

RESEARCH ARTICLE

Performance of Hitchens-Pike-Todd-Hewitt Medium for Group B Streptococcus Screening in Pregnant Women

Simone Cristina Castanho Sabaini de Melo^{1,2*}, Angela Andréia França Gavena¹, Flávia Teixeira Ribeiro Silva², Ricardo Castanho Moreira², Regiane Bertin de Lima Scodro³, Rosilene Fressatti Cardoso³, Vera Lúcia Dias Siqueira³, Rúbia Andreia Faleiros de Pádua³, Maria Dalva de Barros Carvalho⁴, Sandra Marisa Peloso^{1,5}

1 Postgraduate Program in Health Sciences, State University of Maringá, Maringá, Paraná, Brazil, **2** Nursing Sector, State University of Northern Paraná, Bandeirantes, Paraná, Brazil, **3** Department of Clinical Analysis and Biomedicine, State University of Maringá, Maringá, Paraná, Brazil, **4** Department of Medicine, State University of Maringá, Maringá, Paraná, Brazil, **5** Department of Nursing, State University of Maringá, Maringá, Paraná, Brazil

* simonecastanho@uenp.edu.br



Abstract

Group B streptococcus (GBS), which commonly colonizes the female genital tract and rectum, can cause infections in newborns with varying severity, possibly leading to death. The aim of the present study was to evaluate Hitchens-Pike-Todd-Hewitt (HPTH) medium performance for GBS screening in pregnant women. A descriptive analytical cross-sectional study was performed with 556 pregnant women, of which 496 were at 35-37 weeks of gestation and 60 were at ≥ 38 weeks of gestation. The study was conducted from September 2011 to March 2014 in northern Paraná, Brazil. Vaginal and anorectal clinical specimens from each pregnant woman were plated on sheep blood agar (SBA) and seeded on HPTH medium and Todd-Hewitt enrichment broth. Of the 496 pregnant women at 35-37 weeks of gestation, 141 (28.4%) were positive for GBS, based on the combination of the three culture media and clinical specimens. The GBS colonization rates that were detected by each medium were 22.2% for HPTH medium, 21.2% for SBA, and 13.1% for Todd-Hewitt enrichment broth. Of the 60 pregnant women at ≥ 38 weeks of gestation, seven (11.7%) were positive for GBS. These results demonstrate that HPTH medium and SBA were more sensitive than Todd-Hewitt enrichment broth for GBS screening in pregnant women and good GBS recovery in culture, indicating that the two media should be used together for vaginal and anorectal specimens.

OPEN ACCESS

Citation: de Melo SCCS, Gavena AAF, Silva FTR, Moreira RC, de Lima Scodro RB, Cardoso RF, et al. (2015) Performance of Hitchens-Pike-Todd-Hewitt Medium for Group B Streptococcus Screening in Pregnant Women. PLoS ONE 10(4): e0123988. doi:10.1371/journal.pone.0123988

Academic Editor: Adam J Ratner, Columbia University, UNITED STATES

Received: October 30, 2014

Accepted: February 25, 2015

Published: April 16, 2015

Copyright: © 2015 de Melo et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: The authors received no specific funding for this work.

Competing Interests: The authors have declared that no competing interests exist.

Introduction

Group B streptococcus (GBS) or *Streptococcus agalactiae* is a Gram-positive cocci that commonly colonizes the female genital tract and rectum [1]. GBS can cause infections in women and men, presenting as cystitis, skin infections, among others. There can be major implications

for pregnant women that cause impairments in pregnancy, chorioamnionitis, abortion, intra-uterine fetal death, premature membrane rupture, preterm delivery, postpartum endometritis, and sepsis [2].

The prevalence of GBS colonization in pregnant women worldwide has been reported to vary from 3% to 45% (3). In Brazil, the GBS colonization rates feature variations according to demographic region. The rates reported by some author showed ranged from 5% to 30% [3–6].

An estimated 1–2% of newborns from colonized mothers develop some diseases caused by GBS, which can vary in severity and cause death [7]. In developing countries, such as Brazil, the incidence of neonatal GBS infection ranges from 0.80 to 3.06 per 1,000 live births [8].

GBS infection can be transmitted to the newborn during labor or in utero from the mother's vaginal and anorectal colonized mucosa. Screening for GBS carriage is imperative in pregnant women. Recommendations have been made to screen for GBS at 35–37 weeks of gestation [9] because this time period correlates with the delivery timeframe.

The U.S. Centers for Disease Control and Prevention (CDC) recommends using selective enrichment medium for GBS screening, such as Todd-Hewitt broth supplemented with antibiotics, followed by subculture on sheep blood agar [9]. However, some studies have demonstrated success using other media to detect GBS in pregnant women [10–14].

Although Todd-Hewitt enrichment broth is widely used because it is recommended by the CDC for GBS screening in pregnant women, the sensitivity and specificity of such methods still need to be evaluated. A previous study in Brazil [12] compared the sensitivity of Hitchens-Pike-Todd-Hewitt (HPTH) medium, Todd-Hewitt enrichment broth, and blood agar direct plating and found that HPTH medium had the best performance for GBS screening in anorectal and vaginal samples. However, the above study was performed with a small number of pregnant women, and GBS colonization rates are unknown in southern Brazil. Therefore, the aim of the present study was to evaluate the performance of HPTH medium for GBS screening in pregnant women.

Materials and Methods

Study population

The study was approved by the Ethics and Human Research Committee, State University of Maringá (no. 236/2011). The term of consent was read and signed by all study participants. A descriptive analytical cross-sectional study was performed with 556 pregnant women, of which 496 were at 35–37 weeks of gestation and 60 were at ≥ 38 weeks of gestation. The study was conducted from September 2011 to March 2014 in 21 municipalities in northern Paraná, Brazil. The gestational age was determined based on the last menstrual period. When the last menstrual period was indeterminate, we used fetal ultrasound in the first trimester of pregnancy. The exclusion criteria were previous use of antibiotics and/or vaginal cream in the last 7 days by the pregnant women.

Clinical specimens and culture

Clinical specimens from the vaginal distal third were collected by introducing sterile swabs labeled 1, 2, and 3, without using a speculum. Anorectal specimens were collected by introducing sterile swabs labeled 4, 5, and 6 in the anorectal region.

Vaginal swab 1 and anorectal swab 4 specimens were immediately plated on 5% desfibrinated sheep blood agar-SBA (Himedia, Curitiba, Paraná, Brazil) and incubated at 35–37°C for 18–24 h.

Vaginal swab 2 and anorectal swab 5 were each submerged in 2ml of HPTH medium [12], and 100 μ l of sterile defibrinated sheep blood (Laborclin, Pinhais, Paraná, Brazil) was added,

followed by incubation at 35–37°C for 18–24 h. Afterward, subculturing was performed on SBA, with incubation at 35–37°C for 24–48 h.

Vaginal swab 3 and anorectal swab 6 were submerged in Todd-Hewitt medium (Himedia, Curitiba, Paraná, Brazil) supplemented with 8 µg/ml gentamicin (Inlab, São Paulo, Brazil) and 15 µg/ml nalidixic acid (Inlab, São Paulo, Brazil) according to the manufacturer's instructions and incubated at 35–37°C for 18–24 h. Afterward, an aliquot of each culture was plated on SBA (Himedia, Curitiba, Paraná, Brazil) and incubated at 35–37°C for 24–48 h.

GBS biochemical and serological identification

The culture interpretation and identification of GBS were performed in the Laboratory of Clinical Bacteriology, Department of Clinical Analyses and Biomedicine, State University of Maringá, Paraná, Brazil. Colonies that were suggestive of GBS (beta- and non-hemolytic) were subjected to microscopy (Gram stain), biochemical identification (catalase, bile esculin, and hippurate hydrolysis), and latex agglutination using a streptococcal grouping kit (Oxoid, Hampshire, UK) according to the manufacturer's instructions.

Data analysis

The data were analyzed by chi-square using Epidata software version 3.1. Values of $p < 0.05$ were considered statistically significant.

Results

Of the 496 pregnant women at 35–37 weeks of gestation who participated in the study, 141 (28.4%) were positive for GBS based on the combination of the three culture media for the two clinical specimens (S1 Table). The GBS colonization rate detected was 22.2% for HPTH medium, 21.2% for SBA, and 13.1% for Todd-Hewitt enrichment broth (Table 1). Of the pregnant women who were positive for GBS, based on HPTH medium, 8.1% were positive exclusively in vaginal specimens, 3.8% were positive exclusively in anorectal specimens, and 10.3% were positive in both specimens. SBA plating revealed GBS-positive cultures in 5.2% of the vaginal specimens and 6.0% of the anorectal specimens, and 9.9% were positive in both specimens. Plating with Todd-Hewitt enrichment broth revealed GBS-positive cultures in 10.1% of the vaginal

Table 1. Prevalence of *Streptococcus agalactiae* in pregnant women at 35–37 weeks of gestation, based on clinical specimens tested with Todd-Hewitt enrichment broth (TODD), sheep blood agar (SBA), and Hitchens-Pike-Todd-Hewitt (HPTH) culture media.

Test	n	%	95% Confidence interval	Chi-square	p value
Vaginal				8.1473	0.0170
HPTH	40	8.1	5.66–10.46		
SBA	26	5.2	3.28–7.20		
TODD	50	10.1	7.43–12.73		
Anorectal				14.7231	0.0006
HPTH	19	3.8	2.14–5.52		
SBA	30	6.0	3.95–8.15		
TODD	7	1.4	0.37–2.45		
Anorectal and vaginal				35.2594	<0.0001
HPTH	51	10.3	7.61–12.95		
SBA	49	9.9	7.25–12.51		
TODD	8	1.6	0.50–2.72		

doi:10.1371/journal.pone.0123988.t001

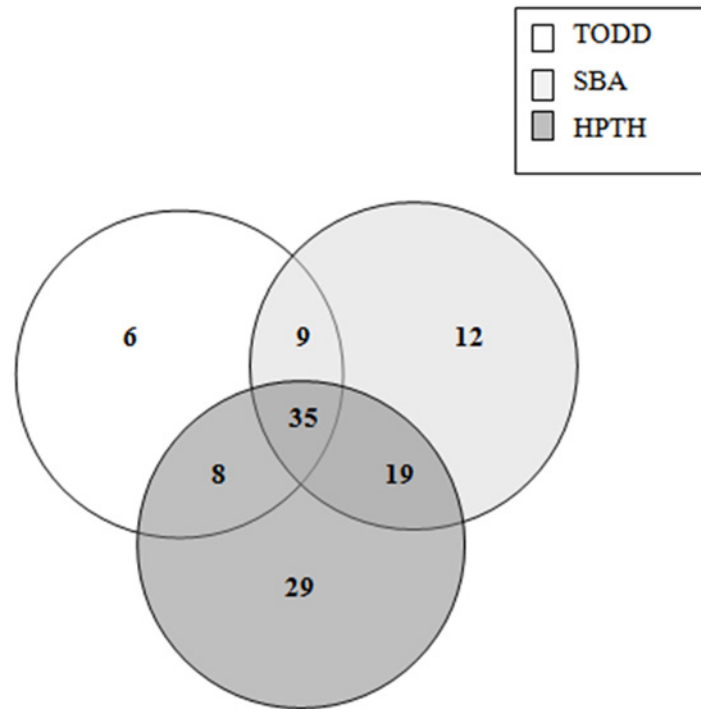


Fig 1. Number of *Streptococcus agalactiae* positive pregnant women (35–37 weeks of gestation) detected by HPTH medium, Todd-Hewitt enrichment broth (TODD), and sheep blood agar (SBA) plating in vaginal samples.

doi:10.1371/journal.pone.0123988.g001

specimens and 1.4% of the anorectal specimens, and 1.6% were positive in both specimens (Figs 1 and 2, Tables 1 and 2).

HPTH had higher performance in detecting GBS in vaginal specimens, which accounted for 91 (18.3%) pregnant women. For anorectal specimens, which include patients with positive culture only in anorectal and anorectal plus vaginal specimens, the best performance for GBS screening was observed with SBA direct plating (15.9%) (Figs 1 and 2, Tables 1 and 2).

Of the 60 pregnant women at ≥ 38 weeks of gestation, seven (11.7%) were positive for GBS. Of these, one was detected only by HPTH, one only by SBA plating, and five were detected by both media. No GBS detection in vaginal or anorectal samples occurred with Todd-Hewitt enrichment broth.

Discussion

In our previous study conducted in southern Brazil, the best performance for GBS screening in pregnant women was found when we used an enrichment culture medium that is not commonly used for this purpose (i.e., HPTH medium). However, some concerns arose about the reproducibility of our results in populations that have different lifestyles, live in different geographical locations, different ages, socioeconomic levels, schooling, and gestational ages. To improve GBS screening performance in pregnant women and considering the benefits of this detection protocol, we extended our study of GBS screening with HPTH medium to another geographical region of Parana state in Brazil.

The percentage of pregnant women (28.4%), which were GBS carriers at 35–37 weeks of gestation in the present study, corroborates with study conducted by Feuerschuetz et al. [1]. Two other studies reported a lower prevalence: 17.4% [15] and 15.0% [16]. This variation in

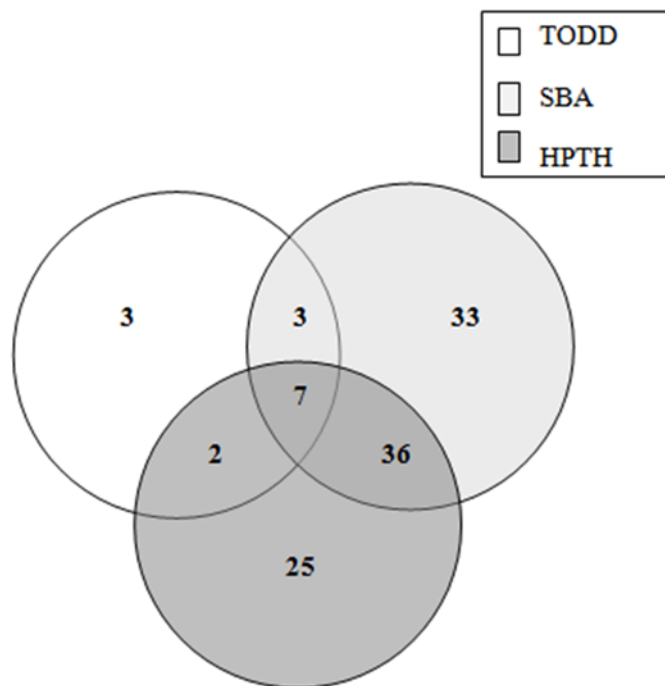


Fig 2. Number of *Streptococcus agalactiae* positive pregnant women (35–37 weeks of gestation) detected by HPTH medium, Todd-Hewitt enrichment broth (TODD), and sheep blood agar (SBA) plating in anorectal samples.

doi:10.1371/journal.pone.0123988.g002

GBS maternal detection may be attributable to differences in the applied methodologies to detect GBS and characteristics of the studied populations.

The CDC recommends GBS screening in both vaginal and anorectal specimens from pregnant women [9]. The criteria suggested by the CDC were perfectly met in our study, in which the percentage of GBS detection were 23.5% and 21.9% in vaginal and anorectal specimens using the three media, respectively.

Our study corroborates the good HPTH performance that was previously reported in a study that evaluated a population in a medium-sized city [12]. In the previous study, 24.5% of pregnant women were GBS carriage, detected using HPTH medium, Todd-Hewitt enrichment

Table 2. Comparison of *Streptococcus agalactiae* detection by Todd-Hewitt enrichment broth (TODD), sheep blood agar (SBA), and Hitchens-Pike-Todd-Hewitt (HPTH) culture media in pregnant women at 35–37 weeks of gestation.

	n	95% Confidence interval	Chi-square	p value
Media			16.0464	0.0003
TODD	65/496	13.1 (10.13–16.07)		
SBA	105/496	21.2 (17.57–24.77)		
HPTH	110/496	22.2 (18.52–25.84)		
Test post-hoc				
TODD versus SBA	65 versus 105	13.1 versus 21.2	10.7974	0.0010
TODD versus HPTH	65 versus 110	13.1 versus 22.2	13.4325	0.0002
SBA versus HPTH	105 versus 110	21.2 versus 22.2	0.0950	0.7579

doi:10.1371/journal.pone.0123988.t002

broth, and antibiotic SBA plating. The present study with a larger study sample ($n = 496$), which was from a different geographic area in the same state (Paraná) in Brazil, the HPTH medium showed better performance in detecting GBS compared to Todd-Hewitt enrichment broth ($p = 0.0002$) (Table 2). The addition of HPTH clearly increased in 10.9% ($n = 54$) the GBS detection in both specimens. For vaginal samples, the increase was 5.8% ($n = 29$) and for anorectal, the increase was 5.1% ($n = 25$).

The addition of Todd-Hewitt enrichment broth presented less improvement in GBS screening in both vaginal and anorectal specimens ($n = 9$, 1.8%), 1.2% ($n = 6$) in vaginal, and 0.6% ($n = 3$) in anorectal (Figs 1 and 2).

None difference was found between HPTH medium and SBA direct plating ($p = 0.7579$) for GBS screening (Table 2). The detection of GBS in anorectal and vaginal isolated samples by SBA direct plating was 6.7% ($n = 33$) and 2.4% ($n = 12$), respectively (Figs 1 and 2). These results clearly demonstrate the applicability of using SBA direct plating, which increases GBS detection mainly in anorectal samples.

The use of HPTH and SBA media together is promising because of their specificities. The HPTH has the ability to recover lower GBS inoculums, and the SBA is associated with faster detection, which is of paramount importance.

Given the reliability of using SBA direct plating, we may conclude that it can identify GBS 24h earlier than HPTH medium and Todd-Hewitt enrichment broth. Expert microbiologists can perform bacterial identification by serology in selected colonies that are suggestive of GBS and release preliminary results within this time. This shorter time for identification can provide faster results once GBS colonized pregnant women could take the newborn babies at risk for early-onset disease.

One difference in our study's methodology was the use of SBA without antibiotics. Chaves et al. [12] added gentamicin (8 $\mu\text{g}/\text{ml}$) and nalidixic acid (15 $\mu\text{g}/\text{ml}$) to the SBA in an attempt to not compromise GBS detection by the microbiota. This difference in the medium composition, used in our study, resulted in an increase in the detection of GBS in both vaginal and anorectal specimens. One explanation for the findings may be the susceptibility of GBS to the antibiotic that was added to the SBA in the previous study [12].

Similar to the pregnant women at 35–37 group, in the sixty pregnant women at ≥ 38 weeks of gestation group the addition of the HPTH and SBA direct plating increased the sensitivity of GBS screening too. Our results corroborate a study that was conducted in Iran [17], which detected a high percentage of pregnant women with GBS at more than 38 weeks of gestational age.

We conclude that neither HPTH nor SBA direct agar plating can be recommended as a sole medium for GBS screening in pregnant women because both media alone failed to detect GBS in some of the pregnant women. For adequate GBS recovery in culture, the two media should be used together for vaginal and anorectal specimens. Using HPTH medium would be interesting in future studies from different laboratories to detect GBS in pregnant women from different countries.

Supporting Information

S1 Table. Results of cultures, from vaginal and anorectal specimens, in pregnant women at 35–37 weeks of gestation to detect *Streptococcus agalactiae* by Todd-Hewitt enrichment broth (TODD), sheep blood agar (SBA), and Hitchens-Pike-Todd-Hewitt (HPTH) culture media.

(PDF)

Acknowledgments

We thank all the nurses of the Health Unit and their team for supporting, which were essential to the execution of this work. We greatly appreciate the former students, Aline Balandis Costa and Mariana Digieri Cavalheiro by their assistance in the data collection.

Author Contributions

Conceived and designed the experiments: SCCSM SMP. Performed the experiments: SCCSM RAFP. Analyzed the data: AAFG RCM. Contributed reagents/materials/analysis tools: SCCSM. Wrote the paper: SCCSM RFC SMP. The data compilation: SCCSM FTRS RBLs RFC VLDS MDBC SMP.

References

1. Feuerschuette OM, Serratine AC, Bazzo ML, Martins TR, Silveira SK, Silva RM. Performance of RT-PCR in the detection of *Streptococcus agalactiae* in the anogenital tract of pregnant women. Arch Gynecol Obstet. 2012; 286: 1437–1442. doi: [10.1007/s00404-012-2498-9](https://doi.org/10.1007/s00404-012-2498-9) PMID: [22872028](https://pubmed.ncbi.nlm.nih.gov/22872028/)
2. Choi SJ, Park SD, Jang IH, Uh Y, Lee A. The prevalence of vaginal microorganisms in pregnant women with preterm labor and preterm birth. Ann Lab Med. 2012; 32: 194–200. doi: [10.3343/alm.2012.32.3.194](https://doi.org/10.3343/alm.2012.32.3.194) PMID: [22563554](https://pubmed.ncbi.nlm.nih.gov/22563554/)
3. Kiss FS, Rossato JS, Graudenz MS, Gutierrez LLP. Prevalência da colonização por *Streptococcus agalactiae* em uma amostra de mulheres grávidas e não grávidas de Porto Alegre, Estado do Rio Grande do Sul. Scientia Medica. 2013; 23: 169–174.
4. Oliveira MV, Teles MF, Viana TA. Prevalência e fatores de risco associados à colonização por *Streptococcus agalactiae* em gestantes atendidas no hospital municipal Esaú Matos em Vitória da Conquista—BA. C&D-Revista Eletronica da Fainor. 2013; 6: 172–184.
5. Melin P. Neonatal group B streptococcal disease: from pathogenesis to preventive strategies. Clin Microbiol Infect. 2011; 17: 1294–1303. doi: [10.1111/j.1469-0691.2011.03576.x](https://doi.org/10.1111/j.1469-0691.2011.03576.x) PMID: [21672083](https://pubmed.ncbi.nlm.nih.gov/21672083/)
6. Chaves M Jr. Prevalência de Streptococcus do grupo B em gestantes. M.Sc. Thesis, State University of Maringá. 2007.
7. Cortés JIA, Domingo AA, Mir LA, Roura LC, López MC, Sastre JL et al. Prevención de la infección perinatal por estreptococo del grupo B. Recomendaciones españolas. Actualización 2012. Documento de consenso. SEIMC/SEGO/SEN/SEQ/SEMFYC. Enferm Infecc Microbiol Clin. 2013; 31: 159–172. doi: [10.1016/j.eimc.2012.03.013](https://doi.org/10.1016/j.eimc.2012.03.013) PMID: [22658283](https://pubmed.ncbi.nlm.nih.gov/22658283/)
8. Dagnev AF, Cunnington MC, Dube Q, Edwards MS, French N, Heyderman RS et al. Variation in reported neonatal group B streptococcal disease incidence in developing countries. Clin Infect Dis. 2012; 55: 91–102. doi: [10.1093/cid/cis395](https://doi.org/10.1093/cid/cis395) PMID: [22523262](https://pubmed.ncbi.nlm.nih.gov/22523262/)
9. Verani JR, McGee L, Schrag SJ. Prevention of perinatal group B streptococcal disease: revised guidelines from CDC, 2010. Morb Mortal Wkly Rep. 2010; 59: 1–36.
10. Verhoeven PO, Noyel P, Bonneau J, Carricajo A, Fonsale N, Ros A et al. Evaluation of the new brilliance chromogenic medium for screening of *Streptococcus agalactiae* vaginal colonization in pregnant women. J Clin Microbiol. 2014; 52: 991–993. doi: [10.1128/JCM.02926-13](https://doi.org/10.1128/JCM.02926-13) PMID: [24403300](https://pubmed.ncbi.nlm.nih.gov/24403300/)
11. Berg BR, Houseman JL, Garrasi MA, Young CL, Newton DW. Culture-based method with performance comparable to that of PCR-based methods for detection of group B Streptococcus in screening samples from pregnant women. J Clin Microbiol. 2013; 51: 1253–1255. doi: [10.1128/JCM.02780-12](https://doi.org/10.1128/JCM.02780-12) PMID: [23345288](https://pubmed.ncbi.nlm.nih.gov/23345288/)
12. Chaves M Jr, Padua RAF, Campanerut PAZ, Pelloso SM, Carvalho MDB, Siqueira VLD et al. Preliminary evaluation of Hitchens-Pike-Todd-Hewitt medium (HPTH) for detection of group B Streptococci in pregnant women. J Clin Lab Anal. 2010; 24: 403–406. doi: [10.1002/jcla.20419](https://doi.org/10.1002/jcla.20419) PMID: [21089171](https://pubmed.ncbi.nlm.nih.gov/21089171/)
13. Mocelin CO, Carvalho DAF, Brites C, Christofolini D, Mocelin AO, Fracalanza SEL et al. Isolamento de *Streptococcus agalactiae* de gestantes da região de Londrina—PR. Rev Bras Ginecol Obstet. 1995; 105: 915–918.
14. Beraldo C, Brito ASJ, Saridakis HO, Matsuo T. Prevalência da colonização vaginal e anorretal por Estreptococo do grupo B em gestantes do terceiro trimestre. Rev Bras Ginec Obstet. 2004; 26: 543–549.
15. Função JM, Narchi NZ. Pesquisa do estreptococo do Grupo B em gestantes da Zona Leste de São Paulo. Rev Esc Enferm USP. 2013; 47: 22–29. PMID: [23515799](https://pubmed.ncbi.nlm.nih.gov/23515799/)

16. Caetano MSSG Colonização pelo *Streptococcus agalactiae* (EGB) em gestantes atendidas na rede pública de Uberaba-MG. M.Sc. Thesis, Federal University of Triangulo Mineiro. 2008 Available: http://www.ufm.edu.br/pgcs/imagem/Tese_MarioSergioSGCaetME.pdf. Accessed 19 June 2014.
17. Hamed A, Akhlaghi F, Seyedi SJ, Kharazmi A. Evaluation of group B Streptococci colonization rate in pregnant women and their newborn. Acta Med Iran. 2012; 50: 805–808. PMID: [23456521](https://pubmed.ncbi.nlm.nih.gov/23456521/)