

# Prevalence and antimicrobial susceptibility profile of *Streptococcus agalactiae* in pregnant women seen at the University Hospital of Londrina, Paraná, Brazil

## *Prevalência e sensibilidade aos antimicrobianos de Streptococcus agalactiae em gestantes atendidas no Hospital Universitário de Londrina, Paraná, Brazil*

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### Abstract

A retrospective study of pregnant women seen at the University Hospital of Londrina, Paraná, Brazil was performed to determine the prevalence of Group B *Streptococcus* (GBS) vaginal-rectal colonization, and the GBS susceptibility for antimicrobials used in intrapartum antibiotic prophylaxis. A vaginal-rectal swab was collected from 2,901 women between 35 and 37 weeks of gestation. Of these, 527 (18.2%) had a positive culture for GBS, and 0.4%, 10.2% and 10% of the isolates were resistant to penicillin, erythromycin and clindamycin, respectively. These results highlight the importance of continuous surveillance of GBS colonization in pregnant women for preventing GBS infections in neonates.

**Keywords:** *Streptococcus agalactiae*. Colonization. Antimicrobial resistance. Prevalence.

### Resumo

Um estudo retrospectivo foi realizado com gestantes atendidas no Hospital Universitário de Londrina, Paraná, Brasil para determinar a prevalência de colonização vaginal-retal por estreptococos do Grupo B (EGB) e o perfil de sensibilidade de EGB aos antimicrobianos utilizados para a antibioticoterapia profilática intraparto. Swabs vaginais-retais foram coletados de 2.901 mulheres entre a 35<sup>a</sup> e 37<sup>a</sup> semana de gestação. Destes, 527 (18,2%) apresentaram cultura positiva para EGB, e 0,4%, 10,2% e 10% dos

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isolados foram resistentes à penicilina, eritromicina e clindamicina, respectivamente. Estes resultados destacam a importância de vigilância contínua da colonização por EGB em gestantes para a prevenção de infecções em neonatos por EGB.

**Palavras chave:** *Streptococcus agalactiae*. Colonização. Resistência antimicrobiana. Prevalência.

## Introduction

*Streptococcus agalactiae* (group B *Streptococcus* - GBS) can be found as a harmless colonizer of the human microbiota, mainly in the gastrointestinal and genitourinary tract.<sup>(1)</sup> Vaginal-rectal GBS colonization in pregnant women is usually asymptomatic, but increases the risk of preterm birth and vertical transmission to newborns.<sup>(2)</sup> Importantly, around 1-3% of neonates colonized during delivery may develop early-onset GBS diseases,<sup>(3,4)</sup> which are associated with high mortality rates or long-term disabilities such as serious neurological sequelae.<sup>(5,6)</sup>

Preventive universal strategies for detecting GBS vaginal-rectal colonization in pregnant women at 35 and 37 weeks of gestation and administration of intrapartum antibiotic prophylaxis (IAP) led to a significant reduction in the incidence of GBS early neonatal infections in many parts of the world.<sup>(7)</sup> Currently, the antenatal strategy for detecting GBS colonization recommended by Center for Diseases Control of the United States<sup>(4)</sup> is based on culture methods, and penicillin is the first-line for IAP. In penicillin-allergic pregnant women with high risk of anaphylaxis, erythromycin, clindamycin or vancomycin are recommended as alternatives. However, this prevention strategy is yet to be adopted by most of the underdeveloped or developing countries.<sup>(6)</sup> In Brazil, although the Brazilian Society of Gynecology and Obstetrics recommends the GBS screening and IAP in colonized pregnant women, there is no consensus regarding prophylactic measures to reduce the incidence of neonatal GBS infection.

In that sense, GBS detection and determination of susceptibility profile on recommended gestational period should be expanded at health care services to perform the IAP properly, as an attempt to reduce the risk of both bacterial transmission to newborns and development of resistance to commercially available antibiotics. Therefore, the present study aimed to evaluate the prevalence and antimicrobial susceptibility profile of GBS isolated from pregnant women seen at the University Hospital of Londrina in northern Paraná, Brazil.

## Material and Method

### *Patients and study design*

This retrospective study based on medical record review was conducted at the University Hospital of Londrina (UHL) between January 2014 and December 2016. This hospital is the major referral center for the “Sistema Único de Saúde (SUS)”, the Brazilian governmental health system, in northern Paraná, Brazil. Besides the population of Londrina and neighboring municipalities, this hospital is also a reference for tertiary care for individuals from several localities in the states of São Paulo and Mato Grosso do Sul. UHL has adopted the CDC recommendations to prevent GBS early-onset diseases in neonates. Penicillin (first-line), and clindamycin or vancomycin (second-lines) were used for IAP.<sup>(4)</sup> Vaginal-rectal swabs were collected from all pregnant women between the 35<sup>th</sup> and 37<sup>th</sup> weeks of gestational age seen at the hospital. Sampling was performed on the lower third of vagina followed by the rectum using COPAN Transystem Stuart collection device (COPAN Diagnostic, Italy) and immediately transported to the microbiology laboratory. Data from AGTA healthcare information system database, LABHOS® module indicated that 2,901 pregnant women were seen at the UHL during the analyzed period. The study protocols were approved by the Ethics Committee of Universidade Estadual de Londrina (Document 193/12-CEP/UEL).

### *Microbiological analysis*

The swab specimens were inoculated into Granada Biphasic broth (bioMérieux, Brazil) and incubated at 37°C for 24 hours, in accordance with the hospital routine. After incubation, the samples were subcultured on Muller-Hinton agar (MHA) containing 5% sheep blood at 37°C for 24 hours. All isolates were identified to the species level by standard phenotypic methods based on colony morphology, Gram staining, catalase and CAMP (Christie, Atkins, Munch-Petersen) tests. Bacteria were kept at -20°C in TSB containing 20% glycerol and 5% sheep blood.

All GBS isolates were tested for penicillin G, clindamycin and erythromycin (Oxoid™, Brazil)

susceptibility using the disk diffusion method following the recommendations of the Clinical and Laboratory Standards Institute.<sup>(8)</sup> *Streptococcus pneumoniae* ATCC 49619 was used as the quality control of the assays.

## Results and Discussion

Vaginal-rectal GBS colonization in pregnant and non-pregnant women can be transient, intermittent or persistent.<sup>(2)</sup> Accordingly, the risk of maternal GBS transmission to newborn as well as development of early-onset infection have been maintained overtime. Therefore, continuous surveillance of vaginal GBS carriage in pregnant women is essential for prevention of neonatal GBS diseases.

The prevalence rates of GBS colonization in pregnant women vary between geographic regions, with the lowest and highest prevalence of colonization in Asia (11.1%) and Africa (22.4%), respectively. Although this regional heterogeneity is not fully understood, it does not seem to be due to the differences in culture methods or in the timing of vaginal-rectal swab collection in pregnancy.<sup>(9)</sup>

In this study, 2,901 pregnant women (1,137, 820 and 944 during 2014, 2015 and 2016, respectively) with no clinical evidence of streptococcal infection were included. Of these, 528 (18.2%) had a positive culture for GBS, which is in accordance with the average prevalence of 19.7% previously reported for countries in the Americas.<sup>(9)</sup> The GBS colonization prevalence in pregnant women did not vary significantly ( $P > 0.05$ ) over the analysis period, ranging between 17.7% (201/1137), 19.0% (156/820) and 18.0% (170/944) in 2014, 2015 and 2016, respectively (Table 1). Overall, the average age of pregnant women was 27 years (ranging from 13 to 54 years) and there were no significant differences ( $P > 0.05$ ) in the prevalence of GBS colonization between age groups (Table 2). In Brazil, differences in the prevalence of GBS colonization in pregnant women have also been detected according to the geographic region and the data are summarized in Table 3.

**Tabela 1** - Antimicrobial susceptibility profile of GBS isolated from pregnant women seen at UHL from January 2014 to December 2016.

Year	N	n (%)	Antimicrobial						
			Penicilin		Erythromycin		Clindamycin		
			S	R	S	I	R	S	R
		<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)	
2014	1137	201 (17.7)	201 (100)	0	168 (83.6)	5 (2.5)	28 (13.9)	170 (84.6)	31 (15.4)
2015	820	156 (19.2)	154 (98.7)	2 (1.3)	135 (86.0)	6 (3.8)	15 (9.6)	143 (91.7)	13 (8.3)
2016	944	170 (18.0)	170 (100)	0	154 (90.0)	6 (3.5)	11 (6.5)	161 (94.7)	9 (5.3)
Total	2901	528 (18.2)	526 (99.6)	2 (0.4)	457 (86.6)	17 (3.2)	54 (10.2)	475 (90.0)	53 (10.0)

N: number of pregnant woman seen at UHL during the analyzed period; n: number of positive cultures for GBS; S: susceptible; I: intermediate; R: resistant

**Tabela 2** - Age groups of pregnant women positive for vaginal-rectal GBS colonization seen at UHL from January 2014 to December 2016.

Age group	2014		2015		2016	
	N	%	N	%	N	%
< 20 years	41	20.4	29	18.6	24	14.1
20 to 24 years	25	12.4	44	28.2	44	25.9
25 to 29 years	53	26.4	27	17.3	35	20.6
30 to 34 years	39	19.4	22	14.1	36	21.2
> 35 years	43	21.4	34	21.8	31	18.2
Total	201	100	156	100	170	100

N: number of pregnant women seen at UHL during the analyzed period

Currently the use of intravenous penicillin G remains the first-line antibiotic for the IAP.<sup>(7)</sup> The decrease in susceptibility to penicillin in GBS is rare, and 100% of penicillin-sensitive isolates were found in several regions of the world.<sup>(10-13)</sup> However, GBS isolates with decreased susceptibility to penicillin were previously described. This phenomenon occurs due to the accumulation of mutations in the penicillin-binding proteins PBP1, PBP2b and PBP2x, which participate in cell wall biosynthesis.<sup>(14)</sup> In this study, we detected only two isolates (0.4%) resistant to

penicillin. In Brazil, most of the GBS isolates remain sensitive to penicillin. Nonetheless, high-rate resistance to this antibiotic was detected in GBS isolated from

pregnant women seen at a maternity facility in Sobral, Ceará (Table 3).

**Tabela 3** - Prevalence of GBS colonization among pregnant women reported in Brazilian studies.

City/State	N	Site/Timing of swab collection	Enrichment medium	n (%)	Antimicrobial resistance (%)	Reference
Rio de Janeiro/RJ	86	V/NE	TH+5% sheep blood+CN 8µg/mL+NA 15µg/mL	22 (25.6)	NT	Benchetrit et al., 1982 <sup>(15)</sup>
Londrina/PR	309	VR/≥36 weeks	HPTH	46 (14.9)	NT	Beraldo et al., 2004 <sup>(16)</sup>
Florianópolis/SC	273	VR/≥35 weeks	TH+CT 10µg/mL+NA 15µg/mL	59 (21.6)	NT	Pogere et al., 2005 <sup>(17)</sup>
Ribeirão Preto/SP	598	V/35–37 weeks	LIM'S+CT 10µg/mL+NA 15µg/mL	107 (17.9)	NT	Zusman et al., 2006 <sup>(18)</sup>
São Luis/MA	201	VR/≥36 weeks	TH+CT 10µg/mL+NA 15µg/mL	41 (20.4)	E (23.6) DA (25.4) CRO (12.7)	Costa et al., 2008 <sup>(19)</sup>
Campinas/SP	203	V/22–37 weeks	TH	56 (27.6)	NT	Nomura et al., 2009 <sup>(20)</sup>
Rio de Janeiro/RJ	3,929	VR/35–37 weeks	NU	186 (4.7)	NT	Costa et al., 2010 <sup>(21)</sup>
Juiz de Fora/MG	221	VR/< and ≥37 weeks	TH+CN 8µg/mL+NA 15µg/mL+ sodium azide 0.02%	21 (9.5)	E (22.7) DA (50)	Castellano Filho et al., 2010 <sup>(22)</sup>
Maringá/PR	102	VR/≥35 weeks	HPTH TH+CN 8µg/mL+NA 15µg/mL	25 (24.5)	NT	Chaves Jr et al., 2010 <sup>(23)</sup>
Sobral/CE	213	VR/>20 weeks	TH+CN 8µg/mL+NA 15µg/mL	9 (4.2)	AMP, KF, P (44.4) E (33.3) DA (77.8) C (11.1)	Linhares et al., 2011 <sup>(24)</sup>
Tubarão/SC	118	VR/35–37 weeks	NE	32 (27.1)	NT	Kruk et al., 2013 <sup>(25)</sup>
Tubarão/SC	203	VR/≥35 weeks	TH+CN 8µg/mL+NA 15µg/mL	41 (19.7)	NT	Schörner et al., 2014 <sup>(26)</sup>
Maringá/PR	544	VR/≥35 weeks	HPTH TH+CN 8µg/mL+NA 15µg/mL	136 (25)	E (8.1) DA (5.9) C (4.4) TE (82.3)	Melo et al., 2016 <sup>(27)</sup>
Londrina/PR	2,901	VR/35–37 weeks	Granada Broth	528 (18.2)	P (0.4) E (10.2) DA (10.0)	This study

N: Number of pregnant women analyzed; n: number of positive cultures for GBS; V: vaginal; VR: vaginal-rectal; NE: not specified; NT: not tested; NU: not used; TH: Todd-Hewitt Broth; HPTH: Hitchens-Pike-Todd-Hewitt; AMP: ampicillin; P: penicillin G; CRO: ceftriaxone; KF: cephalothin; E: erythromycin; DA: clindamycin; CN: gentamicin; C: chloramphenicol; TE: tetracycline; NA: nalidixic acid; CT: colistin.

Clindamycin, erythromycin, and vancomycin are alternatives for both pregnant women colonized with penicillin-resistant GBS or allergic to penicillin with high risk of anaphylaxis.<sup>(7)</sup> The susceptibility for erythromycin and clindamycin between GBS isolated from these patients can also vary according to geographic location. In African countries, rates of erythromycin and clindamycin resistance ranging from 6.5% to 21.1% and 3.2% to 17.2%, respectively, were reported among GBS isolates from pregnant women.<sup>(11,28)</sup> Data from North America reported rates of resistance to erythromycin and clindamycin ranging from 27.4% to 36% and 27.4% to 33%, respectively.<sup>(11,29)</sup> High rates of GBS resistance for both antimicrobials were reported in Italy (32.2% for erythromycin and 43.8% for clindamycin)<sup>(30)</sup> and China (78.6% for erythromycin and 64.3% for clindamycin) (WANG et al., 2015).<sup>(10)</sup> In contrast, lower rates of resistance were detected in Swiss (14.6% for erythromycin and 8.2% for clindamycin)<sup>(12)</sup> and Saudi Arabia (15.7% for erythromycin and 5.1% for clindamycin).<sup>(31)</sup>

Here, we detected resistance to erythromycin in 54 isolates (10.2%) and, of those, 46 (85.2%) were also resistant to clindamycin. Resistance only to erythromycin was observed in 8 isolates (1.5%), while 7 (1.3%) were resistant only to clindamycin (Table 1). Similar rates of erythromycin and clindamycin resistance between GBS colonizer of pregnant women were detected in a Public Health Service of Maringá city in northern Paraná, Brazil.<sup>(27)</sup> Rates of resistance ranging from 0% to 14.3% and 7.7% to 12.2% for erythromycin and clindamycin, respectively were detected in studies performed in GBS isolated from pregnant women in Rio de Janeiro, Brazil.<sup>(32,33)</sup> In contrast, high rates of resistance for both antimicrobials were detected in other Brazilian studies (Table 3).

## Conclusion

In our region, resistance to penicillin between vaginal-rectal GBS colonizer of pregnant women is rare. However, we identified two penicillin-resistant GBS isolates in this study. In addition, a substantial number of GBS isolates resistant to clindamycin was also detected. These results highlight the importance of continuous surveillance of vaginal-rectal GBS colonization in pregnant women and monitoring antimicrobial resistance for proper IAP. In turn, these measures greatly contribute to the prevention of GBS early-onset diseases in neonates.

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