Colonization Rate of Group B Streptococcus (GBS) in Pregnant Women Using GBS Agar Medium

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Abstract- Group B streptococci (GBS) or *Streptococcus agalactiae* are members of the normal flora of the female genital tract. GBS normally colonizes the vagina in many women asymptomatically. During labor this organism may infect the newborn, leading to neonatal sepsis and meningitis. This study aimed to investigate the prevalence of group B streptococcus in pregnant women by a rapid and easy culture method. It seems that in cases in which GBS carriage is not suspected until the time of labor, using such a quick and specific culture method would be valuable. A total of 330 vaginal swabs were collected from women attending delivery room at Hedayat hospital, Tehran, Iran, from April through July 2008. Cotton swabs contaminated with vaginal fluid were placed into Amies transport medium and transported to the Avicenna laboratory daily. Vaginal specimens were cultured on selective GBS Agar Base medium (ISLAM) for isolation and detection of group B streptococcus. The plates were incubated at 35-37°C under anaerobic condition for 24 hours. Incubated *S.agalactiae* developed orange/red pigmented colonies in GBS agar plates. Among the 330 women, the results of the culture were positive for GBS in 68 women (20.6%). Statistical analyses showed no significant relationship between demographics, reproductive histories and obstetric characteristics of subjects with the test results. Solely the antibiotic therapy was associated with GBS colonization. The results are indicating that the relatively high maternal GBS colonization rate in pregnant women warrants a routine screening and prophylactic treatment of the infected women. Colonization with group B streptococcus can be identified directly by GBS agar medium and decrease the time to detection of GBS.

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Key words: Group B streptococcus (GBS), Perinatal infection, GBS agar medium

Introduction

Group B beta-hemolytic streptococcus (GBS) or *Streptococcus agalactiae* is a species of the normal flora of the gut and female urogenital tract (1). GBS infections occur as early onset disease of the neonate on day 1-7 after birth or late onset disease on day 7-90. Perinatal infection causes septicemia, meningitis or pneumonia, which are associated with a high mortality.

A baby can acquire *S. agalactiae* and develop neonatal GBS infections by contact with the genital tract of the mother during labor and delivery (2). Infants who have such infections may require prolonged hospitalization, and those who survive may have mental retardation or visual loss (3). Therefore, identification of GBS-colonized women is critical for prevention of neonatal GBS infection. Among pregnant women, the prevalence of colonization with group B streptococci varies in countries owing to socioeconomic and ethnic differences (4,5).

The Center of Disease Control (CDC) recommends the screening of all pregnant women for vaginal and rectal GBS colonization between 35 and 37 weeks of gestation (6). The standard method for the diagnosis of group B streptococcal colonization consists of culturing vaginal secretions in a selective broth medium that inhibits the growth of other microorganisms (7). However, this method requires at least 36 hours, because the broth

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must be incubated for 18 to 24 hours and then subcultured on agar plates, and group B streptococci must be identified using bacteriologic criteria. Although widely utilized and considered the gold-standard method, alternative methods have emerged with the goal of improving sensitivity and specificity while reducing the incubation time and need for additional plated media (8-11).

In order to decrease the time to detection GBS using culture, media of Islam type with starch and horse serum have also been developed (9,10). These media have the great advantage of being able to identify the growth of GBS by the production of carotinoid pigment. GBS agar medium allows the direct and easy identification of GBS-colonized pregnant women permitting the use of intrapartum antibiotic prophylaxis to prevent the vertical transmission of GBS from colonized pregnant women to their neonates.

Although great progress has been made in preventing GBS, only a few epidemiological studies have produced comprehensive data on GBS colonization rate in different region of Iran. This study aimed to investigate the prevalence of GBS in our population of pregnant women attending delivery room at Hedayat hospital, Tehran, Iran. We used GBS agar medium for rapid and specific detection of group B streptococcus in women whose GBS status is unknown at delivery. It seems that in cases in which GBS carriage is not suspected until the time of labor, using such a quick and specific culture method would be valuable. This information will contribute to the design of an optimal public health prevention strategy for neonatal sepsis due to GBS infection in Iran.

Patients and Methods

A total of 330 pregnant women attending delivery room at Hedayat hospital, Tehran, Iran, from April through July 2008 were enrolled in this study and after signing a written informed consent which have been evaluated and proved by Avicenna ethical committee. The age of women ranged between 16 and 40 years. Specimen of vaginal fluid was obtained by brushing the lower vagina (vaginal introitus) with a sterile cotton swab before membrane rupture. The swab was immediately placed into Amies transport medium with charcoal (Hi-Media, India) and transported at room temperature to the Avicenna laboratory daily. Vaginal specimens were cultured on selective GBS Agar Base medium (ISLAM) (Oxoid, UK) with 5% horse serum for isolation and detection of group B streptococcus.

The swab was rotated over one-third of the surface of a GBS agar plate and the inoculum was then spread over the plate using an inoculating loop.

Figure 1. In plates of GBS agar medium incubated anaerobically group B streptococcus develop large heavily pigmented colonies after 24 hours at 35-37ºC

The plates were then incubated at 35-37°C under anaerobic conditions in an anaerobic jar (Hi-Media, India) with gas pack and read for the presence of orange pigmented colonies after 18–24 hours. Beta-hemolytic group B streptococci developed orange-red pigmented colonies in GBS agar plates (Figure 1).

The pigment made the colonies readily distinguishable from other organisms that may be grown on the plate. Any degree of orange development would be considered a positive result. Negative plates were reincubated for an additional 24-48 hours before being discarded.

Information concerning host factors associated with maternal GBS colonization was collected using maternal interview forms, while labor and delivery outcomes, including demographics, reproductive histories, gestational age, premature rupture of membranes and duration labor. The rates of colonization calculated on the basis of the results of culture for the total population. Data were analyzed using SPSS software (version 13) and evaluated statistically by T-test, Chi square, Fisher's exact test and multiple logistic regression model. P-values <0.05 were considered as significant.

Results

Among the 330 pregnant women, 68 (20.6 %) (95% CI 16.7-24.5%) were identified as carriers of group B streptococci on the basis of the results of culture on GBS agar. The mean age of participants was 25.83±4.6 years. The mean age of GBS-positive women was 26.0±3.9 and the mean age of GBS-negative women was 25.7±4.8.

Table 1. GBS carriage rates and demographics

Demographics	GBS-positive Number (%)	GBS-negative Number (%)	Total Number (%)	P-value
Education				
Primary & Secondary school	16 (14.7)	93 (85.3)	109 (100)	0.16
High school	41 (23.0)	137 (77.0)	178 (100)	
University	11 (25.6)	32 (74.4)	43 (100)	
Occupation				
Housewife	61 (20.2)	241 (79.8)	302 (100)	0.54
Working	7 (25.0)	21 (75.0)	28 (100)	

There was no statistical relationship between age and GBS colonization (P, 0.07). Also 7 (8.8%) of 80 women with any kind of antibiotic therapy during third trimester and 61 (24.4%) of 250 in those who were not receiving antibiotic, the results of culture were positive. Statistical analysis showed significant correlation between history of antibiotic therapy and GBS colonization status (*P*= 0.003).

Statistical analysis of demographics, reproductive histories and obstetric characteristics related to GBS carriage are shown in Tables 1-3.

Education level and occupation of mothers had no effect on GBS carriage (Table 1).

For the total population none of the following factors that might contribute to colonization were found to be significantly associated with GBS colonization status (Table 2).

GBS colonization as a predictor of labor and delivery outcome variables was also studied. GBS colonization was not associated with gestational age < 37 weeks; prolonged rupture of membrane and mode of delivery (Table 3).

Table 2. GBS carriage rates and reproductive histories

Reproductive histories	GBS-positive Number (%)	GBS-negative Number (%)	Total Number (%)	P-value
Fever				
Yes	6 (18.8)	26 (81.3)	32 (100)	0.78
No	62 (20.8)	236 (79.2)	298 (100)	
Vaginitis				
Yes	16 (19.0)	68 (81.0)	84 (100)	0.68
No	52 (21.1)	194 (78.9)	246 (100)	
Urethritis				
Yes	17 (16.7)	85 (83.3)	102 (100)	0.23
No	51 (22.4)	177 (77.6)	228 (100)	
Abortion				
Yes	11 (19.6)	45 (80.4)	56 (100)	0.84
No	57 (20.8)	217 (79.2)	254 (100)	
Infertility				
Yes	3 (15.0)	17 (85.0)	20 (100)	0.77
No	65 (21.0)	245 (79.0)	31 (100)	
Low birth weigh				
Yes	0 (0)	6 (100)	6 (100)	0.35
No	68 (21.0)	256 (79.0)	324 (100)	

Table 3. GBS carriage rates and obstetric characteristics

Obstetric characteristics	GBS-positive number (%)	GBS-negative number (%)	Total Number (%)	P-value
Gestational age				
< 37 weeks	0 (0)	9 (100)	9 (100)	0.21
> 37 weeks	68 (21.2)	253 (78.8)	321 (100)	
Membrane rupture duration				
Less than 10 h	59 (20.3)	231 (79.7)	290 (100)	0.75
10-18 h	9 (22.5)	31 (77.5)	40 (100)	
Type of birth				
Vaginal	48 (20.2)	190 (79.8)	238 (100)	0.75
Cesarean	20 (21.7)	72 (78.3)	92 (100)	

When all variables were controlled in a multivariate analysis, the association between group B streptococcal colonization and antibiotic therapy was confirmed. Among all variable solely the antibiotic therapy was associated with GBS colonization. GBS colonization tended to be less prevalent in women who received antibiotic during third trimester.

Discussion

Group B Streptococcus (GBS) is an important cause of infection in pregnant women and their newborns; however, it has been little studied in Iran. The published study from Hamadan, central west of Iran provide evidence of maternal GBS colonization. Rabiee *et al.* reported a GBS maternal colonization rate of 26.7% in 544 pregnant women in Hamadan (12). Aali *et al.* studied 101 laboring women with a gestation age of 24-37 weeks and 105 women admitted for term delivery at maternity of Afzalipour hospital in Kerman, Iran. Colonization was detected in 9.2% of all women. Although GBS colonization was found more frequently in preterm than term patients (12 v/s 7 cases), the difference was not statistically significant (13).

Several special media have been introduced to rapidly detect GBS by pigment production (10,14). The sensitivity of the standard method using selective Todd-Hewitt broth for detecting vaginal and rectal carriage of GBS was 97 and 90.9%, the corresponding values for GBS agar were 93.9 and 92.2%, and those of GBS broth were slightly lower at 89.4 and 87%, respectively. Therefore inoculation of vaginal specimens onto GBS agar or into GBS broth can be substituted for the standard CDC method for detecting GBS in women in labor (15). The clear advantages of this substitution are the reduced costs involved and a decreased time to detection, which can be at least 24 h earlier than the standard method. Production of an orange carotenoid pigment on GBS agar medium is unique to the beta-hemolytic group B streptococci isolated from humans. The use of GBS agar as a primary isolation medium can enable the detection of those organisms with the ability to produce pigment, making further subcultures unnecessary (10,16). These features make GBS agar a highly sensitive, accurate, and faster method of detecting beta-hemolytic group B streptococci. Non-hemolytic group B streptococci are infrequently (1-2%) found in clinical specimens. Although these strains do not produce the orange pigment, they grow perfectly in GBS agar.[17,18] Methods that do not rely on either hemolytic or pigment production must be used to detect them.

In the USA, two strategies are recommended to prevent perinatal GBS disease-the screening method and the risk-based approach. Intrapartum antibiotic prophylaxis is offered to all pregnant women who are found to be carrying GBS, either vaginally and/or rectally, at gestational week 35-37 or to women in labor who have risk factors for GBS transmission, e.g., fever, prolonged rupture of the membranes, or preterm delivery. Since up to 50% of the infants who develop GBS disease are born to carriers without risk factors, the screening approach seems to be more reliable (2).

Maternal GBS colonization is a risk factor for adverse pregnancy outcomes, including Prematurity (<37 weeks), low birth weight, longer duration of labor and prolonged rupture of membranes. No significant relationship between GBS colonization status and delivery outcomes were noted in this study.

In conclusion, our results are indicating that the relatively high maternal GBS colonization rate in pregnant women warrants a routine screening and prophylactic treatment of the infected women. All pregnant women should be screened for vaginal GBS colonization during labor and intrapartum chemoprophylaxis should be given to all pregnant women identified as GBS carriers. This work has confirmed that human beta-hemolytic GBS can be detected directly by using GBS agar after overnight incubation at least as reliably as they can be detected after 2 to 3 days of incubation in a selective enrichment broth and subculture onto blood agar plates. GBS agar medium would be an accurate and rapid method for identifying colonized women at the time of delivery. In addition, identification of GBS using this medium is straightforward (because of its characteristic red-orange color), resulting in an important savings the cost of reagents otherwise necessary for the accurate identification of GBS.

We found that GBS colonization was not affected by demographics and host risk factors, nor did GBS status influence the labor and delivery outcomes. Therefore, a prevention strategy in this population cannot safely rely on risk factor approach for the identification of GBS-colonized mothers. By identifying GBS colonized women who did not present with obstetric risk factors, screening reached more of the population at risk than did the risk-based approach. Benefit studies and data on the prevalence of GBS neonatal disease, preventative measures and outcome of infected infants are greatly needed in our country to allow the most appropriate preventive strategy to be selected.

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Conflict of interests

The authors declare that they have no competing interests.

References

1. Brooks GF, Butel JS, Morse SA. Jawetz, Melnick, & Adelberg's medical microbiology. 23rd ed. 2004, New York; London: Lange Medical Books/McGraw Hill. xi, 818 p.
2. Schuchat A. Epidemiology of group B streptococcal disease in the United States: shifting paradigms. Clin Microbiol Rev. 1998 Jul; 11(3): 497-513.
3. CDC. Prevention of perinatal group B streptococcal disease: a public health perspective. Centers for Disease Control and Prevention. MMWR Recomm Rep.1996 May; 45: 1-24.
4. Tsolia M, Psoma M, Gavrili S, Petrochilou V, Michalas S, Legakis N, Karpathios T. Group B streptococcus colonization of Greek pregnant women and neonates: prevalence, risk factors and serotypes. Clin Microbiol Infect. 2003 Aug; 9(8): 832-8.
5. Feikin DR, Thorsen P, Zywicki S, Arpi M, Westergaard JG, Schuchat A. Association between colonization with group B streptococci during pregnancy and preterm delivery among Danish women. Am J Obstet Gynecol. 2001 Feb; 184(3): 427-33.
6. Schrag S, Gorwitz R, Fultz-Butts K, Schuchat A. Prevention of Perinatal Group B Streptococcal Disease. MMWR Recomm Rep. 2002 Aug; 51: 1-22.
7. Baker C. Summary of the workshop on perinatal infections due to group B Streptococcus. J Infect Dis. 1977 Jul; 136(1): 137-52.
8. Rosa-Fraile M, Sampedro A, Varela J, Garcia-Pena M, Gimenez-Gallego G. Identification of a peptide from mammal albumins responsible for enhanced pigment production by group B streptococci. Clin Diagn Lab Immunol. 1999 May; 6(3): 425-6.
9. Rosa-Fraile M, Rodriguez-Granger J, Cueto-Lopez M, Sampedro A, Gaye EB, Haro JM, Andreu A. Use of Granada medium to detect group B streptococcal colonization in pregnant women. J Clin Microbiol. 1999 Aug; 37(8): 2674-7.
10. Gil EG, Rodriguez MC, Bartolome R, Berjano B, Cabero L, Andreu A. Evaluation of the Granada agar plate for detection of vaginal and rectal group B streptococci in pregnant women. J Clin Microbiol. 1999 Aug; 37(8): 2648-51.
11. de la Rosa M, Perez M, Carazo C, Pareja L, Peis JI, Hernandez F. New Granada Medium for detection and identification of group B streptococci. J Clin Microbiol. 1992 Apr; 30(4): 1019-21.
12. Rabiee S, Arab M, Yousefi Mashouf R. Epidemiologic pattern of vaginal colonization by group B Streptococcus in pregnant women in Hamadan, Central west of Iran. Iran J Med Sci. 2006 Jun; 31: 106-8.
13. Aali BS, Abdollahi H, Nakhaee N, Davazdahemami Z, Mehdizadeh A. The association of preterm labor with vaginal colonization of group B streptococci. IJRM. 2007 Dec; 5(4): 191-4.
14. Islam AK. Rapid recognition of group-B Streptococci. Lancet. 1977 Jan; 1: 256-7.

15. Votava M, Tejkalova M, Drabkova M, Unzeitig V, Braveny I. Use of GBS media for rapid detection of group B streptococci in vaginal and rectal swabs from women in labor. Eur J Clin Microbiol Infect Dis. 2001 Feb; 20(2): 120-2.
16. Forbes BA, Sahm DF, Weissfeld AS, Bailey WRDm. Bailey & Scott's diagnostic microbiology. 10th ed. 1998, St. Louis, Mo; London: C.V. Mosby Company.
17. Cueto M, Sa´nchez MJ, Serrano J, Aguilar JM, Martinez R, Rosa M. Bacteremia caused by nonhemolytic group B streptococci. Clin Microbiol News. 1996 Apr; 18(7): 55-6.
18. Miranda C, Gamez MI, Navarro JM, Rosa-Fraile M. Endocarditis caused by nonhemolytic group B streptococcus. J Clin Microbiol. 1997 Jun; 35(6): 1616-7.