Research Square Preprint: Please note that this article has not completed peer review.

Group B Streptococcus detection in pregnant women: comparison of real-time PCR assay, culture, and the Xpert GBS rapid test

CURRENT STATUS: ACCEPTED

BMC Pregnancy and Childbirth BMC Series

Laura Vieira
HCPA

Amanda Vilaverde Perez
HCPA

amandavperez@gmail.com*Corresponding Author*
ORCiD: https://orcid.org/0000-0002-3150-6675

Monique M Machado
HCPA

Michele L Kayser
HCPA

Daniela V Vettori
HCPA

Ana Paula Alegretti
HCPA

Charles Francisco Ferreira
HCPA

Janete Vettorazzi
HCPA

Edimárlei G Valério
HCPA

DOI:
10.21203/rs.2.11969/v1

SUBJECT AREAS
Maternal & Fetal Medicine

KEYWORDS

Group B Streptococcus; Streptococcus agalactiae; Xpert GBS; real-time polymerase chain reaction; antenatal care

Abstract

Background

Group B Streptococcus (GBS) is one of the most important causative agents of neonatal sepsis. As administration of prophylactic antibiotics during labor can prevent GBS infection, routine screening for this bacterium in prenatal care before the onset of labor is recommended. However, many women present in labor without having undergone such testing during antenatal care, and the turnaround time of detection methods is insufficient for results to be obtained before delivery.

Methods

Vaginal and anorectal specimens were collected from 270 pregnant women. Each sample was tested by Xpert GBS, PCR, and culture for GBS detection.

Results

The overall prevalence of maternal GBS colonization was 30.7% according to Xpert GBS, 51.1% according to PCR, and 14.3% according to cultures. Considering the PCR method as the reference, the Xpert GBS had a sensitivity of 53% and specificity of 93%. Positive Xpert GBS results were associated with marital status (married or cohabitating) and with prematurity as a cause of neonatal hospitalization. Positive cultures were associated with ischemic–hypoxic encephalopathy requiring therapeutic hypothermia.

Conclusions

Combined enrichment/PCR and the Xpert GBS rapid test found a high prevalence of GBS colonization. The Xpert GBS technique gives faster results and could be useful for evaluating mothers who present without antenatal GBS screening results and are at risk of preterm labor, thus allowing institution of prophylactic antibiotic therapy. Trial Registration: The study was approved by the institutional Research Ethics Committee (CAAE: 59688316.0.0000.5327)

Background

Group B *Streptococcus* (GBS) is a gram-positive bacterium associated with the colonization of human body's mucous membranes. GBS is one of the most important causative agents of neonatal sepsis, which can be prevented by administration of prophylactic antibiotics during labor. Women can be

transiently, intermittently, or persistently colonized by GBS in their vaginal or anorectal mucosae [1]. A prevention strategy based on bacterial screening and intrapartum antimicrobial prophylaxis in those pregnant women identified as carriers has led to a reduction in the incidence of neonatal diseases attributable to GBS [2]. The U.S. Centers for Disease Control and Prevention (CDC) [3] recommend routine screening for GBS as an integral part of antenatal care. Ideally, this should be done at a gestational age of 35 to 37 weeks, or earlier in women at risk of premature labor.

Routine GBS screening is done by polymerase chain reaction (PCR)-based tests and cultures. A 2011 study that evaluated a combination of enrichment culture and PCR versus conventional cultures at Hospital de Clínicas de Porto Alegre (HCPA), a tertiary care hospital in Southern Brazil [4], found that enrichment culture/PCR had 87% specificity, with a positive predictive value of 59% and a negative predictive value of 100%. Since 2015, enrichment culture with real-time PCR has become the standard method for GBS detection at HCPA [4-6]. However, these methods usually take around 48-72 hours to complete, which has prompted the search for a more rapid test, especially to support urgent decision-making regarding administration of antibiotic prophylaxis.

The Xpert® GBS (Cepheid) is a rapid test based on PCR technology whereby rectal and vaginal swabs are collected and a result is obtained in approximately 50 minutes. This method is commercially available in Brazil and has demonstrated high sensitivity for GBS detection in other studies [7, 8]. Considering that many women in Brazil present in labor without having undergone GBS screening during antenatal care, and that many women at risk of preterm labor are admitted to maternity units before GBS screening can be performed on an outpatient basis, the turnaround time of conventional GBS detection methods is too slow for results to be obtained before delivery. Within this context, the present study was designed to evaluate the diagnostic accuracy of the Xpert GBS rapid test and compare it with that of combined enrichment/PCR (currently used for GBS screening at HCPA) and with the conventional vaginal/rectal discharge culture method.

Methods

This prospective study was carried out between March and September 2017. Pregnant women who presented for medical appointments at the outpatient, antenatal, and labor and delivery units of the

HCPA Department of Obstetrics and Gynecology were recruited. The study was approved by the institutional Research Ethics Committee (CAAE: 59688316.0.0000.5327) and conducted in accordance with the provisions of the Declaration of Helsinki. All patients provided written informed consent prior to enrollment. The inclusion criterion was gestational age ≥24 weeks, while the exclusion criterion was any use of antibiotics in the 30 days preceding enrollment.

Among 300 enrolled women, 30 were excluded: 5 refused to participate, 10 had already undergone GBS screening and received their results at the time of study inclusion, and 15 had used antibiotics in the last 30 days (Figure 1). Thus, the final sample comprised 270 women.

Three vaginal and rectal swabs (one sample for each screening method – Xpert GBS, real-time PCR, and culture) – were collected from each patient and immediately stored in Stuart transport medium, according to CDC recommendations. The swabs collected for PCR were sent to the HCPA microbiology and molecular biology laboratories; the culture swabs were sent to an outside laboratory (Endocrimeta®); and the Xpert GBS samples were analyzed on specific equipment provided by Cepheid. All samples were sent for evaluation within 24 hours of collection.

-

-

Xpert GBS (Cepheid®)

The collected swab was transferred to the designated chamber of the Xpert GBS cartridge, which was loaded into a Cepheid GeneXpert device, as recommended by the manufacturer. A trained physician performed all Xpert GBS assays. The result could be negative or positive based on the detection of the target gene sequence adjacent to the GBS *cfb* gene, as defined by the GeneXpert software. Xpert GBS performs automation and integration of sample lysis, amplification and purification of nucleic acids, and detection of the target sequence using real-time PCR. The total assay runtime was around 50 minutes.

GBS culture

The collected swab was inoculated on blood agar plates and incubated at 37°C for 48 hours in a

microbiological incubator. After incubation, the plates were inspected for the presence of beta-hemolytic colonies. If there was any suspicion of beta-hemolytic plaque growth after 48 hours, plaques were reincubated for another 24 hours and inspected again. Beta-hemolytic colonies whose morphology were consistent with GBS were subcultured and CAMP-tested [9]. CAMP-positive colonies were deemed presumably positive for GBS.

-

Real-time polymerase chain reaction

Sample preparation and DNA (deoxyribonucleic acid) extraction

The swabs were incubated for 18 to 24 hours into Todd Hewitt selective medium containing gentamicin and nalidixic acid. After centrifugation of the broth, the precipitate was washed with 1X PBS (phosphate-buffered saline) solution (pH = 7.2) and centrifuged again. Then, the precipitate was washed with 1X Tris-EDTA (TE) buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH = 7.5), and DNA extracted by thermal lysis. The thermal lysis protocol was performed using TE solution for 15 min at 100°C followed by 15 min at −80°C to lyse bacterial cell walls. The quality and quantity of DNA extracted from samples were estimated spectrophotometrically in a Nanodrop ND-1000 system (Thermo Fisher Scientific, USA), at 260 nm (A260) and 280 nm (A280) absorbance, with the sample diluted to 5 ng/μL.

-

Real-time polymerase chain reaction

For the real-time PCR reaction, we used the *cfb* gene region that encodes the CAMP factor present in GBS. The primers used were 5′-TTT CAC CAG CTG TAT TAG AAG TA-3′ and 5′-GTT CCC TGA ACA TTA TCT TTG AT-3′. For internal control, a synthetic single-chain DNA (5′-ATC GCT GAT CCG GCC ACA TAT CGC GTT TAT GCG AGG TCG GGT GGG CGG GTC GTT AGT TTC GTT TTG GGC CTA CGT GGC CTT TGT CAC CGA-3′) was used to detect amplification inhibition in all samples using the primers 5′-ATC GCT GAT CCG GCC ACA-3′ and 5′-TCG GTG ACA AAG GCC ACG TA-3′.

The amplification reagents were prepared as follows: Platinum® SYBR® Green (Invitrogen) concentrated mix 6.25 μL, SBG primers 1.25 μL, ROX 1:50 0.25 μL, and DNAse- and RNAse-free water

2.5 µL; 0.5 µL of internal control (IC) solution and 0.75 µL of primers were added to the IC tube. The extracted DNA solution was added to 10 µL of amplification reagents. Amplification and fluorescent detection were measured by real-time quantitative reverse-transcription polymerase chain reaction (RT-qPCR) in a 7500 Real-Time PCR System (Applied Biosystems). The amplification was performed with one cycle at 50°C for 2 minutes for DNA polymerase activation, followed by one cycle at 95°C for 10 minutes for initial denaturation, than 40 cycles at 95°C for 15 sec and 60°C for 1 min for amplification, followed by two cycles at 95°C for 15 sec and 60°C for 15 sec for fluorescence detection and melting temperature (Tm) measurement. Samples are considered positive when the amplification curve is detected and the Tm is in the acceptable range (GBS Tm 76-78°C and IC Tm 82-84°C). The negative control should not have an amplification curve for the GBS target, while the positive control should be positive for the two targets tested. To ensure high sensitivity in the PCR, the cutoff point was set at a threshold cycle value of $C_t = 40$ [10, 11].

Statistical analysis

Sample size was calculated in WinPEPI Version 11.63, based on the findings of a previous study [4]. Considering a 16% prevalence of GBS positivity with the gold-standard method (culture) and 95% power to detect a 5% difference in prevalence of a positive result, with an estimated 10% attrition rate, the final sample size required was 230 participants. Agreement between assays was determined using the kappa and Cronbach's alpha coefficients. Statistical analyses were performed in PASW Statistics for Windows, Version 18.0. The sensitivity, specificity, negative predictive value, and positive predictive value of the tests were evaluated in accordance with STARD (Standards for Reporting of Diagnostic Accuracy) initiative recommendations.

Results

The maternal characteristics and main neonatal outcomes are listed in Table 1 and Table 2. The median maternal age was 29 years (95% CI 27.6-29.5); 39% were nulliparous, 73% were white, and 83% were single or not living with a partner. Women with a gestational age <35 weeks and any risk of preterm labor represented 48.5% of the sample.

Xpert GBS testing was performed in samples from 270 women; 75 (27.8%) were positive, 169 (62.6%) were negative, 1 (0.4%) was inconclusive, 21 (7.8%) yielded errors, and 4 (1.5%) had no result available, as shown in Table 3. The percentage of errors is justified by a known and reported problem with a specific batch of cartridges, while four samples were lost due to a power outage (no result). Only the positive and negative results were included in the analysis. Considering these results alone, the overall prevalence of maternal GBS colonization was 51.1% according to real-time PCR, 30.7% according to Xpert GBS, and 14.3% according to cultures (Table 3).

We compared the performance of the three tests considering valid results alone (Table 4). A total of 239 women were screened with both the Xpert GBS and real-time PCR. GBS colonization was detected in 124 (51.9%) with real-time PCR versus 74 (31.0%) with the Xpert GBS. Considering real-time PCR as the gold standard, the Xpert GBS had a sensitivity of 53.2% and a specificity of 93.0%. The positive predictive value was 89.2%, and the negative predictive value was 64.8%. The kappa coefficient between the two techniques indicates moderate agreement (kappa = 0.46), with apparent low sensitivity and high specificity for the rapid test.

A total of 220 women were screened with both the Xpert GBS and culture methods with valid results. GBS colonization was detected in 66 patients (30.0%) by the Xpert GBS versus 34 (15.5%) with the culture method (Table 4). Considering culture as the gold standard, the Xpert GBS had a sensitivity of 61.8% and a specificity of 75.8%. The positive predictive value was 31.8%, and the negative predictive value was 91.6%. The kappa coefficient between the two techniques indicates fair agreement (kappa = 0.27).

In this study, GBS colonization detected by real-time PCR was not associated with maternal characteristics, such as age, marital status, ethnicity, education attainment, and parity, nor with maternal or neonatal complications, such as chorioamnionitis and sepsis. Positive results with the Xpert GBS rapid test were associated with marital status (married or cohabitating) and with preterm delivery as a cause of neonatal hospitalization. Finally, ischemic–hypoxic encephalopathy and need for therapeutic hypothermia were associated with positive cultures (Table 5).

Discussion

The overall prevalence of GBS colonization varies depending on the studied population and the test used for screening. This variability may be related to various climatic, biological, sociocultural, geographic, and methodological determinants [12, 13]. In Brazil, prevalence has been reported to range from 9 to 36% [13-15]. In one Brazilian study [14] that compared culture and PCR, only 9.5% of samples were positive for GBS by culture, while 32.6% were positive when using PCR methods. Our study population was restricted to patients of a public hospital that serves as a referral center for high-risk pregnancies, which may explain our finding of a much higher prevalence (51.1%) than is usually reported in the literature, considering the real-time PCR method. In fact, this is one of the highest prevalence values ever reported among Brazilian women. In a 2010 study [4] conducted at the same hospital as the present investigation but using the conventional PCR agarose gel technique, the prevalence of GBS was 27.0%—much lower than that found in the present study. However, this difference can be justified by the higher sensitivity of real-time PCR.

In a study [16] carried out among women in the Southeast region of Brazil, the prevalence of GBS by the culture method was around 18%, although only vaginal samples were collected. In an Italian sample of pregnant women [17], about 20% were GBS-positive with the culture method. The prevalence of GBS by culture in the present study (14.3%) was similar to the results of previous studies [4, 14, 16]. The low prevalence of GBS positivity by culture methods as compared to other modalities may be justified by issues of technical execution, as the culture technique does not always follow CDC recommendations[3], or perhaps because this method has a much lower sensitivity than PCR-based methods.

According to the CDC [3], cultures are the gold-standard method for GBS screening in pregnant women at 35-37 weeks of gestational age. The CDC guidelines also cite other laboratory tests for GBS detection, including PCR methods. PCR-based approaches are gaining a promising role in GBS detection, largely due to their higher sensitivity [6, 13, 18]. A European consensus statement noted that failure to treat GBS-positive mothers could lead to serious adverse neonatal outcomes. Thus, it seems reasonable to consider methods with higher sensitivity even if they are associated with more

false-positive results.

On comparison of the Xpert GBS to real-time PCR, we found a high degree of agreement on negative results (93% specificity), but only reasonable agreement on positive results (53% sensitivity). These discordant results can be justified by the higher sensitivity of the combined sample enrichment/PCR method. As the maternal pathogen load that characterizes actual risk of neonatal infection is unknown, it is unclear whether a real need exists to increase the sensitivity of rapid tests or if their current parameters are sufficient to support clinical decision-making.

Considering culture as the gold standard, the Xpert GBS showed a sensitivity of 62% and a specificity of 76% in this study. According to Gavino [19], the sensitivity and specificity of the Xpert GBS were 95.8% and 64.5% respectively, while those of antenatal cultures were 83.3% and 80.6% respectively. Mueller [20] found a sensitivity of 85.7% and a specificity of 95.6% for the Xpert GBS compared to culture. These divergent results suggest that additional studies are needed to evaluate this method.

In a previous evaluation [17] of the performance of the Xpert GBS rapid test when performed intrapartum, 13.4% of performed tests failed to yield a valid result on the first attempt (7.3% erroneous, 4.4% invalid, and 1.6% yielded no result). Another study [21] reported an invalid result rate of 13.6%, while Mueller [20] reported 13.4% after a 2-hour training session on thermocycler operation. In the present study, 90.4% of tests were valid; the remainder were 0.4% inconclusive, 7.8% erroneous, and 1.5% yielded no result. Although some of the errors found in this study are justified by known problems with a batch of Xpert cartridges, the percentage of invalid tests is consistent with previous reports in the literature.

Positive GBS test results were not associated with neonatal sepsis in this study. Considering that GBS infection can be very serious and affects approximately 2% of newborns whose mothers are colonized, a larger sample would almost certainly be needed to demonstrate this association; more probably, the absence of association found in this sample suggests that intrapartum antibiotic prophylaxis is effectively preventing neonatal infection by GBS.

Conclusion

We found a high prevalence of GBS colonization with PCR-based tests. According to real-time PCR

with prior sample enrichment, 51.1% of samples were positive for GBS. This is among the highest prevalence values ever reported in Brazilian women; additional studies using the same technique are warranted to confirm these findings.

On the other hand, in this study the Xpert GBS test detected a prevalence of GBS colonization among Brazilian women similar to that found in the literature (around 30%). We conclude that the Xpert GBS test may be an option for rapid diagnosis, especially in women at risk for preterm labor and women presenting in labor who did not undergo prenatal GBS testing. This would allow initiation of appropriate antibiotic therapy, as well as reduce hospital costs and prevent development of bacterial resistance to antimicrobials. Furthermore, this would protect asymptomatic newborns whose mothers do not have GBS results available from undergoing unnecessary investigations.

Bacterial cultures for GBS detection, which are currently considered the gold-standard method by the CDC, may be replaced by more sensitive and specific methods, such as different PCR-based techniques.

Several factors interfere with the results of the different tests available for GBS detection. Additional studies are needed to compare the performance of these tests, as well as to compare their findings with clinical outcomes.

List Of Abbreviations

CDC - Centers for Disease Control and Prevention

DNA - deoxyribonucleic acid

GBS - Group B *Streptococcus*

HCPA - Hospital de Clínicas de Porto Alegre

IC - internal control

PBS - phosphate-buffered saline

PCR - polymerase chain reaction

RT-qPCR - real-time quantitative reverse-transcription polymerase chain reaction

STARD - Standards for Reporting of Diagnostic Accuracy

Tm - melting temperature

Declarations

Ethics approval and consent to participate: The study was approved by the institutional Research Ethics Committee (CAAE: 59688316.0.0000.5327) and conducted in accordance with the provisions of the Declaration of Helsinki. All patients provided written informed consent prior to enrollment.

Consent for publication: Not applicable

Availability of data and materials: All data generated or analysed during this study are included in this published article [and its supplementary information files]

Competing of Interest: The authors declare that they have no competing interests.

Funding: This work was supported by *Fundo de Incentivo à Pesquisa e Ensino, Hospital de Clínicas de Porto Alegre* (FIPE/HCPA) and *Programa de Apoio à Pós-Graduação*, *Universidade Federal do Rio Grande do Sul* (PROAP/UFRGS).

Authors' contributions: JV, EGV and LLV conceived/designed the study and worked on data collection. LLV, AVP, MLK and MMM worked on data collection. LLV, JV, EGV, CFF, DVV, AVP and APA carried out the initial analyses, drafted the initial manuscript, and critically reviewed and revised the manuscript. All authors read and approved the final manuscript as submitted.

Acknowledgements: Not applicable

References

1. Kwatra G, Cunnington MC, Merrall E, et al. Prevalence of maternal colonisation with group B streptococcus: a systematic review and meta-analysis. Lancet Infect Dis, 2016; 16: 1076-1084.
2. Verani JR, Schrag SJ. Group B streptococcal disease in infants: progress in prevention and continued challenges. Clin Perinatol, 2010; 37: 375-392.
3. Verani JR, McGee L, Schrag SJ, et al. Prevention of perinatal group B streptococcal disease--revised guidelines from CDC, 2010. MMWR Recomm Rep, 2010; 59: 1-36.
4. de-Paris F, Machado AB, Gheno TC, et al. Grouping B Streptococcus detection: comparison of PCR assay and culture as a screening method for pregnant women.

Braz J Infect Dis, 2011; 15: 323-327.

5. Ke D, Ménard C, Picard FJ, et al. Development of conventional and real-time PCR assays for the rapid detection of group B streptococci. Clin Chem, 2000; 46: 324-331.
6. Munari FM, De-Paris F, Salton GD, et al. A combined enrichment/polymerase chain reaction based method for the routine screening of Streptococcus agalactiae in pregnant women. Braz J Microbiol, 2011; 43: 253-260.
7. El Helali N, Nguyen JC, Ly A, et al. Diagnostic accuracy of a rapid real-time polymerase chain reaction assay for universal intrapartum group B streptococcus screening. Clin Infect Dis, 2009; 49: 417-423.
8. Edwards RK, Novak-Weekley SM, Koty PP, et al. Rapid group B streptococci screening using a real-time polymerase chain reaction assay. Obstet Gynecol, 2008; 111: 1335-1341.
9. Phillips EA, Tapsall J.W, Smith DD. Rapid tube CAMP test for identification of Streptococcus agalactiae (Lancefield group B). J Clin Microbiol, 1980; 12: 135-137.
10. Yeung SW, Cheung PT, Chau SL, et al. Evaluation of an in-house real-time polymerase chain reaction method to identify group B streptococcus colonization in pregnancy. J Obstet Gynaecol Res, 2015; 41: 1357-1362.
11. Meehan M, Cafferkey M, Corcoran S, et al. Real-time polymerase chain reaction and culture in the diagnosis of invasive group B streptococcal disease in infants: a retrospective study. Eur J Clin Microbiol Infect Dis, 2015; 34: 2413-2420.
12. Sharmila V, Joseph NM, Arun Babu T, et al. Genital tract group B streptococcal colonization in pregnant women: a South Indian perspective. J Infect Dev Ctries, 2011; 5: 592-595.
13. Wollheim C, Sperhacke RD, Fontana SKR, et al. Group B Streptococcus detection in pregnant women via culture and PCR methods. Rev Soc Bras Med Trop, 2017; 50:

179-183.

14. Castellano-Filho DS, da Silva VL, Nascimento TC, et al. Detection of group B Streptococcus in Brazilian pregnant women and antimicrobial susceptibility patterns. Braz J Microbiol, 2010; 41: 1047-1055.

15. Melo SCCS, Costa AB, Silva FTRD, et al. Prevalence of Streptococcus agalactiae colonization in pregnant women from the 18th Health Region of Paraná State. Rev Inst Med Trop Sao Paulo, 2018; 60: e2.

16. Zusman AS, Baltimore RS, Fonseca SNS. Prevalence of maternal group B streptococcal colonization and related risk factors in a Brazilian population. Braz J Infect Dis, 2006; 10: 242-246.

17. Picchiassi E, Coata G, Babucci G, et al. Intrapartum test for detection of Group B Streptococcus colonization during labor. J Matern Fetal Neonatal Med, 2017; 31: 3293-3300.

18. Feuerschuette OHM, Silveira SK, Cancelier ACL, et al. Diagnostic yield of real-time polymerase chain reaction in the diagnosis of intrapartum maternal rectovaginal colonization by group B Streptococcus: a systematic review with meta-analysis. Diagn Microbiol Infect Dis, 2018; 91: 99-104.

19. Gavino M, Wang E. A comparison of a new rapid real-time polymerase chain reaction system to traditional culture in determining group B streptococcus colonization. Am J Obstet Gynecol, 2007; 197: 388.

20. Mueller M, Henle A, Droz S, et al. Intrapartum detection of Group B streptococci colonization by rapid PCR-test on labor ward. Eur J Obstet Gynecol Reprod Biol, 2014; 176: 137-141.

21. Plainvert C, El Alaoui F, Tazi A, et al. Intrapartum group B Streptococcus screening in the labor ward by Xpert® GBS real-time PCR. Eur J Clin Microbiol Infect Dis, 2018; 37:

265-270.

Tables

Due to technical limitations, tables are only available as a download in the supplemental files section.

Figures

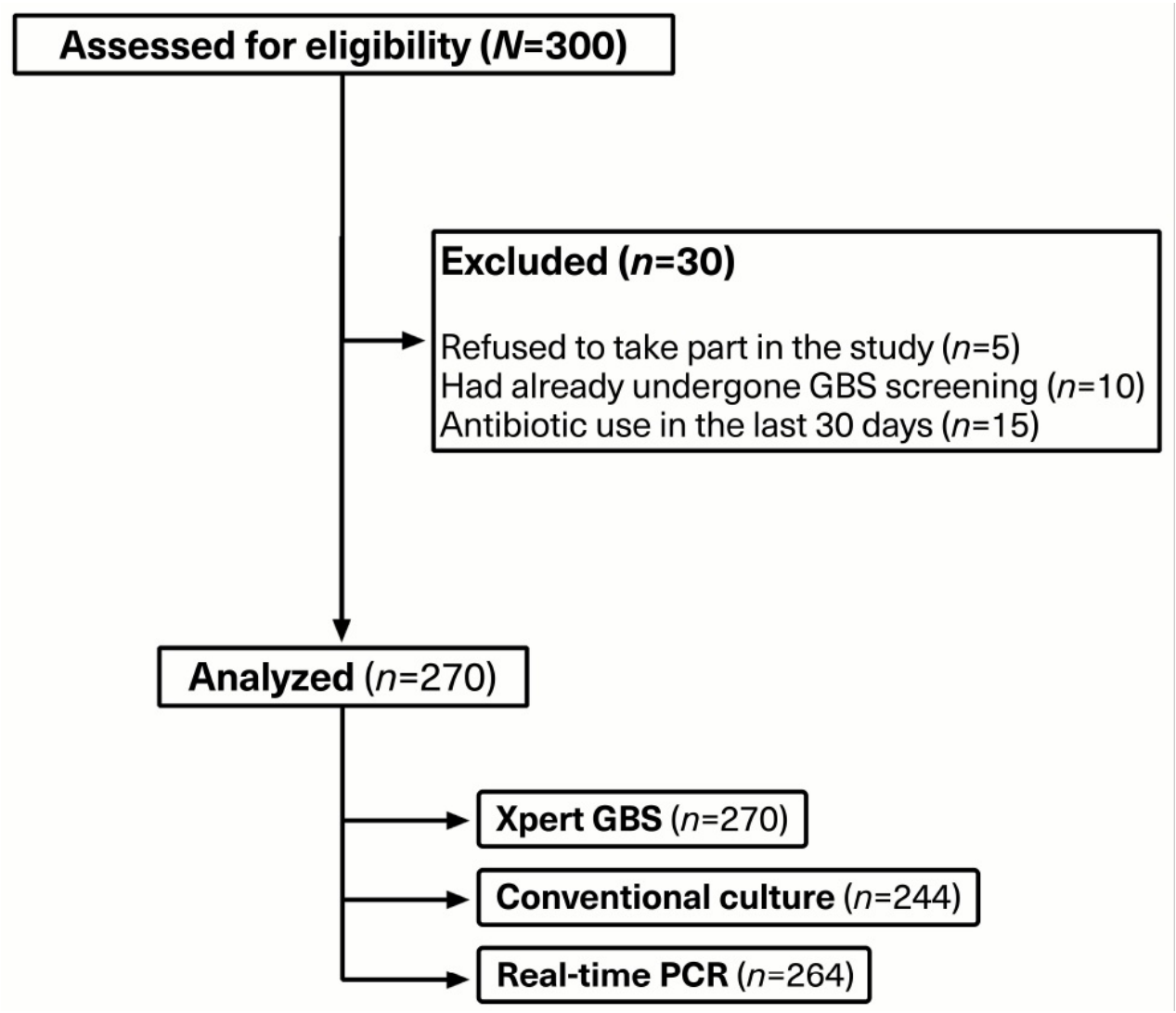

Figure 1

Flow diagram of study participants. GBS, Group B Streptococcus; PCR, polymerase chain reaction

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

Tables.pdf