pregnant women, in which colonization by GBS was 4.2% in pregnant women aged between 25 and 29 years, and 27.4% among those aged over 40 years[27].

Table 1 – Sociodemographic profile, clinical-obstetric factors, and frequency of pregnant women colonized by GBS assisted in a regional hospital in Pará State, Brazil, between March and April 2019

Exposure Variables	Pregnant women		Positive exams	
	N = 50	%	N = 17	%
Age (X = 26,4; standard deviation ± 7,2)				
≤ 25	24	48,0	8	33,3
≥ 26	26	52,0	9	34,6
Education attainment				
≤ 8 years	9	18,0	5	55,6
≥ 9 years	41	82,0	12	29,3
Skin color				
White	4	8,0	1	25,0
Non-white	46	92,0	16	34,8
Área de residência				
Rural	7	14,0	4	57,1
Urban	43	86,0	13	30,2
Nº of pregnancies				
Primigravida	21	42,0	6	28,6
Multigravida	29	58,0	11	37,9
Gestational age (time of *swab* collection)				
(X = 31,9; standard deviation ± 5,9)				
Preterm < 37 weeks	35	70,0	10	28,6
Full term from 37 to 42 weeks	15	30,0	7	46,7
Postterm > 42 weeks	–	–	–	–
STI diagnosis (in the current pregnancy)				
Yes	3	6,0	–	–
No	47	94,0	17	36,2

Conventional sign used: – Numeric data equal to zero, not resulting from rounding.

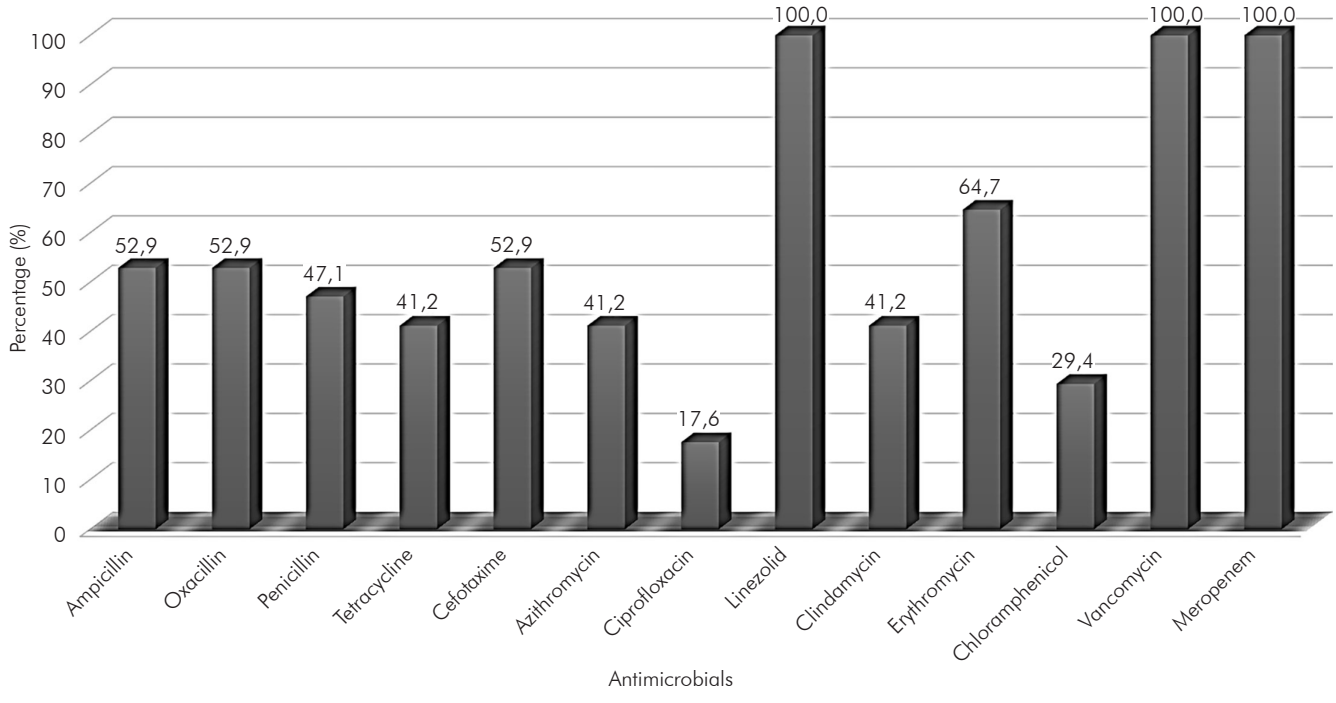

Figure 1 – Sensitivity profile against antimicrobials tested in disk-diffusion (*in vitro*) of the strains of *Streptococcus agalactiae* isolated from high-risk pregnant women in southeastern Pará state, Brazil, between March and April 2019

Positive samples	Antimicrobials												
	AMP	OXA	PEN	TET	CFO	AZI	CIP	LNZ	CLI	ERY	CHL	VAN	MER
A1	S	S	S	S	S	R	S	S	R	S	R	S	S
A2	S	S	S	S	S	R	S	S	R	R	R	S	S
A6	R	R	R	R	S	S	R	S	S	S	S	S	S
A8	S	S	S	S	S	S	R	S	S	S	R	S	S
A9	R	R	R	R	S	S	R	S	S	S	S	S	S
A11	S	S	S	R	S	R	S	S	S	S	S	S	S
A12	S	R	R	S	S	S	R	S	R	S	R	S	S
A15	R	R	R	S	R	S	R	S	R	S	R	S	S
A16	S	S	S	R	R	S	R	S	R	S	R	S	S
A21	S	S	S	S	R	S	R	S	R	S	R	S	S
A23	R	R	R	S	R	R	R	S	R	S	R	S	S
A24	S	S	S	R	R	R	R	S	R	R	R	S	S
A25	R	R	R	R	R	R	R	S	S	R	S	S	S
A28	R	R	R	R	R	R	R	S	S	S	R	S	S
A36	R	S	R	R	R	R	R	S	S	R	S	S	S
A38	S	S	S	R	S	R	R	S	R	R	R	S	S
A45	R	R	R	R	S	R	R	S	R	R	R	S	S

A: Demonstrative data representing positive samples for *Streptococcus agalactiae*, named according to the identification received during analysis; S: Sensitive; R: Resistant. AMP: Ampicillin; OXA: Oxacillin; PEN: Penicillin; TET: Tetracycline; CFO: Cefotaxime AZI: Azithromycin; CIP: Ciprofloxacin; LNZ: Linezolid; CLI: Clindamycin; ERY: Erythromycin; CHL: Chloramphenicol; VAN: Vancomycin; MER: Meropenem.

Table 2 – Sensitivity profile of *Streptococcus agalactiae* isolated from 17 high-risk pregnant women in Pará State, Brazil, between March and April 2019

The present research results agree with those of a study carried out in Rio de Janeiro, which demonstrated that arterial hypertension and gestational diabetes were the most observed maternal pathologies among high-risk pregnant women[8]. The pathology that most predisposes to maternal colonization is still not well understood; however, it is assumed that the change in immune status, as occurs in diabetes during pregnancy, enables invasive GBS infection[28,29,30].

Drugs used for the treatment of GBS must have the ability to inhibit cell wall synthesis, protein synthesis, and DNA gyrase. However, some antimicrobials cannot be prescribed during pregnancy because they could break the placental barrier and harm the health of the mother and fetus. Thus, penicillin, amoxicillin, ampicillin, cephalexin, and nitrofurantoin are recommended during pregnancy and, in case of allergies to penicillin, clindamycin or erythromycin[4,31].

However, when analyzing the effectiveness of these antimicrobials for the isolates in this research, it was possible to observe that the penicillins and cephalosporins tested did not show good sensitivity (*in vitro*). Corroborating this data, a study conducted in Ceará State showed that among isolated GBS strains, some were resistant to ampicillin (44.4%), cephalotin (44.4%), clindamycin (77.8%), chloramphenicol (11, 1%), erythromycin (33.3%), and penicillin (44.4%)[32]. Low rates of resistance of this bacterium to erythromycin had been reported in other studies, revealing sensitivity rates for this drug of 85.7% and 92.5%[33,34].

Indices of reduced sensitivity of GBS to penicillin have been described in the international literature[35,36]. These indices can be associated with several factors, such as its large-scale use and mutations that alter the binding site of penicillin binding proteins; those mutations can be carried by plasmids and transferred horizontally between different species[35,36]. However, the description of GBS resistance to this drug in Brazil remains very low, and this may be related to the low rates of research focused on this subject and the lack of structure to carry out the test[16,32]. As in this research, Linhares et al.[32] reported that the GBS test for pregnant women was not performed in prenatal routines.

In addition, as demonstrated in a study carried out in Amazonas State, penicillins and cephalosporins are among the main drugs used, especially cephalexin (39.7%), amoxicillin (29.4%), followed by benzathine benzylpenicillin (4.4%), ciprofloxacin (3.7%), sulfadiazine (3.7%), tetracycline (3.7%), azithromycin (2.9%) and levofloxacin (2.9%), which may influence the increased detections of strains resistant to these drugs[37].

The bacteria analyzed in this research showed high levels of resistance to the quinolone tested. According to research carried out in Argentina, this fact is associated with point mutations in *gyrA* and *parC genes*[38]. The importance of monitoring the sensitivity of this bacterium

is highlighted, as, in addition to presenting virulence factors that facilitate the development of an infectious process, it may present resistance mechanisms that hinder the therapeutic approach[38,39].

The indiscriminate use of medications can promote the emergence of multidrug-resistant bacteria associated with high morbidity and mortality[40,41]. With the advent of bacterial resistance, the identification of the pathogen and knowledge about its sensitivity profile in infection/colonization during pregnancy became important for efficient intrapartum prophylaxis. In this regard, the CDC recommends screening based on microbiological results, with detection of colonization by *swab* collection routinely performed between the 35th and 37th gestational week[4,41].

Similarly, the American College of Obstetricians and Gynecologists recommends performing GBS screening between the 36th and 37th week of pregnancy, with appropriate intrapartum antibiotic prophylaxis[42]. However, in this study, pregnant women from the 22nd gestational week were considered, considering the risk of premature birth, as they are high-risk pregnant women; the fact that colonization by GBS during pregnancy is transient, intermittent, or constant; and that, in the hospital under study, the test for the presence of GBS is not carried out at any stage of pregnancy. Thus, it is noteworthy that there is scientific evidence that suggests the association between colonization by this bacterium and prematurity, resulting in increased mortality rates[43,44].

In the hospital where the present study was carried out, despite specifically attending high-risk pregnant women, the exam for the detection of GBS is not performed as part of prenatal care, which can increase the risks to the health of the mother and fetus. On the other hand, in a hospital in São Paulo State, preventive strategies were implemented considering correct periods for collection, higher positivity rates, and risks inherent to the health of the mother and fetus for screening and detecting GBS, making it possible to mitigate the number of infections from 57.8% to 55.1%, which shows the importance of public policies and strategies to improve health care[44,45].

It is also important to emphasize that the interpretation of the results was made in light of limitations. The number of participants was small, thus limiting the possibility of representativeness of resistance detections. This may be associated with the fact that the hospital only attends high-risk pregnant women.

CONCLUSION

It was possible to evidence the presence of GBS among high-risk pregnant women attended at a regional hospital in a city in southeastern Pará and to detect multidrug-resistant strains, including those with resistance to penicillins and cephalosporins. The frequencies of positivity for colonization by GBS and of multidrug resistance detected highlight the importance of screening for microbiological detection of this bacterium during pregnancy and the beginning of antibiotic prophylaxis when indicated, demonstrating the need to adapt prenatal care practice to current recommendations. Knowledge about the clinical-epidemiological profile described in this study can contribute to the development of local preventive measures.

It is also recommended to conduct further studies with a larger sample size and an epidemiological characterization following current recommendations to correlate colonization by maternal GBS with damage to the fetus's health.

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CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest.

AUTHORS' CONTRIBUTION

All authors contributed to the idealization of the study, the analysis and interpretation of data, and the writing of the manuscript, approving the final published version. They declare that they are responsible for the entire article content, ensuring its accuracy and integrity.

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