JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Antenatal group B streptococcus detection in pregnant women: culture or PCR?

Gerasimos Gerolymatos[1], Paraskevi Karlovasiti[1], Argiri Sianou[1], Emmanuel Logothetis[1], George Kaparos[1], Charalampos Grigoriadis[2], Stavroula Baka[1]

[1] Department of Biopathology - Microbiology and Biochemistry, Aretaieion Hospital, Medical School, National and Kapodistrian University of Athens, Athens, Greece
[2] 2nd Department of Obstetrics and Gynecology, Aretaieion Hospital, Medical School, National and Kapodistrian University of Athens, Athens, Greece

Abstract

Introduction: Group B streptococcus (GBS) is an important cause of neonatal infections. Maternal GBS colonization screening and intrapartum antimicrobial prophylaxis of colonized women can prevent neonatal diseases. The aim of this study was to assess the prevalence of GBS colonization in pregnant and non-pregnant women and to compare the performance of a polymerase chain reaction (PCR) assay with the established as gold standard technique, culture method, used for the detection of this microorganism.

Methodology: Vaginal and rectal samples collected from 857 pregnant and 370 non-pregnant women were examined through cultures, while the samples collected from 452 pregnant women between 35 and 37 weeks of gestation were assayed by culture and PCR method targeting the *cfb* gene.

Results: GBS colonization was present in both pregnant and non-pregnant women. The colonization rate was similar in non-pregnant and first trimester pregnant women and then increased from first to the third trimester of pregnancy. GBS cultures for vaginal and rectal samples were positive in 13.2% and 14.3% in non-pregnant women, while in pregnant women 13.2% and 13.7% in the first trimester, and 15.0% and 16.5% in the second trimester, respectively. In third trimester pregnant women, compared to culture method, PCR identified a significantly increased number of GBS positive vaginal (18.4% vs 22.6%, p = 0.0006) and rectal (18.1% vs 21.2%, p = 0.01) samples.

Conclusions: GBS colonization rate was higher in the third trimester. PCR proved to be a rapid and useful GBS screening method allowing a shorter detection time, while identifying more colonized women than culture.

Key words: Group B streptococcus; pregnancy; vaginal culture; polymerase chain reaction; *cfb* gene; neonatal disease.

J Infect Dev Ctries 2018; 12(8):631-635. doi:10.3855/jidc.10367

(Received 19 March 2018 – Accepted 29 June 2018)

Introduction

Streptococcus agalactiae or Lancefield group B streptococcus (GBS) has been acknowledged as a leading cause of severe and possibly life-threatening neonatal infections, including pneumonia, meningitis and sepsis [1,2]. Pregnant women are often colonized with this microorganism in their gastrointestinal and genitourinary tracts, putting the neonates at risk through vertical transmission. In order to prevent these severe complications, revised CDC guidelines recommend universal culture-based screening of vaginal and rectal samples obtained from all pregnant women at 35-37 weeks of gestation to identify those at risk, who should receive prophylactic intrapartum antibiotic treatment [1]. Through the implementation of screening programs and the introduction of prophylactic antibiotic therapy of colonized mothers, a significant decline in GBS neonatal infections has been noted [3,4].

Although in the CDC guidelines culture is still the preferred method as gold standard method, other laboratory methods are encouraged [1]. In the last decade, molecular techniques and in particular, polymerase chain reaction (PCR) tests have been developed for the rapid detection of GBS even after admission in the delivery room. Characterized by higher sensitivity and specificity, the rapid PCR assays have gained increased attention over the time-consuming culture-based methods [5-8].

This study aimed to evaluate the prevalence of GBS in non-pregnant and pregnant women during the course of gestation and to compare the effectiveness of a PCR test, if any, when compared to the gold standard culture method.

Methodology

Pregnant women presenting to our hospital for antenatal check-up and a group of non-pregnant, reproductive age women, presenting for Pap smear test, were invited to participate in this study. Patients who had received antimicrobial therapy were not included in this study. Approval from the Ethics Committee of Aretaieion Hospital was obtained prior to the beginning of this study and informed consent was obtained from all the participants.

Vaginal and rectal swab specimens were collected from all subjects, immediately placed in Stuart's bacterial transport medium and sent to the microbiology laboratory for processing. All swab specimens were inoculated in Todd-Hewitt broth (Difco, Detroit, MI, USA) supplemented with nalidixic acid and gentamicin and incubated at 36° C in 5% CO_2 for 24 hours. The following day, the broths were subcultured onto 5% sheep blood agar plates (Bioprepare, Keratea-Attiki, Greece) and incubated for 24 hours in the same conditions. The plates were inspected daily for two days and suspected colonies, beta-haemolytic or non-haemolytic, were identified as GBS based on colony morphology, Gram staining, catalase test, bile-esculin test and confirmed by CAMP test, latex agglutination test AVIPATH STREP (Omega Diagnostics Ltd., Omega House, Hillfoots Business Village, Alva, Scotland, UK) and, finally, with the VITEK2 automated system (BioMerieux, Marcy l'Etoile, France) using the AST-ST01 card.

Furthermore, the 452 specimens collected between 35-37 weeks of gestation were tested by a PCR assay targeting the *cfb* gene which encodes the Christie-Atkins-Munch-Petersen (CAMP) factor. After incubation into Todd-Hewitt medium, broths were centrifuged and each precipitate was washed with 1X PBS solution and then resuspended in TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 7.5). This solution was submitted for DNA extraction and the extraction protocol was performed using the commercial kit QIAmp DNA mini kit (Qiagen, Valencia, USA), according to manufacturer's instructions. We used 50 μL of the PCR mixture [20 mM Tris-HCl (pH 8.4), 50 mM KCl, 2.5 mM $MgCl_2$, 0.4 mM of each deoxynucleotide, 0.4 μM of each primer and 1 U Platinum *Taq* polymerase (Invitrogen)] that were added to 22 μL of DNA extract. The primers used were Sag 59 and Sag 190 as previously presented [9]. Amplification was accomplished after a period of 5 min at 94ºC, followed by 45 cycles of 30s at 94ºC, 30s at 55ºC, and 30s at 72ºC, using the GeneAmp PCR System 9700 (Applied Biosystems Inc., Foster City, USA). The amplification products were detected by electrophoresis using 10 μL of the amplified reaction mixture on 2% agarose gels containing 0.4 mg/mL ethidium bromide. The samples presenting a 153 amplicon were considered positive for GBS.

The results of the PCR assay were not disclosed to the personnel performing the cultures and vice-versa. The performance of the PCR assay was analysed by comparison with the gold standard culture method. Statistical analysis was performed using the SPSS 22.0 edition (Chicago, IL, USA). Descriptive statistical analysis (frequencies and cross tabulations) and custom non parametric binomial test for proportions for each sampling and testing technique were used to analyze data. A p-value <0.05 was considered statistically significant.

Results

A total of 205 pregnant women in the first trimester, 200 in the second and 452 women in the third trimester, specifically at 35 to 37 weeks of gestation, aged from 22 to 38 years old, were screened for GBS colonization. A group of 370 reproductive age women, asymptomatic, non pregnant, presenting for their routine Pap smear test, and aged from 25 to 36 years old, was also included.

Positive samples identified by culture and PCR methods are summarized in Table 1. There were no statistical significant differences between GBS colonization rates in pregnant and non-pregnant women in regard to pregnancy or gestational age at the time of examination. Furthermore, no significant differences were found between the vaginal and rectal colonization rates in all groups.

All samples found positive by culture method were confirmed to be positive by PCR assays. However, PCR

Table 1. Group B streptococcus colonization rate determined by culture and polymerase chain reaction (PCR) method.

Sample	Culture				PCR
	Non-pregnant (n = 370)	1st trimester (n = 205)	2nd trimester (n = 200)	3rd trimester (n = 452)	3rd trimester (n = 452)
Vaginal	49 (13.2 %)	27 (13.2 %)	30 (15.0 %)	83 (18.4 %)*	102 (22.6 %)*
Rectal	53 (14.3 %)	28 (13.7 %)	33 (16.5 %)	82 (18.1 %)#	96 (21.2 %)#

*p = 0.0006; #p = 0.01.

identified more colonized women in the third trimester of pregnancy than culture method. Specifically, among the 452 women at 35 to 37 weeks of gestation, positive results were obtained in 102 (22.6%) vs. 83 (18.4%) vaginal samples (p = 0.0006) and 96 (21.2%) vs. 82 (18.1%) rectal samples (p = 0.01), by PCR compared to culture, respectively.

All GBS colonized pregnant women at 35-37 weeks of gestation received intrapartum antimicrobial prophylaxis and no colonized neonates were identified or neonatal diseases in the population studied.

Discussion

GBS remains a common cause of neonatal diseases, although preventive actions have decreased their incidence in many countries [2]. CDC recommends culture-based GBS colonization screening at 35 to 37 weeks of gestation and adequate intrapartum antimicrobial prophylactic therapy which has been shown to stop the vertical transmission from mother to her offspring and to decrease the risk of neonatal GBS infections [1]. Accurate and rapid identification of this pathogen is of outmost importance in order to reduce the number of women unnecessary receiving antimicrobial prophylaxis and, as a result, the percentage of neonates exposed to these compounds and their possible adverse effects including allergy and asthma [3,4].

In this study, the colonization rate in non-pregnant women was 14.3% while during pregnancy ranged from 13.7% in the first trimester, to 16.5% in the second trimester and 18.4% in the third trimester by culture method. At 35-37 weeks of gestation the PCR assay identified 22.6% subjects with positive GBS samples. GBS colonization rate demonstrated by culture in this study correlates well with previous studies [5,8]. The rate of vaginal colonization ranges from 6.5% to 36% in Europe [7,10]. A previous report in Greece has demonstrated that the maternal colonization reached 6.6%, while the incidence of neonatal GBS invasive disease was very low (0.5%) [11]. However, in the second trimester of pregnancy the maternal colonization rate was 12.5% [12]. Different factors such as geographic location, socio-economic status, age, parity, gestational age and genetic factors might explain the GBS colonization rates that vary significantly in different populations [13].

The ability to identify and isolate GBS is highly dependent on the specimen type, the time of collection and the implemented method [8,14]. CDC recommends that GBS colonization screening should be determined through the collection and adequate laboratory processing of both vaginal and rectal samples [1]. Even a single combined rectovaginal specimen can be used, since sampling both anatomical sites can enhance antenatal detection rate, compared to a vaginal sample only [1,5,15]. In this study, both vaginal and rectal samples were collected from all subjects. Previous data have demonstrated that vaginal samples alone provide lower yield than combined vaginal and rectal samples [1,2,4,15,16] and that cultures performed with samples collected early in the third trimester have a low predictive value in identifying GBS colonization at term [17].

Interestingly, the GBS colonization status can change, a positive result at 35 weeks can turn negative at delivery, leading to unnecessary prophylactic treatment [18,19] and vice-versa, probably leaving these women exposed to the risk of vertical transmission [20]. Maternal GBS colonization can be transitory or intermittent as previously demonstrated [4,21,22] and this observation raises the question regarding the adequacy of screening at 35-37 weeks instead of performing the test at the time of delivery. Since GBS colonization is present before 34-35 weeks of gestation, as demonstrated in this study, when screening is recommended, preterm neonates born before 34 weeks are still at increased risk for GBS infections. When reviewing all the patients' journals after delivery and since all colonized women included in this study received intrapartum antibiotic prophylaxis, no cases of neonatal GBS infections have been recorded.

The use of selective enrichment broths and subsequent culture is currently proposed as gold standard method detecting a significant number of colonized women [1,22]. However, culture requires up to 48 to 72 hours to yield results. Except for being time consuming, culture requires trained personnel for optimal culture conditions, recognition of colonies, proper handling and identification [15]. Nevertheless, members of the genital tract normal flora can interfere with the isolation of GBS by inhibiting its growth, resulting in false negative results [15]. In fact, negative cultures have been obtained from pregnant women whose neonates developed GBS infection [1].

Although the revised CDC guidelines [1] recommend universal culture-based GBS screening between 35 and 37 weeks of pregnancy, the use of molecular methods as an additional rapid method of detection is encouraged. In concordance to our data, increased GBS detection rates by PCR assays compared to culture have been previously reported [15,23] with comparable results [5,6,8,24] or with lower

colonization rates than the ones observed in this study [25]. However, previous data showed comparable performance of PCR method to culture [14]. El Helali *et al*. [18] reported that screening by PCR at delivery reduced the number of neonatal GBS infections as well as their severity together with the hospital costs when compared to culture based screening method at 35-37 weeks of gestation. However, PCR needs sophisticated and expensive setup and is not available in all laboratories [14]. Furthermore, its inability to provide antimicrobial susceptibility testing constitutes a significant disadvantage, especially with the increased resistance to antimicrobials exhibited by GBS. It can be assumed that until cheaper PCR will be available, together with the possibility of antimicrobial susceptibility testing, cultures will still be needed in resource constrained laboratories to provide information regarding the susceptibility to antimicrobials for the best management options, especially for penicillin-allergic patients [3].

Conclusion

This study demonstrated that PCR is a fast and efficient screening method for GBS. Vaginal together with rectal samples can improve detection rates. Since GBS represents a significant pathogen that can colonize the female genital tract at any point during pregnancy, its presence should be carefully monitored. Although further research is needed in order to find the ideal screening method and timing, a rapid, yet sensitive and specific test for GBS detection, ready to use at any given point during pregnancy, would be the best option for early detection and effective prevention, especially in patients presenting for delivery with unknown GBS colonization status.

References

1. Centers for Disease Control and Prevention (2010) Prevention of perinatal group B streptococcal disease. Revised guidelines from CDC. MMWR Recomm Rep 59: 1-32.
2. Jordan JA, Hall G, Davis T (2010) Multicenter study evaluating performance of the Smart Group B Streptococcus (GBS) assay using an enrichment protocol for detecting GBS colonization in patients in the antepartum period. J Clin Microbiol 48: 3193-3197.
3. Capanna F, Emonet SP, Cherkaoui A, Irion O, Schrenzel J, Martinez de Tejada B (2013) Antibiotic resistance patterns among group B streptococcus isolates: implication for antibiotic prophylaxis for early-onset neonatal sepsis. Swiss Med Wkly 143: w13778.
4. Poncelet-Jasserand E, Forges F, Varlet MN, Chauleur C, Seffert P, Siani C, Pozzetto B, Ros A (2013) Reduction of the use of antimicrobial drugs following the rapid detection of *Streptococcus agalactiae* in the vagina at delivery by real-time PCR assay. BJOG 120:1098-1109.
5. Rallu F, Barriga P, Scrivo C, Martel-Laferriere V, Laferriere C (2006) Sensitivities of antigen detection and PCR assays greatly increased compared to that of the standard culture method for screening for group B streptococcus carriage in pregnant women. J Clin Microbiol 44: 725-728.
6. Shabayek S, Abdalla S, Abouzeid AM (2010) Comparison of scpB gene and cfb gene polymerase chain reaction assays with culture on Islam medium to detect Group B Streptococcus in pregnancy. Indian J Med Microbiol 28: 320-325.
7. De Tejada BM, Pfister RE, Renzi G, Francois P, Irion O, Boulvain M, Schrenzel J (2011) Intrapartum group B streptococcus detection by rapid polymerase chain reaction assay for the prevention of neonatal sepsis. Clin Microbiol Infect 17: 1786-1791.
8. Konikkara KP, Baliga S, Shenoy S, Bharati B (2014) Evaluation of culture, antigen detection and polymerase chain reaction for detection of vaginal colonization of group B streptococcus (GBS) in pregnant women. J Clin Diagn Res 8: 47-49.
9. Ke D, Menard C, Picard FJ, Boissinot M, Ouellette M, Roy PH, Bergeron MG (2000) Development of conventional and real-time PCR assays for the rapid detection of group B streptococci. Clin Chem 46: 324-331.
10. Khan MA, Faiz A, Ashshi AM (2015) Maternal colonization of group B streptococcus: prevalence, associated factors and antimicrobial resistance. Ann Saudi Med 35: 423-427.
11. Tsolia M, Psoma M, Gavrili S, Petrochilou V, Michalas S, Legakis N, Karpathios T (2003) Group B streptococcus colonization of Greek pregnant women and neonates: prevalence, risk factors and serotypes. Clin Microbiol Infect 9: 832-838.
12. Daskalakis G, Papapanagiotou A, Mesogitis S, Papantoniou N, Mavromatis K, Antsaklis A (2006) Bacterial vaginosis and group B streptococcal colonization and preterm delivery in a low-risk population. Fetal Diagn Ther 21: 172-176.
13. Valkenburg-van den Berg AW, Sprij AJ, Oostvogel PM, Mutsaers JA, Renes WB, Rosendaal FR, Joep Dorr P (2006) Prevalence of colonization with group B streptococci in pregnant women of a multi-ethnic population in The Netherlands. Eur J Obstet Gynecol Reprod Biol 124: 178-183.
14. Berg BR, Houseman JL, Garrasi MA, Young CL, Newton DW (2013) 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 51: 1253-1255.
15. De-Paris F, Machado AB, Gheno TC, Ascoli BM, Oliveira KR, Barth AL (2011) Group B Streptococcus detection: comparison of PCR assay and culture as a screening method for pregnant women. Braz J Infect Dis 15: 323-327.
16. Håkansson S, Källén K, Bullarbo M, Holmgren PÅ, Bremme K, Larsson A, Norman M, Norén H, Ortmark-Wrede C, Petterson K, Saltvedt S, Sondell B, Tokarska M, von Vultee A, Jacobsson B (2014) Real-time PCR-assay in the delivery suite for determination of group B streptococcal colonization in a setting with a risk-based antibiotic prophylaxis. J Matern Fetal Neonatal Med 27: 328-332.
17. El Aila NA, Tency I, Claeys G, Saerens B, Cools P, Verstraelen H, Temmerman M, Verhelst R, Vaneechoutte M (2010) Comparison of different sampling techniques and of different culture methods for detection of group B streptococcus carriage in pregnant women. BMC Infect Dis 10: 285.
18. El Helali N, Giovangrandi Y, Guyot K, Chevet K, Gutmann L, Durand-Zaleski I (2012) Cost and effectiveness of intrapartum

group B streptococcus polymerase chain reaction screening for term deliveries. Obstet Gynecol 119: 822-829.
19. Tanaka K, Iwashita M, Matsushima M, Wachi Y, Izawa T, Sakai K, Kobayashi Y (2016) Intrapartum group B screening using real-time polymerase chain reaction in Japanese population. J Matern Fetal Neonatal Med 29: 130-134.
20. Towers CV, Rumney PJ, Asrat T, Preslicka C, Ghamsary MG, Nageotte MP (2010) The accuracy of late third trimester antenatal screening for group B streptococcus in predicting GBS colonization at delivery. Am J Perinatol 27: 785-790.
21. El Helali N, Nguyen JC, Ly A, Giovangrandi Y, Trinquart L (2009) Diagnostic accuracy of a rapid real-time polymerase chain reaction assay for universal intrapartum group B streptococcus screening. Clin Infect Dis 49: 417-423.
22. Alfa MJ, Sepehri S, De Gagne P, Helawa M, Sandhu G, Harding GKM (2010) Real-time PCR assay provides reliable assessment of intrapartum carriage of group B streptococcus. J Clin Microbiol 48: 3095-3099.
23. Wollheim C, Sperhacke RD, Fontana SKR, Vanni AC, Kato SK, de Araujo PR, Barth AL, Madi JM (2017) Group B streptococcus detection in pregnant women via culture and PCR methods. Rev Soc Bras Med Trop 50: 179-183.
24. Helmig RB, Gertsen JB (2017) Diagnostic accuracy of polymerase chain reaction for intrapartum detection of group B streptococcus colonization. Acta Obstet Gynecol Scand 96: 1070-1074.
25. Bakhtiari R, Soltan Dallal MM, Mehrabadi JF, Heidarzadeh S, Pourmand MR (2012) Evaluation of culture and PCR methods for diagnosis of group B streptococcus carriage in Iranian pregnant women. Iranian J Publ Health 41: 65-70.

Corresponding author

Assoc. Prof. Stavroula Baka, M.D.
76 Vas. Sofias Avenue, 11528 Athens, Greece
Phone/Fax: +30-210-7286150;
E-mail address: sbaka@aretaieio.uoa.gr

Conflict of interests: No conflict of interests is declared.