

Maternal Carriage and Neonatal Colonization of *Streptococcus agalactiae* in Tabriz, Northwest Iran

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Abstract

Group B Streptococcus (GBS) is a major cause of severe systemic and local infections in newborns. This study was performed to evaluate the presence of GBS in pregnant women and their newborns in Tabriz, Iran. Vaginal specimens were collected from 965 women who were candidate for vaginal delivery for bacteriological study of group B streptococci. Several samples from various sites of the newborns' body (ear canal, nose, navel, and groin) were also obtained to study the colonization rate in newborns after vaginal delivery. Identification of GBS strains was accomplished by bacteriological and serological tests. During the study period, 327 microorganisms were isolated from vaginal specimens of pregnant women from which 5.2% were *Streptococcus agalactiae*. Serotypes of *S. agalactiae* strains in our study were Ia (17.6%), Ib (13.4%), II (14.2%), III (9.5%), IV (8.2%), V (19.5%) and nontypable (17.6%). All of the newborns were followed up for eight weeks. Low carriage rate of GBS in vaginal canal of women in this area and probably lack of more virulent serotypes of GBS may explain the rarity of disease due to *Streptococcus agalactiae* in our region.

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Keywords • *Streptococcus agalactiae* • pregnancy, septicemia • meningitis

Introduction

Lancefield group B β -hemolytic streptococci were first described as etiologic agents of septicemia in three patients in 1938.¹ Despite sporadic cases reported in 1940s to 1960s, the bacterium was frequently isolated in late 1960s in the United States and Europe. Subsequently this bacterium became a substitute for *E coli* as the most prevalent pathogen in bacteremia and meningitis in the first two months of life.

Results collected from different geographic regions indicated heterogeneous prevalence.² According to the reports from western countries, especially the United States, 5-35% of pregnant women are carriers of GBS. And 29-70% of newborns are colonized by this bacterium,³⁻⁷ which predisposes the neonates to infections such as septicemia, meningitis, and pneumoniae.^{7,8}

The frequency of maternal carriage in vagina have been reported from some developing countries, including Kuwait (14.6%),⁹ India (5.8%), Libya (5%), Saudi Arabia (13.9%),² Brazil (26%),¹⁰ Nigeria (19.5%),¹¹ and Ivory Coast (19.3%).¹² The prevalence of GBS in septicemia and meningitis reported

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as 1-5 in 1000 live births in industrialized countries.² However because of limited studies, such rate has not been reported from Iran yet. Therefore this study was designed to evaluate the frequency of maternal carriage of GBS in pregnant women referred to obstetric teaching hospitals affiliated to Tabriz University of Medical Sciences and to clarify the role of this carriage in newborns' infections.

Materials and Methods

Of a total of 1146 pregnant women referred to the obstetric teaching hospitals affiliated to Tabriz University of Medical Sciences (northwest Iran) from April 2001 to February 2002, 965 women who were candidate for vaginal delivery were selected for bacteriological study of group B streptococci in their vaginal specimens. Their neonates were also followed up for possible occurrence of septicemia, meningitis and pneumonia for eight weeks. One hundred eighty one cases were excluded because of cesarean section, discharge without labor, and stillbirth.

After filling a questionnaire, three vaginal samples were taken by sterile swabs. The first swab was used to inoculate blood agar plate (Oxoid), chocolate agar plate (Oxoid), McConkey agar plate (PVI), V-agar and Todd Hewitt broth (BBL). The second swab was used to prepare slides for microscopic evaluation, and the third swab was used for a wet mount to search for *Trichomonas vaginalis* and fungi. The growth yield in Todd Hewitt broth after 24h incubation at 37°C, was studied after Gram staining and subculturing onto blood agar plates for isolation of GBS.

To study the colonization rate in newborn infants several samples from various sites of the body (ear canal, nose, navel, and groin) were also obtained within 3 hours after delivery for culture using sterile swabs soaked in Todd Hewitt broth medium and inoculated onto sheep blood agar plates. The plates were incubated at 37°C for 48h and then studied for GBS. Streptococci isolated from test plates were identified using conventional bacteriological methods, including colonial morphology, hemolysis, bacitracin sensitivity, sodium hippurate hydrolysis, CAMP test, and serologic latex agglutination test (Bio Merieux).

Serotypes of GBS were determined by agglutination method using antisera for serotypes Ia, Ib, II, III, IV and V (Denka Seiken, Japan). The procedure was performed in accordance with the manufacturer's instructions. Todd Hewitt broth was inoculated with the test organism and incubated at 37°C overnight. After centrifugation, swine pancreatic extract and phenol-red solution were added to 0.5 ml of sediment. The

pH was adjusted to 8-8.5, and the mixture was incubated at 37°C for 1h. After centrifugation, 0.5 ml of phosphate-buffered saline (pH 7.2) was added to the sediment. The bacterial suspension was treated at 120°C for 30 minutes and agglutination tests were performed on glass slides using specific antisera. Strong agglutination within 1 minute was recorded as positive.

Statistical Analyses

SPSS software version 13 was used and the data were analysed using the Chi-square test for pair differences. $P < 0.05$ was considered to be significant. Descriptive summarization of data consisted of frequency counts and percentages. Fisher exact test was used for non-categorical variables.

Results

The microorganisms isolated from vaginal samples are listed in table 1. As for the order of microbial isolation, *S. agalactiae* (GBS) ranked sixth, with incidence of 17 cases (5.2%). And only 1.7% of the newborns were colonized by *S. agalactiae*.

Table 1: Microorganisms isolated from vaginal canal of pregnant women prior to labor

Organisms	No (%) of isolated organisms	% of colonization (in newborns)
<i>Escherichia coli</i>	92 (28.1)	9.5
<i>Candida albicans</i>	54 (16.5)	5.6
<i>Streptococcus spp.</i>	48 (14.7)	4.9
<i>Klebsiella spp.</i>	30 (9.2)	3.1
<i>Staphylococcus aureus</i>	21 (6.4)	2.1
<i>Streptococcus agalactiae</i>	17 (5.2)	1.7
<i>Enterobacter spp.</i>	16 (4.9)	1.6
<i>Proteus spp.</i>	12 (3.7)	1.2
Non-ferment Gram – ve bacilli	11 (3.4)	1.1
<i>Gardnerella vaginalis</i>	10 (3.1)	1.0
<i>Trichomonas vaginalis</i>	9 (2.7)	0.9
<i>Hafnia alveii</i>	7 (2.1)	0.7

Table 2 shows GBS positive women and the sites of isolation of *S. agalactiae* from their newborn infants. The higher rates of isolation had been from ear canal (80%) and nose (60%). Of the 17 GBS positive mothers, 5 transmitted *S. agalactiae* to their newborns. Colonization of infants with micro-organisms present in mothers' vagina is shown in figure 1.

Despite the absence of maternal carriage in two cases their newborns had been colonized by GBS. Serotypes of *S. agalactiae* strains isolated in this study were Ia (17.6%), Ib (13.4%), II (14.2%), III (9.5%), IV (8.2%), and V (19.5%). Remaining strains (17.6%) were non-typable.

Table 2: *Streptococcus agalactiae* positive pregnant women and the sites of isolation of GBS from their newborn infants

Pregnant women				Newborn infants				
No	Age (yr)	Parity	Abortion history	Ear	Nose	Navel	Groin	Weight (kg)
1	18	2	1	+	+	-	-	3.00
2	30	4	-	+	-	-	-	3.10
3	19	-	-	+	+	-	-	2.90
4	24	3	1	+	+	+	-	1.60
5	20	-	-	-	-	-	-	3.40

+ isolation of GBS; - lack of GBS

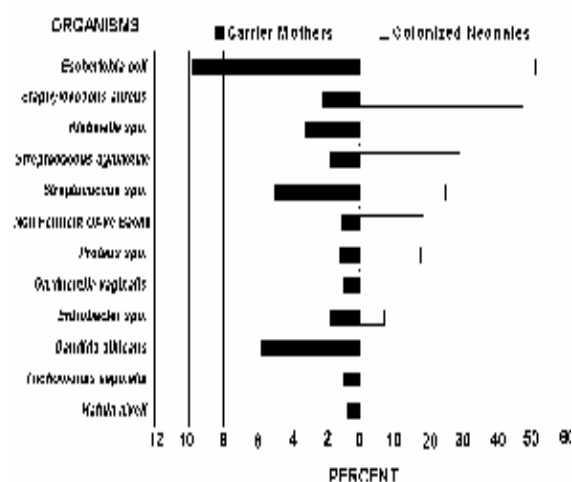


Figure 1: Colonization of newborns by organisms present in their mothers' vagina.

The relation between premature rupture membrane and colonization of newborns was examined using Fisher exact test, which was statistically significant ($p=0.009$).

Discussion

In this study various micro-organisms such as *E coli*, *Candida albicans*, and *Streptococcus spp* were isolated from vaginal canal of pregnant women. These findings were in concord with other studies.¹³⁻¹⁵ GBS was isolated from 17 cases (5.2%). The frequency of 1.7% colonisation in the newborns was indicative of a low carrier rate in our pregnant women. Studies performed in other countries have reported an overall higher carriage rates including 26% in Brazil,¹⁰ 22% in Gambia,⁴ 18.6% in the United States,¹⁵ 11.6% in Canada,¹⁶ 10.5% in England,¹⁷ 9.2% in Saudi Arabia,¹⁸ 6.6% in Greek,¹⁹ 6.5% in Israel,²⁰ 5.8% in India,²¹ and 2.9% in Japan.²² The low carrier rate found in the present study may account for the rare infections caused by GBS.

In some studies carried out to examine the invasive infections by *S. agalactiae*, the potential of invasiveness has been related to serological types of the organism. *S. agalactiae* strains have been divided into at least 9 serotypes (Ia, Ib, II to VIII) on the basis of different chain structures of its capsular polysaccharide.^{9,23} Serotype III is the

most invasive.^{3,4} In this study frequencies of the serotypes of isolated *S. agalactiae* strains were 17.6%, 13.4%, 14.2%, 9.5%, 8.2%, 19.5% and 17.6% for Ia, Ib, II, III, IV, V, and nontypable, respectively. So the rarity of GBS diseases in Iranian newborns may be due to the low rates of maternal carriage and the less virulent GBS serotypes.

Because *S. agalactiae* originates from intestinal flora and colonizes individuals,²⁴ hygienic condition of the perineal and vaginal regions is therefore of paramount importance and can reduce the colonization rate. Meanwhile some reports indicated the presence of the bacterium in semen.²⁵ Transmission of the GBS through sexual contact is another route of contamination, but low rate in multi- sexual partnership in Iran may in part explain the rarity of maternal carriage in our country. In our study, *S. agalactiae* was isolated from blood cultures of only one newborn, who contracted early infection, and was born to a GBS⁺ mother. According to our knowledge, this case was a rare report of septicemia in infants due to GBS in Iran.

Information collected from questionnaires showed no significant relationship between maternal carriage and number of parity. Also no significant relationship was found in regard to abortion history ($p>0.05$). Higher isolation of GBS from ear canal of newborns was in agreement with previous findings.²⁶ In two neonates born to GBS⁻ mothers, the bacterium was found in ear canal or in navel and groin, which was suggestive of a hospital-acquired source. Indeed, similar findings with higher rates (22%) have been reported previously in Gambian infants.⁴

Association of occupation in mothers and their husbands, gestation age, presence of fever and discharge during pregnancy and also premature rupture of membranes were studied in GBS⁺ and GBS⁻ mothers and statistical analyses showed no meaningful correlation ($p>0.05$), except in the cases who had vaginal discharge during pregnancy and those with premature rupture of membranes ($p<0.05$). The recent finding underlines the control of *S. agalactiae* in pregnant women, especially in the third trimester to prevent its transmission to infants.²⁷ Membranes and its rupture showed a

good correlation with colonization rate in infants, as only one GBS colonized infant was born to 12 pregnant women with intact membranes. While four GBS colonized infants were born to five pregnant women with ruptured membranes, which underlined the role of membranes in prevention of colonization in newborn infants.

Conclusion

The results of this study indicated the presence of *S. agalactiae* in vaginal canal of women from northwest of Iran, but the status of maternal carriage and subsequent low colonization rate of their newborns accounted for rarity of septicemia and other consequences of this important bacterium in Iranian newborns.

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