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Serotype distribution, virulence factors, and antimicrobial resistance profiles of *Streptococcus agalactiae* (Group B *Streptococcus*) isolated from pregnant women in the Brazilian Amazon

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Abstract

Background *Streptococcus agalactiae* (Group B *Streptococcus*, GBS) is a leading cause of neonatal infections, exhibiting remarkable adaptability. This study aimed to characterize the virulence and resistance profiles of GBS isolates obtained from pregnant women in Porto Velho, Rondônia, within the Brazilian Amazon. GBS strains were isolated and identified from rectovaginal cultures using phenotypic and genotypic methods. Capsule typing and the detection of virulence and resistance genes were performed through polymerase chain reaction (PCR). Antimicrobial susceptibility profiles and in vitro biofilm formation were also assessed.

Results A total of 85 GBS strains were characterized. The most prevalent serotypes were Ia (36.5%) and V (15.3%), followed by Ib (14.1%), II (14.1%), III (11.8%), and VI (8.2%). A high prevalence of virulence genes was observed, including *scpB* (100%), *lmb* (97.6%), *bca* (84.7%), *hylB* (64.7%), *cylE* (60%), *bac* (57.6%), and *hvgA* (11.8%). All strains were susceptible to penicillin, ampicillin, cefazolin, ceftriaxone, and vancomycin. Non-susceptibility was identified for tetracycline (77.6%), erythromycin (18.9%), chloramphenicol (4.7%), clindamycin (3.5%), and levofloxacin (1.2%). Among the 17 strains non-susceptible to erythromycin and/or clindamycin, the detected phenotypes included M (82.4%), $cMLS_B$ (11.8%), and L (5.9%), with associated resistance genes *erm(A)* (5.9%), *erm(B)* (11.8%), and *mef(A/E)* (64.7%). Additionally, over 90% of the strains demonstrated strong biofilm formation capacity.

Conclusions This is the first characterization of GBS in this region, revealing notable virulence and high susceptibility to first-line antibiotics. Six serotypes were identified, including Brazil's second report of serotype VI, emphasizing the genetic and epidemiological diversity of the Amazonian region. Moreover, given that the serotype distribution in this population corresponds to the most prevalent types worldwide, the hexavalent GBS conjugate vaccine could

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represent a promising strategy for this population, as it would theoretically cover 91.8% of the analyzed strains, if proven effective and made available in Brazil.

Keywords Maternal and child health, Antimicrobial resistance, Neonatal infections, Capsule typing, Epidemiological surveillance

Background

Streptococcus agalactiae, commonly referred to as Group B *Streptococcus* (GBS), is a leading etiological agent of neonatal infections. This microorganism asymptomatically colonizes the human gastrointestinal and genitourinary tracts. Rectovaginal GBS colonization during late pregnancy constitutes a significant risk factor for the development of neonatal infections. GBS infections are categorized into early-onset disease (EOD), which occurs within the first 7 days of life, and late-onset disease (LOD), manifesting between 8 and 90 days of age [1].

Early-onset disease is primarily associated with vertical transmission, wherein GBS ascends through the vaginal tract before or during delivery, resulting in neonatal colonization. The most common clinical presentations of EOD are sepsis and pneumonia. Conversely, late-onset disease can arise from maternal sources or other exposures, such as nosocomial or environmental transmission [2]. Meningitis is the predominant clinical manifestation of LOD, with 32–44% of surviving neonates suffering from cognitive or neurological sequelae. The World Health Organization (WHO) estimates that GBS causes 518,000 preterm births, 392,000 neonatal infections, and 91,000 neonatal deaths annually [3].

The pathogenicity of GBS is driven by its virulence mechanisms, with the sialic acid-rich polysaccharide capsule (CPS) being its primary factor for persistence and survival within the host. Based on the variable composition of CPS, GBS is classified into ten serotypes (Ia, Ib, and II–IX), which differ in prevalence, geographic distribution, and virulence [4]. Additionally, GBS expresses several surface-anchored structures that facilitate adhesion, invasion of host tissues, and immune evasion. These include laminin-binding protein (Lmb), C5a peptidase (ScpB), protein C antigens α and β (Bca and Bac), and hypervirulent GBS adhesin (HvgA). The pathogen also produces β-hemolysin (CylE) and hyaluronidase (HylB), which further enhance its virulence [1, 4]. Another critical factor contributing to GBS pathogenicity is its ability to form biofilms, which promote colonization and persistence within the host [5].

Currently, in many countries, screening for GBS colonization in pregnant women, combined with intrapartum antimicrobial prophylaxis (IAP), constitutes the primary strategy for preventing neonatal GBS infections. However, some nations, such as the United Kingdom, adopt risk-based approaches rather than universal screening. Despite these differences in prevention strategies, there is growing concern about increasing rates of antimicrobial resistance [6]. While GBS remains highly susceptible to first-line β-lactam antibiotics, resistance to macrolides and lincosamides has been reported globally. This rising resistance has led the Centers for Disease Control and Prevention (CDC) to classify GBS as a pathogen with a concerning resistance threat. Resistance in GBS is mediated by methylation of the ribosomal binding site (*erm* genes) or efflux pumps (*mef* gene), resulting in various resistance phenotypes [7, 8].

Although the early detection of GBS is critical for preventing neonatal infections, GBS is not widely recognized as a significant etiological agent of neonatal diseases in Brazil. This has resulted in substantial gaps in the national understanding of the pathogen. Brazil lacks a standardized protocol for GBS screening or the administration of IAP for pregnant women [9]. The state of Rondônia, located in the Brazilian Amazon and the site of this study, consistently reports one of the highest infant mortality rates in Brazil [10]. According to national health records, neonatal bacterial septicemia has been the leading cause of neonatal deaths in the region over the past ten years. However, local surveillance systems do not specify the etiological agents involved, which limits targeted interventions and hinders the development of effective public health strategies [11]. Given the global recognition of GBS as a major cause of early-onset neonatal sepsis, it is plausible that GBS contributes significantly to the burden of neonatal morbidity and mortality in Rondônia [1, 2]. The absence of local data on maternal colonization and circulating GBS strains represents a critical knowledge gap—particularly in underserved regions such as the Amazon.

In this context, the present study aimed to characterize GBS strains isolated from pregnant women receiving care through the public health system in Porto Velho, the capital of Rondônia. This characterization included species identification, capsular typing, and the detection of virulence and antimicrobial resistance factors—key elements that can support future surveillance efforts and inform prevention strategies such as maternal screening and vaccination.

Methods

Bacterial strains

This study analyzed *Streptococcus agalactiae* strains cryopreserved at -80 °C in the Microbiology Laboratory of the Oswaldo Cruz Foundation Rondônia (FIOCRUZ-RO).

These strains were obtained from rectal and vaginal samples of pregnant women between 35 and 37 weeks of gestation who attended primary healthcare facilities in Porto Velho, Rondônia, Brazil, from April 2018 to March 2021. Detection of GBS in clinical samples was performed via PCR amplification of the *cfb* gene, which encodes the CAMP factor [12].

Microbiological isolation

Cryopreserved strains were reactivated in Todd Hewitt broth (THB; Sigma-Aldrich, Missouri, USA) supplemented with gentamicin (8 µg/mL; Interlab, São Paulo, Brazil) and nalidixic acid (15 µg/mL; Sigma-Aldrich, Missouri, USA). After incubation at 37 °C with 5% CO_2 for 24 h, the cultures were streaked onto Columbia Agar supplemented with 5% defibrinated sheep blood (EBE FARMA, Rio de Janeiro, Brazil). Plates were incubated for 24 h, and colonies suggestive of GBS were analyzed. Colony morphology, Gram staining, and catalase assays were utilized for presumptive identification.

Molecular identification

Genomic DNA was extracted using the phenol-chloroform method [13]. Amplification of the 16 S rRNA gene was carried out to confirm bacterial species identification [14]. The amplicons were purified using the QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany), following the manufacturer's instructions. After purification, DNA concentration and purity were measured using a NanoDrop 1000 Spectrophotometer (Thermo Scientific, Waltham, Massachusetts, USA). Sequencing was performed using an ABI 3100 Prism automated DNA sequencer (Applied Biosystems, series 1592-015). Consensus sequences were analyzed with BioEdit software (v7.0) and aligned with the BLAST database for species confirmation. The DNA sequences generated in this study have been deposited in the NIH GenBank database under accession numbers PV111954–PV112038 and are accessible at: https://www.ncbi.nlm.nih.gov/nuccore/?term=PV111954:PV112038accn.

Capsular typing

Capsular typing was performed using a multiplex PCR assay to identify serotypes Ia, Ib, and II–IX, following previously described methods [15]. Positive controls for the most prevalent serotypes in Brazil (Ia, Ib, II, III, IV, V, and VI) were included.

Detection of virulence genes

Virulence determinants were identified via PCR using primers targeting *hylB* (hyaluronidase protein), *cylE* (beta-hemolysin/cytolysin) [16], *lmb* (laminin-binding protein) [17], *bac* and *bca* (subunits of protein C), and *scpB* (fibronectin-binding protease) [18]. The *hvgA* gene, which encodes hypervirulent adhesin, was evaluated exclusively in serotype III strains [19].

Phenotypic profile of antimicrobial susceptibility

Antimicrobial susceptibility was assessed using the disk diffusion method on Müeller-Hinton blood agar (Kasvi, Paraná, Brazil). Tested antibiotics included ampicillin (10 µg), cefazolin (30 µg), ceftriaxone (30 µg), clindamycin (2 µg), chloramphenicol (30 µg), erythromycin (15 µg), levofloxacin (5 µg), penicillin (10 µg), tetracycline (30 µg), and vancomycin (30 µg) (all obtained from CECON, São Paulo, Brazil). Interpretations were based on the Clinical Laboratory Standards Institute (CLSI) guidelines [20].

Detection of macrolide-lincosamide-streptogramin resistance phenotypes

Inducible clindamycin resistance was evaluated using the double-disk diffusion test (D-test). Erythromycin (15 µg) and clindamycin (2 µg) disks were placed 12–16 mm apart on the agar surface. Plates were incubated at 35 °C for 16–18 h. The presence of a "D"-shaped inhibition zone around the clindamycin disk, adjacent to the erythromycin disk, was interpreted as indicative of inducible resistance ($iMLS_B$). Strains resistant to both erythromycin and clindamycin were classified as having a constitutive resistance phenotype ($cMLS_B$). The M phenotype was assigned to strains resistant to erythromycin but susceptible to clindamycin, while the L phenotype was identified in strains resistant only to clindamycin [20].

Detection of antimicrobial resistance genes

The presence of resistance genes (*ermA*, *ermB*, *mefA/E*) was detected via PCR in strains exhibiting non-sensitivity to clindamycin and/or erythromycin [21–23].

Biofilm production assays

Biofilm production was quantified following established protocols [24]. Each strain was tested in triplicate across three independent biological replicates, ensuring reproducibility of the results. Assays were conducted alongside positive controls (*Streptococcus agalactiae* ATCC 25956, *Escherichia coli* EAEC 042, and *Pseudomonas aeruginosa* ATCC 27853) and a negative control (sterile Todd-Hewitt Broth). Biofilm formation was assessed by measuring the mean optical density at 570 nm (OD_{570}), and the classification of isolates was based on a calculated cutoff value, as previously described [24].

Statistical analysis

Categorical variables were analyzed using Fisher's exact test at a 95% significance level. A p-value ≤ 0.05 was considered statistically significant. Statistical analyses were performed using GraphPad Prism 7.

Table 1 Distribution of virulence genes among the GBS serotype strains analyzed

	Serotype						
	Ia	**Ib**	**II**	**III**	**V**	**VI**	**Total**
	31 (36.5%)	12 (14.1%)	12 (14.1%)	10 (11.8%)	13 (15.3%)	7 (8.2%)	85 (100%)
bac	17 (54.8%)	9 (75.0%)	7 (58.3%)	4 (40.0%)	9 (69.2%)	3 (42.9%)	49 (57.6%)
bca	23 (74.2%)	12 (100%)	10 (83.3%)	8 (80%)	12 (92.3%)	7 (100%)	72 (84.7%)
lmb	29 (93.5%)	12 (100%)	12 (100%)	10 (100%)	13 (100%)	7 (100%)	83 (97.6%)
scpB	31 (100%)	12 (100%)	12 (100%)	10 (100%)	13 (100%)	7 (100%)	85 (100%)
hylB	18 (58.1%)	9 (75%)	7 (58.3%)	8 (80%)	8 (61.5%)	5 (71.4%)	55 (64.7%)
clyE	16 (51.6%)	10 (83.3%)	6 (50.0%)	7 (70%)	9 (69.2%)	3 (42.9%)	51 (60%)
hvgA	-	-	-	10 (100%)	-	-	10 (11.8%)

Table 2 Prevalence and profiles of virulence genes evaluated in GBS strains

	Virulence genes						
Profiles	**bac** **49 (57.6%)**	**bca** **72 (84.7%)**	**lmb** **83 (97.6%)**	**scpB** **85 (100%)**	**hylB** **55 (64.7%)**	**cylE** **51 (60%)**	***N*(%)**
1	+	+	+	+	+	+	24 (28.2%)
2	-	+	+	+	+	+	17 (20%)
3	+	+	+	+	-	-	16 (18.8%)
4	+	+	+	+	+	-	2 (2.4%)
5	-	+	+	+	+	-	6 (7.1%)
6	-	+	+	+	-	-	3 (3.5%)
7	-	-	+	+	+	+	6 (7.1%)
8	+	-	-	+	+	+	2 (2.4%)
9	+	+	+	+	-	+	2 (2.4%)
10	+	-	+	+	-	+	2 (2.4%)
11	-	-	+	+	-	-	2 (2.4%)
12	+	-	+	+	-	-	3 (3.5%)
Total							85 (100%)

Subtitle: (+) Positive for the gene, (-) Negative for the gene

Results

From the 496 pregnant women included in the study, 96 were detected as GBS-positive by PCR targeting the *cfb* gene, and 85 (17.1%) GBS strains were isolated and identified from the selective broth and agar plating. These strains were confirmed as *S. agalactiae* through 16 S rRNA gene sequencing and subsequently subjected to both phenotypic and genotypic characterization.

Capsular typing

Serotype Ia was the most prevalent, accounting for 36.5% (31/85) of the strains, followed by serotype V at 15.3% (13/85). Serotypes Ib and II each represented 14.1% (12/85) of the strains, while serotypes III and VI were identified in 11.8% (10/85) and 8.2% (7/85) of the strains, respectively (Table 1).

Virulence profile

A high frequency of virulence genes was observed among the GBS isolates: *scpB* (100%), *lmb* (97.6%), *bca* (84.7%), *hylB* (64.7%), *clyE* (60%), and *bac* (57.6%). The distribution of these genes across serotypes is detailed in Table 2. No statistically significant associations were found between virulence genes and serotypes. All serotype III strains carried the *hvgA* gene, classifying them as hypervirulent.

Based on the presence of virulence genes, the strains were categorized into 12 profiles (Table 2). Among these, 28.2% (24/85) of the strains contained all the evaluated genes, and every strain harbored at least two of the investigated genes.

Antimicrobial susceptibility profile

A total of 75.3% (64/85) of the isolates were non-susceptible to at least one of the tested antimicrobials. Tetracycline resistance was observed in 77.6% of isolates (66/85), followed by erythromycin (18.9%, 16/85), chloramphenicol (4.7%, 4/85), clindamycin (3.5%, 3/85), and levofloxacin (1.2%, 1/85). Additionally, 2.4% (2/85) of the strains were classified as multidrug-resistant (MDR), exhibiting resistance to tetracycline, erythromycin, and clindamycin.

All isolates were susceptible to penicillin, ampicillin, cefazolin, ceftriaxone, and vancomycin. Resistance to tetracycline and erythromycin was significantly associated with serotype Ia ($p = 0.0004$ and $p < 0.0001$, respectively). Conversely, serotypes V and VI demonstrated lower

Table 3 Distribution of antimicrobial resistance and resistance genes among the GBS serotype strains analyzed

	Serotype						
	Ia	**Ib**	**II**	**III**	**V**	**VI**	**Total**
	31 (36.5%)	12 (14.1%)	12 (14.1%)	10 (11.8%)	13 (15.3%)	7 (8.2%)	85 (100%)
Antimicrobial non-susceptibility							
Tetracycline	31 (100%)*	11 (91.7%)	8 (66.7%)	9 (90%)	6 (46.2%)**	1 (14.3%)***	66 (77.6%)
Erythromycin	12 (38.7%)****	-	2 (16.7%)	-	1 (7.7%)	1 (14.3%)	16 (18.9%)
Clindamycin	-	-	-	-	2 (15.4%)	1 (14.3%)	3 (3.5%)
Chloramphenicol	2 (6.5%)	1 (8.3%)	1 (8.3%)	-	-	-	4 (4.7%)
Levofloxacin	-	1 (8.3%)	-	-	-	-	1 (1.2%)
Resistance genes							
erm(A)	-	-	-	-	1 (7.7%)	-	1 (1.2%)
erm(B)	-	-	-	-	1 (7.7%)	1 (14.3%)	2 (2.4%)
mef(A/E)	10 (32.3%)	-	-	-	1 (7.7%)	-	11 (12.9%)

Subtitle: Statistical significance by the chi-square test. * $p < 0.0001$; ** $p = 0.0049$; *** $p = 0.0005$; **** $p = 0.0004$

Table 4 Prevalence and resistance gene profiles identified in GBS strains

	Resistance genes			
Profiles	***erm(A)*** **1 (5.9%)**	***erm(B)*** **2 (11.8%)**	***mef(A/E)*** **11 (64.7%)**	***N*(%)**
1	-	+	+	1 (5.9%)
2	+	-	-	1 (5.9%)
3	-	+	-	1 (5.9%)
4	-	-	+	10 (58.8%)
5	-	-	-	4 (23.5%)
				17 (100%)

Subtitle: (+) Positive for the gene, (-) Negative for the gene

Table 5 Relationship between resistance phenotypes to macrolides and Lincosamides and the associated resistance genes

Resistance	***n* (%)**	**Resistance phenotypes to macrolide-lincosamide**	**Resistance genes**
Erythromycin/ Clindamycin	2 (11.8%)	$cMLS_B$	*erm(B)* (1) *erm(B)* + *mef(A/E)* (1)
Erythromycin	14 (82.4%)	M	*mef(A/E)* (10) non-gene (4)
Clindamycin	1 (5.9%)	L	*erm(A)* (1)

resistance rates to tetracycline ($p = 0.0049$ and $p = 0.0005$, respectively) (Table 3).

MLS_B phenotypes

Among all strains, 20% (17/85) were non-susceptible to erythromycin and/or clindamycin. Of these, 82.4% (14/17) exhibited the M phenotype, 11.8% (2/17) displayed the constitutive clindamycin-resistant phenotype ($cMLS_B$), and 5.9% (1/17) showed the L phenotype. The inducible resistance phenotype ($iMLS_B$) was not observed.

Resistance gene profiles

Among strains non-susceptible to erythromycin and/or clindamycin, 5.9% (1/17) carried the *erm(A)* gene, 11.8% (2/17) carried *erm(B)*, and 64.7% (11/17) carried *mef(A/E)*. No resistance genes were detected in 23.5% (4/17) of these strains. Table 4 summarizes the five resistance profiles, while Table 5 details the macrolide- and lincosamide-resistant phenotypes and their associated resistance genes.

Biofilm production

A total of 91.8% (78/85) of the strains demonstrated strong or moderate biofilm-forming ability, while 8.2% (7/85) were weak or non-producers. Among the serotypes, all strains of serotype VI (100%, 7/7) exhibited strong or moderate biofilm formation. Serotype III displayed the lowest percentage of biofilm producers (80%, 8/10). There were no statistically significant differences in biofilm production (strong/moderate) among serotypes (Fig. 1). However, strong and moderate biofilm production was significantly associated with the presence of the *hylB* and *clyE* genes ($p = 0.0070$ and $p = 0.0150$, respectively).

Discussion

GBS is a significant pathogen associated with neonatal infections. Colonization of the rectovaginal site by GBS is a major risk factor for the development of these pathologies [1]. Consequently, substantial efforts are underway to characterize isolates from colonized pregnant women globally. Brazil, with its vast size, exhibits diverse climatic and socioeconomic conditions across different regions. The Brazilian Amazon, in particular, lacks sufficient data on GBS compared to the southern and southeastern regions. This study represents the first extensive characterization of the phenotypic and genotypic profiles of GBS strains from colonized pregnant women in an Amazonian city in Brazil.

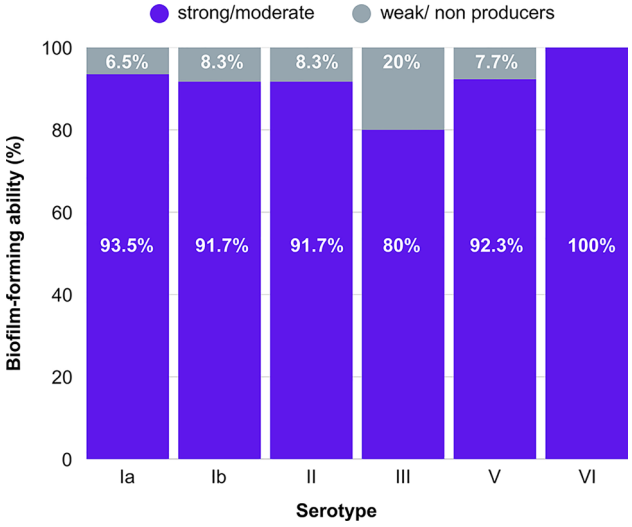

Fig. 1 Distribution of biofilm-forming ability among the GBS serotype strains analyzed

Capsular typing of GBS has been widely used to understand the prevalence of maternal colonization and neonatal disease, as well as the potential impact of a maternal GBS vaccine [25]. Globally, serotypes Ia, Ib, II, III, IV, and V account for 98% and 97% of maternal colonization and neonatal diseases, respectively. The remaining serotypes (VI, VII, VIII, and IX) are predominantly found in South, Southeast, and East Asia [26].

This study identified six different serotypes, with Ia and V being the most prevalent, followed by Ib, II, III, and VI. CPS Ia, which is widely distributed globally, is the second most prevalent serotype associated with neonatal disease. In South America, CPS Ia predominates over CPS III in EOD cases. Other Brazilian studies have similarly identified CPS Ia as the most common type. An increasing prevalence of CPS V has been observed in Brazil, warranting attention due to its association with invasive diseases in adults, pregnant women, and neonates. CPS V strains are also more prone to acquiring exogenous DNA, contributing to greater genetic and antigenic diversity [26–31].

Serotype VI has a more restricted distribution and is more frequently reported in Asian countries such as Japan and Malaysia [26, 31]. In Brazil, its occurrence is extremely limited, having been reported for the first time in 2017 [29]. The present study therefore represents the second report of serotype VI in the country. This finding is noteworthy and may be associated with multiple epidemiological and biogeographic factors. Such evidence suggests either a recent introduction or underreporting of this serotype in Brazilian populations and raises the hypothesis of a still poorly explored regional distribution. Its presence in this region may have been historically overlooked due to the scarcity of local studies on the capsular typing of GBS [32].

Additionally, the detection of this rare serotype in the Amazon may reflect the influence of international travel, human migration, or the importation of strains carrying capsular type VI—a phenomenon previously observed in GBS strains isolated from tilapia [33]. Another plausible explanation is capsular switching, a phenomenon mediated by horizontal gene transfer in which recombination events lead to the replacement of the capsular polysaccharide synthesis (*cps*) operon. This mechanism has been documented in GBS and can lead to the emergence of new serotype profiles within clonal backgrounds, driven by selective pressures such as host immunity. The possibility that a strain originally belonging to a different serotype may have acquired the *cps* genes encoding serotype VI through horizontal transfer underscores the dynamic nature of the GBS population structure [34–36]. In light of this, further investigations involving whole-genome sequencing and phylogenomic analysis will be essential to elucidate the origin, dissemination pathways, and genetic context of serotype VI isolates identified in the Amazon region.

Understanding serotype distribution is essential for evaluating the theoretical impact of multivalent vaccines. Among the advanced vaccine proposals, the hexavalent GBS conjugate vaccine (GBS6), targeting serotypes Ia, Ib, II, III, IV, and V, stands out. Based on the serotypes identified in this study, GBS6 would theoretically cover 91.8% of the analyzed strains, highlighting its potential effectiveness in this context. Despite the possibility of capsular substitution—similar to what has been observed following the introduction of pneumococcal conjugate vaccines—vaccination of pregnant women remains the most comprehensive and promising strategy for preventing neonatal GBS disease [37].

Another widely used tool for GBS characterization is the detection of virulence factors. This study revealed a high prevalence of these genetic determinants, identifying 12 distinct profiles. No associations were found between specific genes and serotypes, indicating a widespread distribution of these virulence factors in strains from this region, consistent with previous findings [27, 38].

The *scpB* and *lmb* genes were present in nearly all strains. The *scpB* gene encodes a C5a peptidase that facilitates immune evasion and fibronectin binding, while the *lmb* gene promotes laminin binding, enabling GBS dissemination within the host [1, 4]. Both genes were observed in colonizing and invasive human strains at nearly 100% prevalence rates [39, 40]. Approximately half of the strains harbored the hyaluronidase (*hylB*) and hemolysin/cytolysin (*cylE*) genes, which contribute to colonization and infection by neutralizing immune

factors [41]. However, global studies have reported higher prevalence rates (> 80%) for these determinants [16–18, 27, 42].

The presence of *bac* and *bca* genes was also evaluated. The *bac* gene encodes a protein that binds to human immunoglobulin A, playing a crucial role in resistance to mucosal immune defenses, while the *bca* gene modulates cervical epithelial cell invasion [43]. Prevalence rates for these genes vary widely in the literature [17, 42]. In contrast to this study, other investigations have reported associations between these genes and specific serotypes [42, 44].

Sequence type (ST) 17 of GBS serotype III is classified as hypervirulent due to its heightened ability to cause neonatal invasive diseases, particularly meningitis [45]. All serotype III strains in this study carried the *hvgA* gene, an adhesin exclusively encoded by ST17, and were considered hypervirulent [46, 47]. This finding is noteworthy, as most prior studies have primarily identified ST17/*hvgA* + in invasive strains [45, 48].

Bacterial biofilms are a significant virulence factor, playing a crucial role in immune system evasion, colonization, and infection. Most strains in this study demonstrated a high capacity for biofilm formation. Consistent with previous findings, no association was observed between capsule type and collection site [24, 44]. Brazilian studies have reported biofilm formation rates exceeding 60%, whereas significantly lower rates have been documented in Asia [49, 50].

Regarding antimicrobial susceptibility, all samples were sensitive to penicillin, ampicillin, cefazolin, ceftriaxone, and vancomycin. No GBS strains resistant to β-lactams or glycopeptides have been reported in Brazil [51]. However, reports of reduced susceptibility to β-lactams in countries such as the USA, Canada, Japan, Korea, Italy, and Kenya highlight the importance of ongoing national monitoring [52, 53]. Reports of vancomycin resistance remain rare [53]. Consistent with this study, high sensitivity to chloramphenicol and levofloxacin has been observed [27, 54, 55].

Tetracycline exhibited the highest resistance rate, consistent with national and international studies [16, 28, 56, 57]. Erythromycin demonstrated considerable non-sensitivity, significantly associated with serotype Ia, while clindamycin remained highly sensitive. Brazilian studies have reported non-sensitivity rates ranging from 4 to 25% for erythromycin and 1.9–18.8% for clindamycin, which are lower than the rates observed in the USA, Europe, and China [7, 51].

Regarding macrolide-lincosamide-streptogramin B (MLS_B) resistance phenotypes, the M phenotype was the most common, followed by $cMLS_B$ and L phenotypes. These findings align with previous investigations, which also reported the $iMLS_B$ phenotype—not observed in this study [27, 40]. The L phenotype, an uncommon resistance mechanism to lincosamides, involves enzymatic drug inactivation via nucleotidyltransferases encoded by *lnu* genes or efflux pumps mediated by the *lsa* gene [53, 58]. Two Brazilian studies reported this phenotype, with only one investigating genetic determinants, without success [51, 55].

The *mef(A/E)* gene was the most prevalent among the strains in this study, associated with the M phenotype, while the *erm* gene exhibited low prevalence. Resistance phenotypes correlated well with genotypes; however, four strains exhibiting the M phenotype lacked the evaluated genes, suggesting the involvement of alternative molecular mechanisms [8]. In addition to these commonly assessed genes, other resistance determinants previously described in GBS—such as additional variants of the erm family (*ermC* and *ermTR*) and genes associated with efflux pumps, including *msr(D)* and *lsa(C)*—may contribute to macrolide resistance [53, 58]. The absence of these genes in resistant isolates underscores the need for more comprehensive approaches, such as whole-genome sequencing, which allows for precise identification of resistance determinants and supports a deeper understanding of emerging resistance, as well as improved surveillance and control strategies.

One limitation of the present study is that the preliminary identification and isolation of clinical samples were not conducted simultaneously with the subsequent phenotypic and genotypic characterization of the GBS isolates. As a result, nine isolates were lost during the storage period and could not be included in the serotyping, antimicrobial susceptibility testing, or virulence profiling. Although this loss reduced the total number of fully characterized isolates, it represented a small proportion of the overall dataset. The remaining dataset provides a comprehensive and representative overview of the GBS strains identified in this population, allowing for reliable conclusions to be drawn.

Conclusions

This study provided a comprehensive characterization of GBS in pregnant women from Porto Velho, Rondônia, highlighting the high prevalence of virulence genes and significant biofilm-forming capacity. GBS remains susceptible to β-lactam antibiotics, while resistance to macrolides and lincosamides, although lower compared to international studies, still warrants attention. Six distinct serotypes were identified, including the second report of serotype VI in Brazil, emphasizing the genetic and epidemiological diversity of the Amazonian region. Notably, the serotype distribution in this population aligns with the most prevalent types worldwide. Therefore, if the hexavalent GBS conjugate vaccine demonstrates clinical efficacy and becomes available in the region, it may offer

substantial coverage—potentially encompassing up to 91.8% of the strains identified in this study.

Abbreviations

GBS	Group B *Streptococcus*
EOD	Early-onset disease
LOD	Late-onset disease
WHO	World Health Organization
CPS	Capsular polysaccharide
Lmb	Laminin-binding protein
ScpB	C5a peptidase
Bca	Protein C antigenα
Bac	Protein C antigenβ
HvgA	Hypervirulent GBS adhesion
CylE	β-hemolysin/cytolysin
HylB	Hyaluronidase
IAP	Intrapartum antimicrobial prophylaxis
CDC	Centers for Disease Control and Prevention
PCR	Polymerase chain reaction
CAMP	Christie-Atkins-Munch-Petersen
THB	Todd Hewitt broth
QIAGEN	Nome comercial (QIAquick PCR Purification Kit)
BLAST	Basic Local Alignment Search Tool
CLSI	Clinical Laboratory Standards Institute
MLS_B	Macrolide-Lincosamide-Streptogramin B
$iMLS_B$	inducible Macrolide-Lincosamide-Streptogramin B
$cMLS_B$	constitutive Macrolide-Lincosamide-Streptogramin B
ST	Sequence type
ATCC	American Type Culture Collection
OD_{570}	Optical Density at 570 nm

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Author contributions
AGC, MDR, RLMT and NBM conceived the study. AGC directed all study activities, the statistical analysis and wrote the first draft of the paper. MGLB, NCSL and NWSD led the microbiology testing and result interpretation. AGC led the serotyping analysis and antimicrobial susceptibility tests. RSR, MEPS and TCAP contributed to the drafting and revising of the final manuscript.

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Data availability
The DNA sequences generated and/or analyzed during this study have been deposited in the GenBank database under accession numbers PV111954–PV112038 and are available at: https://www.ncbi.nlm.nih.gov/nuccore/?term=PV111954:PV112038accn. The remaining datasets used and/or analyzed during the study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
The study was approved by the Research Ethics Committee of the Tropical Medicine Research Center (CEP/CEPEM) under approval number 2.788.949. All participants provided written informed consent prior to enrollment. To maintain participant confidentiality, unique study identification numbers were used in lieu of personal identifiers. The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki.

Consent for publication
Not applicable (no individual data).

Competing interests
The authors declare no competing interests.

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