Group B *Streptococcus* colonization prevalence and susceptibility profile in pregnant women in the Brazilian Amazon

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Abstract

Objectives: to assess the prevalence and epidemiological factors associated with group B Streptococcus (GBS) colonization in pregnant women in Porto Velho City, Rondônia.

Methods: GBS was identified and isolated by genotypic and microbiological methods from rectovaginal samples of pregnant women between 35 and 37 weeks of gestation. Epidemiological data were collected using questionnaires and their correlation with colonization was assessed. The antimicrobial susceptibility profile was determined by disk diffusion method.

Results: a total of 22.5% (102/453) pregnant women were colonized with GBS. A higher level of colonization was observed at the vaginal tract (17.6%), compared to the rectal area. We did not find any sociodemographic or obstetric factors associated with an increased risk of GBS colonization. All strains were susceptible to antibiotics penicillin, ampicillin, cefazolin, and ceftriaxone. In contrast, the rates of resistance to tetracycline (74.1%), erythromycin (14.1%), and clindamycin (3.5%) were observed.

Conclusion: the prevalence of GBS as well as the absence of predictors of colonization demonstrated the need for universal screening for GBS in all pregnant women in the region. In addition, we showed that the first-line antibiotics recommended for prophylaxis are still good options for the prevention of neonatal GBS disease in the region.

Key words *Antibiotic resistance, Neonatal diseases, Antibiotic prophylaxis, Public health surveillance, Pregnant women*

http://dx.doi.org/10.1590/1806-9304202400000063-en

Introduction

Group B *Streptococcus* or *Streptococcus agalactiae* (GBS) is the major etiological agent of neonatal infections.[1] GBS mainly colonizes the genitourinary and gastrointestinal tracts of humans; however, colonization in the vaginal and rectal areas of pregnant women poses a risk to the health of newborns because the GBS can be vertically transmitted before, during, and after childbirth. Consequently, GBS can cause a wide range of newborn clinical diseases such as sepsis, meningitis, and pneumonia.[1]

The pathology of GBS can be classified according to the time at which symptoms of the disease first appear. Early onset disease describes the appearance of symptoms within the first seven days of the newborn's life and results from spread of GBS through the ascending pathway of the uterus or at delivery. Severe infections often present as sepsis, pneumonia, cardiovascular instability, or, less frequently, meningitis, and are characterized by adverse clinical evolution.[2] Late onset disease is characterized by symptom onset between the eighth day of life to three months and can also be associated with microorganisms other than GBS, including coagulase-negative staphylococci, *Escherichia coli,* and other gram-negative bacteria[2]. Late onset disease is mainly associated with meningitis, which can lead to cognitive and neurological sequelae.[3] Invasive diseases caused by GBS account for 5–20% of mortality in premature newborns and 1–8.4% of mortality in full-term newborns.[4]

Epidemiological investigations have shown that approximately 18% of women worldwide are colonized by GBS during pregnancy, though the incidence varies between 11% and 35% according to region[1]. GBS is associated with 518,000 preterm births, 392,000 neonatal infections, and 91,000 neonatal deaths worldwide annually.[5] Studies on the prevalence of colonization in pregnant women in Brazil have shown heterogeneous rates of 4.2–28.4%.[6] Concerningly, low-income countries are more susceptible to high morbidity and mortality rates due to GBS than high-income countries, which is associated with a lack of systematic prophylaxis measures, among other factors.[7]

The guidelines for GBS prophylaxis were updated in 2020 by the American College of Obstetricians and Gynecologists. Universal GBS screening involves the screening of maternal colonization through rectal and vaginal cultures collected at 36-37 weeks of gestation. Positive results are then managed with intrapartum antimicrobial prophylaxis (IAP). Penicillin G is the first-choice antibiotic for the treatment of such cases. Alternative drugs, such as clindamycin and cefazolin, are used in pregnant women allergic to penicillin. The majority of GBS isolates remain susceptible to penicillin and other β-lactams; however, resistance to antimicrobial agents used as alternative therapies, primarily lincosamides, has been described previously.[8]

In Brazil, GBS has not been acknowledged as the causative agent of underlying infectious processes affecting newborns and pregnant women owing to failure to isolate the microbe and/or underreporting. This lack of acknowledgement occurs despite the severity of GBS infection and the fact that the population is highly likely to benefit from prophylaxis. In Brazil, there is no recommendation by national health authorities for GBS screening of low-risk pregnant women, which is justified by a shortage of national research substantiating the development of guidelines recommending care provision to GBS carriers.[9]

In the Brazilian Amazon, particularly in the state of Rondônia, there is a lack of epidemiological data on multiple important infectious diseases, including GBS, which is in contrast to the Southeast of Brazil.[6] The Brazilian Amazon covers over 6.3 million km^2 and has a population of around 29.6 million people, and there is substantial variation in many lifestyle factors across the region.[10] There is a pressing need for medical research on topics such as bacteriology in pregnant women in the Amazon. Such research could help to improve medical and social policies affecting both mother and child and thus drive a decrease in child mortality in the Amazon. Given the clinical importance of this pathogenic agent and the lack of research on GBS prevalence in Northern Brazil, this study aimed to assess the prevalence of GBS colonization, and the sociodemographic and clinical characteristics associated with pregnant women attending the Public Healthcare Network in the city of Porto Velho, state of Rondônia, Brazil.

Methods

A prospective, cross-sectional study was conducted from April 2018 to March 2020 in the city of Porto Velho, the capital of the state of Rondônia, which is located in the western part of the Northern Region of Brazil, in the Western Amazon, and has an estimated population of 548.952 people.

The study population comprises pregnant women randomly sampled between 35 to 37 weeks of gestation, according to the 2010 version of the CDC's perinatal GBS guidelines,[11] who were assessed at the Mother and Child Integrative Center, as well as low-risk pregnant women assessed at eleven Basic Healthcare Units in the city of Porto Velho. Pregnant women undergoing treatment with oral or intravaginal antibiotics or at a late delivery stage were excluded from the study. Based on a 20% prevalence of GBS in women and using a sampling error of 5% and

confidence level of 95%, we calculated the optimal sample size as 477 subjects.

Rectal and vaginal secretion samples were collected by healthcare professionals during routine prenatal outpatient visits. Vaginal samples were collected with a sterile swab, which was introduced into the vagina up to its distal third without previous sanitation or use of a speculum. After smooth rotation, each swab was removed and immediately immersed in the Stuart transport medium (CRAL, São Paulo, Brazil). For rectal samples, a sterile swab was introduced into the anal orifice up to the distal wall of the rectum and a smooth rotation movement was used. The swab was then immediately immersed in the transport medium (CRAL). Subsequently, samples were sent to the Microbiology Laboratory of the Oswaldo Cruz Foundation Rondônia and processed within 24 h of collection, as recommended by the Center for Disease Control and Prevention (CDC).[11] Furthermore, data on the age, civil status, ethnicity group, education level, occupation, obstetric history, and sexual activity of each patient, among other variables, were collected through questionnaires.

Swabs were removed from the transport medium and inoculated in tubes filled with 4 mL Todd-Hewitt broth (THB; KASVI, Paraná, Brazil) supplemented with gentamicin (8 µg/mL; Interlab, São Paulo, Brazil) and nalidixic acid (15 µg/mL; Sigma-Aldrich, Missouri, USA). Subsequently, the samples were incubated in 5% CO_2 at 37°C for 24 h. The bacterial DNA was extracted from the culture using the phenol–chloroform method.[12] A further 0.09 mL of broth was transferred to 2 mL cryotubes with 0.06 mL of glycerol (50%; Thermo Fisher Scientific, Massachusetts, USA) and stored in a freezer at -80°C for further bacteriological tests. The in-house GBS PCR was performed according to the method described by Ke *et al*.[13] using *S. agalactiae* ATCC 27956 as a positive control.

Cryopreserved samples were inoculated in THB broth and incubated in 5% CO_2 at 37°C for 24 h. Cultures were then streaked onto Columbia Blood Agar plates (BIOLOG, California, USA) supplemented with 5% defibrinated sheep blood (EBE FARMA, Rio de Janeiro, Brazil) and incubated under the aforementioned conditions.[11] Plates were assessed after 24h to check for the presence of colonies indicative of GBS, namely small (0.5–1 mm) and transparent colonies with discrete beta hemolysis. Colonies presenting these morphological and hemolytic features were subjected to presumptive identification tests for CAMP (*Christie-Atkins-Munch-Peterson*) production, catalase activity, and Gram staining. After the isolation of bacteria, samples were stored in cryotubes and cryopreserved in glycerol at -80°C.[2]

The isolates were tested for susceptibility to the following antimicrobial agents: penicillin G (10µg), ampicillin (10µg), clindamycin (2µg), cefazolin (30µg), ceftriaxone (30µg), erythromycin (15µg), and tetracycline (30µg) (CECON, São Paulo, Brazil) using the Kirby-Bauer disk diffusion method, as recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST). The results were interpreted according to BrCast/EUCAST.[14]

The information from the questionnaires was collated using Excel 2016 (Microsoft, Washington, USA). Descriptive statistics were used to summarize the participants' data. Data were organized in a contingency table and analyzed using Fisher's exact test. P values ≤ 0.05 were considered significant, and 95% confidence intervals were considered.

The study was approved by the Research and Ethics Committee of the Research Center in Tropical Medicine CEP/CEPEM n. 76.812-329.

Results

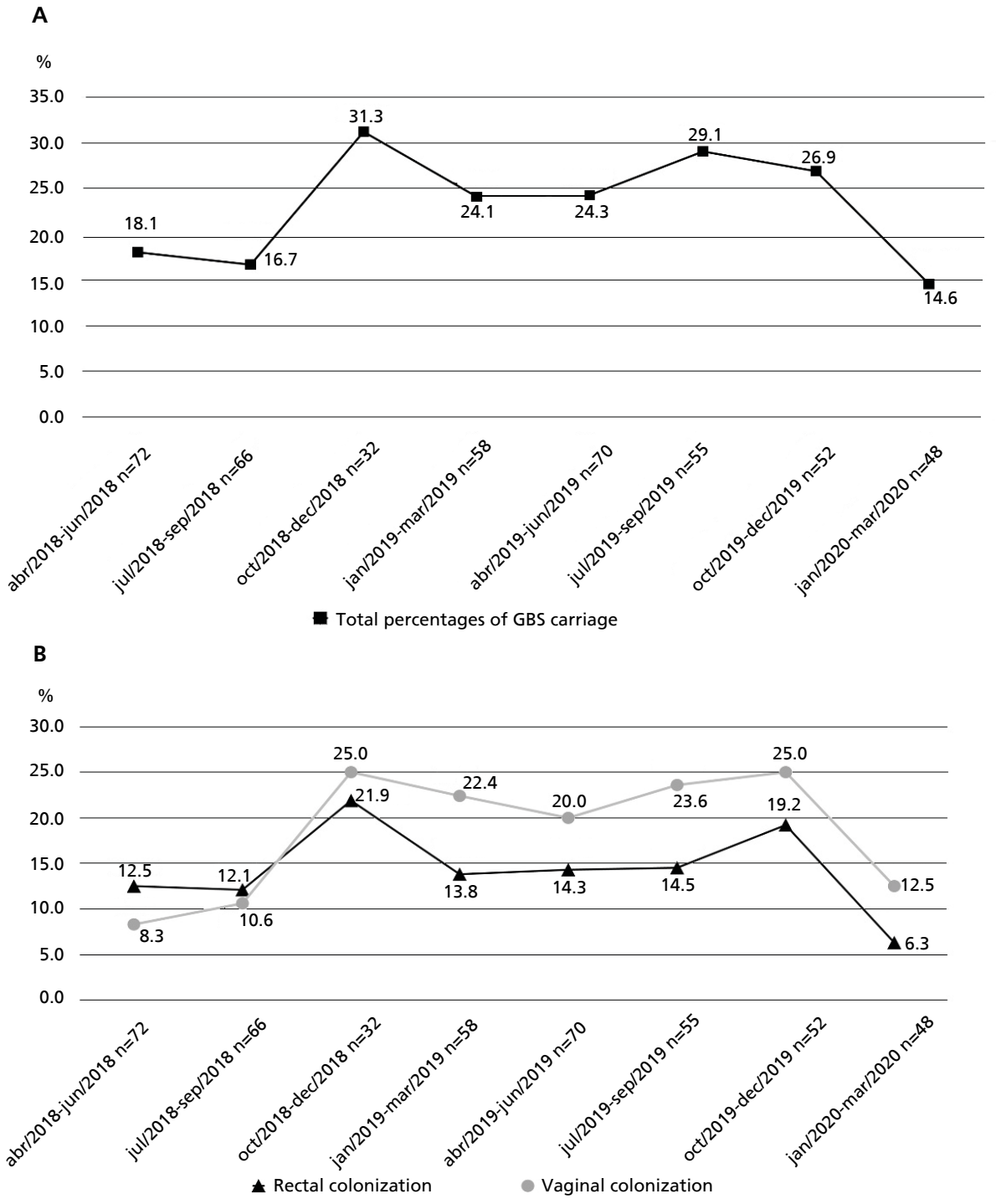
From April 2018 to March of 2020, 453 pregnant women were screened for GBS colonization based on eligibility criteria. The presence of GBS colonization was identified in at least one of the samples (vaginal and/or rectal) in 102 cases, giving a prevalence of 22.5% (CI95%= 19%–26%). No significant fluctuations were observed in colonization rates over the study period (Figure 1A). However, when colonization rate was stratified by the area of collection, we observed a marked growth in the rate of vaginal colonization and a slight decrease in rectal colonization over the study period (Figure 1B).

Table 1 describes the sociodemographic features based on the presence or absence of GBS colonization. Of the 453 screened pregnant women, 54.3% (246/453) were 20–30 years old, 71.7% (325/453) were married, 54.5% (247/453) were housewives, 61.4% (278/453) completed high school, 71.1% (322/453) were self-declared 'brown', and 92.3% (418/453) lived in the urban zone. Rates of colonization by GBS in these pregnant women were as follows: aged 20–30 years (47.1%), married (71.6%), self-declared 'brown' (73.5%), completed high school (62.7%), housewives (47.1%), and living in urban zones (93.1%). However, there were no significant differences in the presence or absence of GBS colonization in patients based on demographic characteristics. Pregnant women younger than 20 years did show a higher tendency for GBS colonization than women in other age groups (odds ratio = 1.648 [CI= 0.9661–2.813]).

The obstetric characteristics assessed in this study are presented in Table 1. In total, 79.4% (81/102) of pregnant women who tested positive for GBS colonization had sexual intercourse during pregnancy, with a reasonable rate of urinary tract infections of 54.9% (56/102) and

Figure 1

Percentages of Group B *Streptococcus* (GBS) carriage among 453 pregnant women living in Porto Velho, Brazil, from April 2018 to March 2020.



The X axis represents the months of collection with the number of samples collected and the Y axis represents the colonization rate; A) Total percentages of GBS carriage (vaginal and/or rectal); B) Percentages of GBS carriage by collection site.

vaginal discharge of 61.8% (63/102). It was also observed that 5.9% (6/102) of these women had preterm delivery in a previous pregnancy, and 4.9% (5/102) reported previous neonatal sepsis. There was no significant difference between the clinical-obstetric variables and GBS colonization. Systemic arterial hypertension was the most prevalent pre-existing syndrome among the investigated patients.

The vaginal area had the highest GBS colonization rate, of 17.6% (80/453 [CI95% = 13.1%–22%]), while

Table 1

Association between sociodemographic factors, obstetric aspects and GBS colonization among pregnant women living in Porto Velho, Brazil, from April 2018 to March 2020.

Characteristics	GBS positive (N=102)		GBS negative (N=351)		*p*
	n	%	n	%	
Age group (years)					
<20	25	24.5	58	16.5	0.0794
20-30	48	47.1	198	56.4	0.1074
30-40	26	25.5	81	23.1	0.5972
>40	0	0.0	4	1.1	0.579
Civil status					
Married	73	71.6	252	71.8	0.8986
Single	2	2.0	5	1.4	0.6597
Divorced	26	25.5	87	24.8	1.0000
Ethnicity group					
White	16	15.7	62	17.7	0.7656
Indigenous	0	0.0	1	0.3	1.0000
Black	10	9.8	38	10.8	0.8565
Brown	75	73.5	247	70.4	0.6158
Education					
Elementary school	28	27.5	75	21.4	0.2852
High school	64	62.7	214	61.0	1.0000
College	10	9.8	52	14.8	0.1943
Occupation					
Autonomous	1	1.0	1	0.3	0.4017
Unemployed	15	14.7	45	12.8	0.6213
Housewife	48	47.1	199	56.7	0.0857
Employed	35	34.3	86	24.5	0.0556
Student	1	0.1	11	3.1	0.3133
Living area					
Peri urban	0	0.0	8	2.3	0.2084
Rural	4	3.9	14	4.0	1.0000
Urban	95	93.1	323	92.0	0.4736
Obstetric aspects					
Sexual intercourse during pregnancy	81	79.4	266	75.8	0.6862
Dysuria	21	20.6	60	17.1	0.5088
UTI	56	54.9	199	56.7	0.9072
Dyspareunia	12	11.8	56	16.0	0.4245
Vaginal Discharge	63	61.8	187	53.3	0.2059
History of premature birth	6	5.9	12	3.4	0.2633
History of neonatal sepsis	5	4.9	27	7.7	0.5067

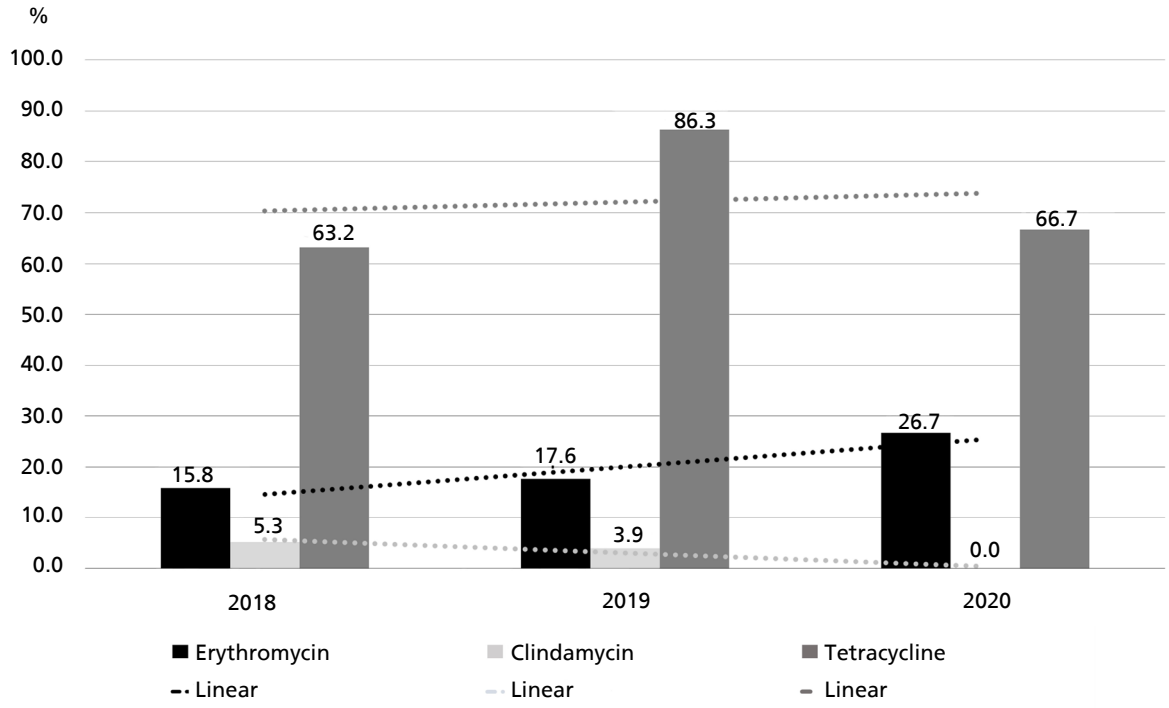
GBS= Group B *Streptococcus*.

the rectal area was colonized in 13.8% (63/453 [CI95%= 10.8%–16.8%]) of pregnant women. Collection site stratification showed that 4.8% (22/453) of pregnant women only tested positive in the rectal area, 8.6% (39/453) only tested positive in the vaginal area, and 9% (41/453) showed colonization at both areas.

Susceptibility to antimicrobial agents was tested in 85 of the viable isolates after cryopreservation in glycerol. A total of 77.9% of the investigated isolates were resistant to at least one of the antimicrobial agents tested, with tetracycline and erythromycin showing the highest percentages of resistance of 74.1% (63/85) and 14.1% (12/85), respectively. The clindamycin resistance rate was 3.5% (3/85), and all isolates were susceptible to penicillin, ampicillin, cefazolin, and ceftriaxone. The level of resistance was evaluated at intervals over the study period. A stable rate of resistance to tetracycline was observed, while the rate of resistance to clindamycin fell and resistance to erythromycin increased considerably (Figure 2). There was no statistical difference in the antimicrobial susceptibility profiles between isolates derived from different anatomical collection areas.

Figure 2

Distribution and tendency of antimicrobial resistant profiles among GBS isolates collected in Porto Velho, Brazil, from April 2018 to March 2020.



GBS= Group B *Streptococcus*.

Discussion

This study represents the first effort to ascertain the prevalence of GBS colonization among pregnant women in Porto Velho, Rondônia, located in the Amazon region of Brazil. Additionally, it evaluates the sociodemographic and clinical characteristics associated with GBS colonization, as well as the susceptibility of the isolated samples to the antimicrobials commonly employed in prophylactic treatment. Research on GBS in pregnant women is particularly significant due to the fact that vaginal and/or rectal colonization by GBS constitutes the primary risk factor for neonatal GBS infection. Newborns, possessing an immature immune system, are consequently more vulnerable to neonatal sepsis, pneumonia, and meningitis caused by GBS.[15]

This study showed a 22.5% prevalence of GBS colonization in women between 35-37 gestational weeks. The rate of GBS colonization determined by previous studies of Brazilian patients was 4.2–28.4%.[6] The prevalence observed in this study was within the upper limit of this range. Brazil is a country of continental proportions and has intrinsic socio-regional differences, which may explain the large range in colonization rates. Another important factor that likely influences this variation is the absence of official recommendations by the Brazilian Unified Health System (SUS – Portuguese acronym) and of universal guidelines for GBS screening.[6,9]

The study indicated that colonization rates for maternal GBS in pregnant women from the Public Healthcare Network of the city of Porto Velho were higher than worldwide estimates (18% [CI95%= 17%–19%]), but remained within regional averages (11%–35%). South Asia and Eastern Asia had the lowest rates of GBS colonization (13% and 11%, respectively). The prevalence in this study was higher than South America's (15.9% [CI95%= 13.5%–18.2%]) and similar to those of Australia and New Zealand (23.3%), North America (23.2%), Northern Europe (22.2%), Eastern Europe (23%), and Northern Africa (22.9%).[1]

The colonization rate varies depending on geographic area, genetic differences in host response, sampling methodologies, and the processing protocol adopted for the screening procedure. Moreover, collection time during pregnancy, the use (or not) of enriched selective culture media, and whether the identification methodology is based on serology, molecular biology, or presumptive tests may also contribute to the variability reported in previous studies.[15]

In addition to the general prevalence of colonization, we also evaluated the differences in GBS load in pregnant women in relation to areas of collection and the time of study. No significant fluctuations were observed in

the general colonization rates over the study period, as has previously been observed in Brazil.[16] Unlike most previous studies, which used a combined swab technique, this study used separate swabs to determine colonization in different anatomical locations, allowing rates to be stratified according to collection site. A higher rate of GBS colonization was observed in the vaginal region, with a marked growth trend over the study period. These data indicate a greater affinity of GBS to the vaginal region, which supports the findings that GBS has numerous surface adhesins and invasins that interact directly with the vaginal epithelium and promote persistent colonization in this niche.[17] Previous studies have disagreed on the superiority of vaginal colonization compared with rectal colonization, with this divergence influenced by the characteristics of the study population itself as well as the identification method used, cited above.[18,19]

The USA CDC recommends universal screening using culture-based methods and subsequent treatment with IAP where colonization is detected. An alternative approach uses risk factors to make decisions about IAP treatment when the colonization status is unknown.[11] Currently, there is no international consensus on which of these two approaches is more effective; however, numerous studies have shown better prophylactic coverage of neonates susceptible to GBS disease using culture screening.[7,20,21] A previous study showed a significant drop in the risk of developing early onset disease among newborns from screened pregnant women compared with that observed in those treated according to risk factors.[11] A study conducted in Rio de Janeiro, Brazil, reported that 14% of women known to be colonized with GBS using the culture-based approach would have been excluded from the IAP recommendation if only risk factors had been considered.[16]

Currently, Brazil does not adopt systematic and standardized universal screening for pregnant women despite global evidence of its efficacy. CDC in the US has shown an 80% decrease in early onset disease as a result of prophylaxis treatment in pregnant women.[11] In countries where there is no recommendation for IAP, there is a 1.1% likelihood of early onset disease development due to GBS colonization in pregnant women, whereas in countries that adopt prophylaxis the risk falls to 0.03%.[22] However, IAP has not affected the disease caused by GBS before childbirth or late onset disease, and there are concerns about effects on the composition of the neonatal and maternal microbiome through selective pressure and the development of antimicrobial resistance.[15,23] As evidence suggests, until more preventative strategies are available, such as a maternal GBS vaccine, universal culture-based screening in conjunction with IAP remains the most effective protocol to prevent neonatal GBS disease.[2,5]

In this study, sociodemographic and obstetric factors were not found to be significantly associated with GBS colonization, indicating a homogeneous distribution of GBS colonization in pregnant women in the region, as observed in previous studies conducted in Brazil and other countries.[24,25] Sociodemographic and obstetric factors may increase the likelihood of GBS colonization. Previous studies demonstrated that certain ethnic groups, ages, and specific obstetric conditions are at a greater risk of GBS colonization, as well as associated risk factors for developing early onset disease and strains with hypervirulent profiles.[2,16,26] We identified a trend towards a higher rate of colonization (though not statistically significant) in pregnant women under 20 years of age. Thus, our results demonstrate the need for universal screening of pregnant women owing to the homogeneous distribution of GBS colonization across widely varying population characteristics. This highlights the importance of continuous surveillance for GBS in the region, since the characteristics associated with colonization may change over time.

Although IAP is the main defense against early GBS infection, non-susceptibility to the antimicrobial agents commonly used in prophylaxis has been reported. Our results showed that 14.1% of the isolates were resistant to erythromycin and that the resistance rate increased over the study period, while 3.5% were resistant to clindamycin. Barros[27] highlighted that the non-susceptibility rate to clindamycin ranged from 1.9 to 18.8% and to erythromycin ranged from 4 to 25% in Brazilian studies carried out in recent decades, with a significant increase after 2010. It is noteworthy that although the degree of susceptibility to erythromycin is commonly assessed in GBS, this antimicrobial is no longer used as an option for IAP due to its pharmacokinetic properties and increasing resistance.[27,28] These temporal trends in antimicrobial susceptibility show the need for continued surveillance of resistance rates to assess whether or not these drugs can be used in IAP in the local context.

The antimicrobial agent with the highest rate of resistance observed in the present study was tetracycline, which is not recommended for use in IAP; however, its resistance levels are being monitored. Other studies carried out in Brazil have also reported high rates of resistance to this antimicrobial agent.[16,27] Additionally, high rates of resistance (>70%) have been reported in other countries.[25,29]

All GBS samples evaluated were susceptible to the first-choice antimicrobial agents penicillin and ampicillin, as well as to cephalosporins. Despite the reports of isolates with decreased susceptibility to penicillin in other countries, there are no reports of β-lactam-resistant GBS in Brazil so far, indicating that the first-line antibiotics recommended for IAP remain good options for the prevention of GBS neonatal disease.[24,27,30] However, this

information may be underreported because penicillin-reduced susceptibility cannot be detected by agar diffusion methods, but only by minimum inhibitory concentration (MIC) tests.[27]

As a limitation of the present study, results relating to antimicrobial susceptibility profiling were obtained from 83.3% (85/102) of the isolates recovered from pregnant women. The preliminary identification and the isolation from clinical samples were not performed simultaneously with the characterization of the isolates, therefore some GBS strains were lost during storage period, situation already described.[16] Despite the high sensitivity and specificity of the PCR assay used to identify GBS, the lack of culture confirmation of all PCR results (both positive and negative) and the non-use of MIC to determine reduced penicillin susceptibility are also recognized as potential limitations of the study.

The findings from this study emphasize the significance of identifying maternal colonization by GBS in the local area as a means of decreasing instances of neonatal infection caused by these bacterial pathogens, due to their prevalence in the region. Furthermore, studies that continue to advance the understanding of epidemiological factors, virulence, and antimicrobial susceptibility of GBS isolates from pregnant women and neonates from the Amazon States of Brazil are crucial for developing surveillance and prevention techniques that target GBS.

This is the first study to investigate GBS colonization in this region. This study revealed a prevalence of GBS colonization in pregnant women in the Public Healthcare Network in Porto Velho, Rondônia, using both microbiological and molecular methodologies. There was no association between sociodemographic and clinical characteristics and colonization, which supports the need for uniform examinations in all pregnant women between the 36th and 37th gestational weeks. Moreover, though the isolates had a high rate of resistance to tetracycline and erythromycin, they also showed high susceptibility to the first-line antimicrobials used in prophylaxis. Considering that Brazil does not adopt systematic and standardized universal screening, despite global evidence of its efficacy, this study provides crucial data for the design of strategies to prevent invasive GBS infection and, consequently, minimize the mortality and morbidity caused by this pathogen in Brazil.

Author's contribution

Carvalho AG: methodology, formal analysis and investigation, writing - original draft preparation, writing of the final version of the manuscript.

Rodrigues RS: writing - original draft preparation, writing of the final version of the manuscript.

Rodrigues MD: conceptualization, methodology, writing - review and editing; writing of the final version of the manuscript.

Oliveira LP, Ricarte MJVG, Dorneles NWS, Lima NCS: methodology, writing of the final version of the manuscript.

Belem MGL: methodology, formal analysis and investigation, writing of the final version of the manuscript.

Rocha PRDA, Pinto TCA: writing - review and editing, writing of the final version of the manuscript.

Lima CM: conceptualization, writing of the final version of the manuscript.

Watanabe M: formal analysis and investigation, writing of the final version of the manuscript.

Taborda RLT: conceptualization, methodology, formal analysis and investigation, funding acquisition, supervision, writing of the final version of the manuscript.

Matos NB: conceptualization, methodology, formal analysis and investigation, writing - review and editing, funding acquisition, supervision, writing of the final version of the manuscript.

The authors approved the final version of the article and declare no conflict of interest.

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Received on February 27, 2023
Final version presented on June 4, 2024
Approved on June 19, 2024

Associated Editor: Leila Katz