Carriage Rate of Group B Streptococci in Pregnant Women in Three Teaching Hospitals in Shiraz, Iran

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Key Words

Carriage rate · Group B streptococci · Pregnant women · Shiraz · Iran

Abstract

Objective: The main goal of this study was to assess differences in the vaginal flora of pregnant women and provide a detailed evaluation of vaginal swabs for the presence of group B streptococcus (GBS) and other organisms in three teaching hospitals in Shiraz, southwest of Iran. **Subjects and Methods:** In a cross-sectional study from April 2006 to March 2007, 310 women with vaginal delivery at three obstetric wards of teaching hospitals in Shiraz were studied. Swab specimens from the vagina and rectum were collected and were examined bacteriologically. All of the newborns were followed up for 3 months. **Results:** Of 310 pregnant women, 43 (13.8%) were colonized with GBS. There were no differences between GBS carriers and GBS-negative women in risk factors like preterm rupture of membranes ($p = 0.77$) and preterm labor ($p = 0.53$). One neonate was hospitalized due to meningitis and GBS was detected in CSF culture and Gram staining. **Conclusion:** Our data showed that the prevalence of GBS carriage rate in pregnant women in Shiraz, Iran was high. The poor correlation between risk factors and GBS carriage requires further investigation in larger groups.

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Introduction

Streptococcus agalactiae, belonging to group B streptococci (GBS), emerged during the 1960s as an important cause of neonatal disease, and by the 1970s, it was already established as a leading cause of infections in the newborn [1, 2]. Also GBS is an important agent of maternal infections, such as chorioamnionitis, endometritis, urinary tract and surgical-site infections [3–5]. Two forms of GBS disease in infants are well recognized: early- and late-onset disease. Early-onset disease is defined as isolation of GBS from a normally sterile site (i.e., blood and/or cerebrospinal fluid) in an infant less than 7 days of age with clinical symptoms and signs compatible with a systemic infection. It accounts for 80–85% of neonatal infections, has a higher mortality rate and it is acquired through vertical transmission from colonized mothers [1, 5, 6]. Exposure of the neonate to the organism occurs either by an ascending route in utero through ruptured or intact membranes or by acquisition during passage through the birth canal. The three most common clinical presentations include sepsis, pneumonia, and meningitis [5].

Late-onset disease usually occurs in infants between 1 week and up to 3 months of age with meningitis being the most common clinical presentation [5, 7]. The source of bacterial strains causing late-onset disease is less well

understood and may involve community or nosocomial acquisition, although there is also evidence that in some infants with late-onset disease, the GBS causing the infection shares the same serotype as the GBS isolated from their mothers, suggesting a maternal source [5, 8].

Results collected from different geographic regions indicate a heterogeneous prevalence. Rates of colonization may vary greatly according to the sample collection site, the culture medium used, the ethnic group, geographical location, socioeconomic status, immunological factors and the age of the population investigated [9, 10].

According to reports from Western countries, especially the United States, vaginal colonization occurs in 11–30% of all pregnant women, and 50–75% of their infants become colonized on their mucosal surfaces and on skin [7, 11, 12]. The frequency of maternal carriage in vagina has been reported from some developing countries, including Kuwait: 14.6% [13], India: 5.8%; Libya: 5%; Saudi Arabia: 13.9% [14], and Brazil: 14.6% [9].

With the objective of reducing neonatal morbidity and mortality due to GBS, different countries have implemented strategies of intrapartum prophylactic antibiotic therapy, resulting in a significant decrease in the incidence of neonatal infection from 2.7 to 0.4 per 1,000 live births [9, 15]. Strategies involve antenatal screening to detect colonization or treatment of women with risk factors such as prolonged rupture of membranes, intrapartum fever, preterm labor and history of maternal colonization during pregnancy which reflects in part the burden of GBS. The Centers for Disease Control (CDC) recommend either risk assessment or screening for group B streptococcal colonization in pregnant women to identify candidates for intrapartum prophylaxis. Screening consists of obtaining vaginal and anal specimens for culture at 35–37 weeks' gestation. Women can be negative in the first 30 weeks and then become positive in the last 10 weeks. Those who are positive in the last trimester could be negative in the first or second [15]. In Iran, there is no uniform policy and many institutions do not adhere strictly to the CDC guidelines. Furthermore, there are few publications on this subject, and some authors have found a GBS isolation rate of 5–25% among Iranian parturient women [16, 17]. Herein, we report the colonization rate of GBS in pregnant women and their neonates and some risk factors in the city Shiraz, Southwest of Iran in order to adopt a preventive strategy for decreasing probable invasive infections in neonates [18].

Subjects and Methods

Study Design

This study was conducted from April 2006 to March 2007 at three obstetric teaching hospitals (Zeinabie, Shooshtari and Shiraz). During the study period, none of the participating hospitals had a written policy for prevention of GBS disease through intrapartum chemoprophylaxis. Three hundred and ten women were studied over a 1-year period (2006–2007), of whom 137 were from Shiraz Hospital, 107 from Zeinabie Hospital and the remaining 66 from the Shooshtari Hospital. Mean age was 25.6 ± 5.4 years (20.2–31.0). The study was approved by the Research Ethics Committee, Shiraz University of Medical Sciences, and all participating women gave written informed consent.

Culture and Identification of GBS

The specimens of 310 pregnant women with gestational age of 20 weeks or more who presented to the labor and delivery room were cultured for GBS. A single vaginorectal swab for culture of GBS was taken from participants. All samples were taken before conducting a pelvic examination and before using any disinfecting materials. The swab was first inserted into the vagina (without using a speculum). For vaginal specimens, excessive secretions or discharge was wiped out, and secretions from the mucosa of the lower third of the vagina were obtained with a swab. Afterwards, a swab was carefully inserted approximately 2.5 cm beyond the anal sphincter and then gently rotated to touch anal crypts.

Swabs were immediately placed in selective enrichment Todd-Hewitt broth with gentamicin (8 mg/l) and nalidixic acid (15 mg/l), as recommended by the CDC, and sent to the Laboratory of Microbiology. At the laboratory, samples were incubated for 24 h at 37°C in a 5% CO₂ atmosphere. Colonies of beta-hemolytic streptococci and Gram-positive diplococci were plated onto blood agar and incubated for 24–48 h, after which they were identified by the negative catalase test process. After obtaining a purified GBS culture, identified both by the presence of hemolysis and negative catalase, colonization was further confirmed by two confirmatory tests: the CAMP and the positive latex agglutination tests [19]. All of the samples also underwent routine microbiological culture and bacterial isolates were identified by using Gram staining and standard biochemical methods. Chlamydia, viruses, and T-strain mycoplasma and *Trichomonas* were not sought in this study, and only simple methods of anaerobic culture were used.

After labor and delivery, all women underwent a bedside postpartum interview using a specific uniform questionnaire. Maternal demographic status was assessed, including age, nationality and origin. Maternal general health variables included verified disease or infections in present and/or past gestations, antibiotic treatment in current pregnancy; obstetric status variables included parity and the number of gestations, past abortions, number of fetuses in the current pregnancy, gestational week of membrane rupture, mode of current labor, history of cesarean sections or premature deliveries and type of contraception used before pregnancy. The medical records of all neonates born to maternal carriers were examined after 12 weeks for neonatal hospitalizations and GBS diseases (meningitis, sepsis).

Table 1. Age, ethnicity, residence, number of pregnancies and contraception method recorded for 310 pregnant women from Shiraz, Iran

Epidemiological information	GBS carrier n (%)	GBS-negative n (%)	p value
Age, years			
<20	7 (16.3)	27 (10.1)	0.98
20–34	32 (74.4)	220 (82.4)	0.65
>34	4 (9.3)	20 (7.5)	0.48
Ethnic origin			
Iranian	38 (88.4)	216 (80.9)	0.35
Afghani	5 (11.6)	51 (19.1)	
Pregnancy			
Primiparous	21 (48.8)	122 (45.7)	0.21
Multiparous	22 (51.2)	145 (54.3)	
Residence			
Urban	31 (72.1)	163 (61.1)	0.42
Rural	12 (27.9)	104 (38.9)	
Contraception			
Hormonal	8 (18.6)	104 (38.9)	0.019
Intrauterine	5 (11.6)	38 (14.2)	0.62
Barrier methods	2 (4.7)	42 (15.8)	0.017
None	28 (65.1)	83 (31.1)	
Previous abortion history			
Yes	4 (9.3)	21 (7.9)	–
No	39 (90.7)	246 (92.1)	

Statistical Analyses

SPSS software version 15 was used to analyze the data and the χ^2 test for pair differences was done. Descriptive summarization of data consisted of frequency counts and percentages. Fisher's exact test was used for noncategorical variables; $p < 0.05$ was considered to be significant.

Results

Epidemiological and Clinical Information

Of the 310 women, 43 (13.8%) were colonized by GBS. There was no significant difference between carriage rates at the three hospitals ($p = 0.366$). None of the women reported having had more than one sexual partner in the previous 12 months; most (254, 81.9%) were Iranians living in urban areas (62.6%) and used hormonal contraception (36.1%) before pregnancy. Further epidemiological data are given in table 1.

With respect to the obstetric characteristics, mean gestational age was 38.3 ± 3.3 weeks; 151 (48.8%) were primiparous and 29 (9.3%) had a history of previous abortion. Of the 43 GBS carriers, 37 (86%) had no risk

Table 2. Occurrence of risk factors for GBS carriage during pregnancy, with results of vaginorectal screening for GBS and level of significance, in 310 women from Shiraz, Iran

Risk factor	GBS carrier (n = 43)	GBS-negative (n = 267)	p value
Intrapartum fever ($>37.5^\circ\text{C}$)	1	9	0.81
Premature rupture of membranes	4	33	0.77
Preterm <37 weeks	2	8	0.53
At least one risk factor			
Present	6	46	–
Absent	37	221	–

factor for GBS colonization (gestational age <37 weeks; premature rupture of membranes; intrapartum fever $>37.5^\circ\text{C}$).

GBS carrier status was not significantly associated with any risk factor. There were no statistically significant differences between risk factors ($p > 0.05$) in colonized and noncolonized women (table 2).

Of the 310 women, 16 (5.2%) admitted to taking antibiotics during pregnancy while approximately 50% could not recall why they were treated and the name of the antibiotic they took. However, urinary infection was the most frequent reason given for taking antibiotics, and amoxicillin or cephalexin was commonly used. Only 1 of the 16 women that received oral antibiotics was a GBS carrier (table 3).

Microbiological Study

Three hundred and nine microorganisms were isolated; 278 (89.7%) women had positive culture and the remaining 32 were negative. The microorganisms isolated from rectovaginal samples are listed in table 4; nine groups or genera were identified. These included *Enterococcus*: 78 (25.1%), coagulase-negative staphylococci: 60 (19.4%), *Escherichia coli*: 46 (14.9%), GBS: 43 (13.8%), *Candida*: 33 (10.6%), coagulase-positive staphylococci: 22 (7%), *Lactobacillus*: 8 (2.6%), streptococci other than GBS: 7 (2.6%), *Gardnerella*: 7 (2.3%), while *Pseudomonas*, *Klebsiella* and *Proteus* constituted less than 2% of the isolates. Gonococci and *Haemophilus* were sought, but not found. Overall, 89.6% of the women yielded significant cultures.

As for the order of microbial isolation, *S. agalactiae* (GBS) ranked 4th, with incidence of 43 (13.8%) cases. From 50 streptococci isolated, 43 (86%) were GBS. Also,

Table 3. Antibiotics given orally at some time during pregnancy to 16 pregnant women and indication for treatment (self-reported by participating women by questionnaire)

Oral antibiotic therapy	n (%)
Antibiotics given	
Amoxicillin	2 (12.5)
Cephalexin	6 (37.5)
Erythromycin	1 (6.3)
Do not remember	7 (43.7)
Total	16 (100)
Indication for antibiotics	
UTI	5 (31.2)
URI	2 (12.5)
Do not remember	9 (56.3)
Total	16 (100)

URI = Upper respiratory tract infection; UTI = urinary tract infection.

Table 4. Microorganisms isolated from rectovaginal samples of pregnant women prior to labor in Shiraz, Iran

Organisms	Isolated organisms n (%)
<i>Streptococcus</i> spp.	50 (16.7)
Streptococcus group B	43 (13.8)
<i>Staphylococcus</i> spp.	82 (26.4)
<i>Staphylococcus aureus</i>	22 (7)
Enterobacteriaceae	48 (15.6)
<i>Escherichia coli</i>	46 (14.9)
<i>Klebsiella</i>	1 (0.3)
<i>Proteus</i>	1 (0.3)
<i>Enterococcus</i> spp.	78 (25.1)
<i>Candida</i> spp.	33 (10.6)
<i>Lactobacillus</i> spp.	8 (2.6)
<i>Gardnerella vaginalis</i>	7 (2.3)
<i>Pseudomonas</i>	3 (1.0)
Total number of isolates per case	1.1

from 82 staphylococcal isolates, 22 (26.8%) were *Staphylococcus aureus*.

Neonatal Population Characteristics

There were 311 infants born to 310 women, of whom 170 (54.5%) were female and the remaining 141 (45.3%) male. The mean birth weight was 2,750 g. At 12 weeks after birth, only 1 neonate with early-onset infection was hospitalized due to poor feeding and lethargy. Of the blood, urine and CSF cultures performed, only CSF culture and Gram staining were positive for GBS.

Discussion

Nine groups or genera of microorganisms were identified in this study. The isolation of *Enterococcus* species and *E. coli* from the vagina is not usual [20–23]. It is known that antibiotics may modify the vaginal flora, but most of the women in this study had had no antenatal care. They were admitted in labor, and only 5.2% had received antibiotics that might have affected the vaginal flora. The presence of many of the organisms which are common in the rectum is most probably due to poor general hygiene.

The absence of lactobacilli from among most isolates in this study is also unusual, although it is known that lactobacilli are less frequently isolated elsewhere from women with disease of the lower genital tract [21–24].

One explanation for this may be the pH of the vagina, which is known to become alkaline in certain morbid states. Isolation of *Streptococcus* and *Staphylococcus* species in this study is not surprising as these organisms are well-known etiological agents of puerperal sepsis. In recent years, there have been many reports on the possible role of *Bacteroides* species and related organisms in gynecological and obstetric infections. In the present study, *Bacteroides* species were not isolated by means of the routine anaerobic method used. It seems surprising that colonization of the lower genital tract with anaerobes has not arisen in this study. As the clinical laboratories are poor in the isolation of anaerobes and also the simple anaerobic culture used in our study did not yield any anaerobic bacteria including *Bacteroides*, a more detailed investigation of the possible role of anaerobic organisms in genital tract infections in this geographical locality is desirable. The frequency of *Candida* species in this study was lower than the findings of other researchers [25, 26]. The association of *Candida albicans* with disease of the lower genital tract in pregnancy is well known.

Perhaps the most important fact that has emerged from this work is that the lower genital tract of most women in Shiraz at the time of labor is heavily colonized by pathogens. In order to prevent genital tract sepsis, therefore, labor should not be prolonged, and unnecessary trauma to the genital tract at the time of delivery should be avoided.

The carriage rate of GBS in pregnant women in Shiraz, Iran was 13.8%, which is similar to that seen in Kuwait [13] and Saudi Arabia [8], but considerably higher than in India [8]. Some authors also found a GBS isolation rate of 5–25% among Iranian parturient women in other parts of Iran [16, 17]. Although our result shows a carriage rate within the aforementioned range, different methodology and geographical or socioeconomic conditions may be the reason for variations seen in GBS carriage rates.

By analysis of risk factors and carriage rates it is estimated that a maximum of 11.9% of women in this study would have been treated with prophylactic antibiotics due to prolonged rupture of membranes and none was under the screening approach of the CDC guidelines [15]. The presence of risk factors did not correlate well with vaginorectal carriage of GBS in this study. However, Oddie and Embleton [27] in a UK-based study did find a correlation between the presence of risk factors in mothers and the occurrence of GBS disease in their neonates, suggesting that analysis of larger groups of pregnant women may be required.

In our study, *S. agalactiae* was isolated from blood cultures of only 1 newborn, which contracted early-onset infection and was born to a GBS-positive mother. According to our knowledge, this case was a rare report of septicemia in infants due to GBS in Iran. This rate of infection is the same as the published rate of 5–10 per 1,000

colonized mothers. Such an attack rate is consistent with the literature [15]. One major limitation of this study is that a majority of the women could not recall if they were treated with antibiotics, hence education of the pregnant woman or the wording of the questionnaire may need to be improved.

Conclusion

This study revealed that 13.8% of pregnant women were colonized vaginorectally with GBS in Shiraz, Iran. There was a poor correlation between risk factors and GBS carriage. Potential future work in Iran should focus on large multicenter carriage studies of GBS in pregnant women, examination of culture versus rapid detection methods for identifying GBS carriage, and randomized controlled trials of strategies for prevention of neonatal GBS infection.

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