# The carriage of group B streptococci in Turkish pregnant women and its transmission rate in newborns and serotype distribution

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**The prevalence of group B streptococci (GBS) colonization was studied in 500 pregnant women and their newborn infants by collecting vaginal and rectal swabs from mothers, and umbilical and throat swabs from their infants. Forty-six isolates of GBS were obtained from mothers' specimens and eight from neonates. Maternal and infant colonization rates were found to be 9.2% and 1.6%, respectively. Vertical transmission rate was 15.2%. Additionally, serotypes and antimicrobial susceptibility of 54 isolates of GBS were determined. Type Ia, II and III were common serotypes among GBS isolates from mothers and infants. When evaluating the factors that affect GBS carriage, age, socio-economic status and education level of pregnant women were important for carriage, while use of intrauterine device and parity were unrelated. No resistance to ampicillin, penicillin, ceftriaxone or vancomycin was found by disk diffusion method. A high level of resistance against tetracycline was noted (91%). Although invasive serotypes are predominant, the rarity of GBS disease in Turkish infants may be due to low rates of maternal carriage or to their possessing protective levels of GBS-specific IgG antibody in their sera.**

***Key words: group B streptococci, carriage, serotype.***

Until the 1930's, group B streptococci (GBS) were known only as a cause of mastitis in cows. In 1935, Lancelfield and Hare[1] isolated some GBS strains from various samples of adult female patients. After having isolated the microorganisms from three puerperal sepsis cases, it was realized that they could also cause some important infections in humans. After the 1960's, an increasing number of newborn and maternal infections caused by these microorganisms were reported[2]. The majority of the infections in newborns occurred via vertical transmission, while others were also acquired from the community. It is now well known that there is a high correlation between maternal anorectal and urogenital carriage and newborn infections. The colonization rate of GBS changes according to age, race, parity and sexual activity. The prevalence of maternal vaginal colonizatilon in industrialized countries is reported to be between 5% and 35%. The vertical transmission rate ranges between 29% and 70%. Low rates of vaginal colonization have been reported from some developing countries, including Libya (5%), Saudi Arabia (13.9%) and India (5.8%)[3], but higher rates have been reported from the Ivory Coast (19.3%)[4] and Nigeria (19.5%)[5]. GBS are classified into serotypes on the basis of structural differences in capsular polysaccharides (Ia, Ib, II-VII). Previous studies have reported a distribution of capsular serotypes Ia, Ib, II and III, with a few nontypeable isolates in neonates with early-onset disease and among pregnant women with vaginal GBS colonization. Late-onset neonatal disease and meningitis among neonates are due primarily to serotype III[3,6-8].

There is an ongoing discussion on the use of antimicrobial therapy for the eradication of GBS[9,10]. The Centers for Disease Control and Prevention (CDC) published guidelines for intrapartum antibiotic prophylaxis in 1996 that

recommended intravenous (iv) penicillin G or ampicillin as the drug of choice for prevention of perinatal GBS disease[11]. Erythromycin and clindamycin are recommended for women who are penicillin-intolerant. Widespread use of antibiotics carries the potential for the emergence of antibiotic resistance. Recent reports of the increasingly frequent resistance of GBS to erythromycin and clindamycin have raised concern about the use of these antibiotics as alternative agents for the prophylaxis of GBS disease[12-14].

We studied Turkish pregnant women and their newborn infants to determine the prevalence of GBS colonization and the rate of vertical transmission, the colonization in infants, serotype distribution, effective factors on carriage and antibiotic susceptibility of isolates.

## Material and Methods

*Bacterial Isolates*: During the period of May 2000 to January 2001, we studied 500 pregnant women who gave birth to their children by vaginal delivery in Zeynep Kamil Women's and Children's Diseases Training and Research Hospital. Vaginal and rectal swabs were taken from mothers at the time of delivery, and umbilical and throat swabs from their infants in the first 24 hours after the birth. All culture swabs wee inoculated directly into the selective Todd-Hewitt Broth (THB) (Oxoid Ltd., U.K.) containing gentamicin sulfate, 8 µg/ml (Schering Corporation), and nalidixic acid, 15 µg/ml (Winthrop Laboratories). The broth cultures were incubated overright at 37°C and then streaked onto 5% sheep blood agar plates and were incubated under the same conditions. Colonies were identified presumptively by morphology, Gram's staining and catalase reactions. Gram-positive cocci with catalase-negative reaction were further identified according to their sensitivities to bacitracin (0.04 IU) sulfamethoxazole-trimethoprim (SXT, 23.75 µg/ml-1.25 µg/ml) and CAMP factor positivities. Isolates were identified serologically by latex agglutination after enzyme extraction (Streptex kit, Wellcome Diagnostics, U.K.). Forty-six isolates of GBS from pregnant women and eight isolates from their offspring were kept frozen in THB supplemented with sheep blood until serotyping and susceptibility testing were performed.

*Serotyping* was done by hemolytic streptococcus group B typing sera (Denka Seiken, Japan). The typing sera used in this study were Ia, Ib, II, III, IV and V. Briefly, a colony of GBS was incubated overnight in 5 ml of THB. The cells were then resuspended for digestion in a mixture of THB, swine pancreatic extract, and pH indicator. The pH was adjusted to range from 8.0 to 8.5, and the mixture was incubated for 1 hour at 37°C for digestion. After digestion, the mixture was suspended in 0.5 ml of phosphate-buffered saline solution. This suspension was heated to 120°C for 30 minutes, and then agglutination tests were carried out on a glass slide with each of the six antisera. Serotype designation was determined by a strong agglutination reaction within 1 minute. If no designation could be made, the isolate was determined to be "nontypeable".

*Statistical Analysis*: To determine interaction between GBS carriage with socio-economic status, parity, educational factors, age and usage of intrauterine device (IUD), an information request form was filled out for each of the pregnant women. Pregnant women were grouped according to their socio-economic status (bad, moderate, good), parity (1, 2, 3, 4 and over), educational factors (elementary, secondary, high school), age (15-20, 21-30, 31-40, older than 40) and usage of IUD (presence or not). While assessing the information request form, pregnant women were first classified as GB positive or negative. These two groups were then evaluated according to their socio-economic status, parity, educational factors, age and usage of IUD by chi-square and Fisher's exact tests.

*Testing for Susceptibility*: The strains were tested for antimicrobial susceptibility by the Kirby Bauer disk diffusion susceptibility test. With Oxoid paper disks were screened for susceptibility to penicillin, ampicillin, tetracycline, clindamycin, erythromycin, chloramphenicol, ofloxacin, ceftriaxone and voncomycin. A small number of colonies obtained from each trypticase soy agar-5% sheep blood plate were incubated in THB for 2h at 37°C to obtain a McFarland level of turbidity of $10^5$ and a logarithmic-growth-phase culture. One loop of inoculum from each 10-fold diluted logarithmic-phase culture was plated uniformly onto Mueller-Hinton agar supplemented with 5% sheep blood with sterile swabs. Paper disks containing antimicrobial agents were placed on the plate. The culture was incubated for 18-20 h in carbon dioxide atmosphere at 37°C. Zones of inhibition were measured and the results were

interpreted as resistant, intermediate, or susceptible according to the range recommendations of National Committee for Clinical Laboratory Standards (NCCLS)[15].

## Results

Among the 500 women screened, 9.2% were colonized with GBS at the time of delivery. Forty-six women had GBS isolated from the vagina, rectum or both; 21 (46%) of the isolates were only from the vagina, 14 (30%) only from the rectum and 11 (24%) from both sites (Table I). Among eight GBS strains isolated from 500 infants, four were recovered from the throat, two from the umbilical swabs and the remaining two from both the throat and the umbilical swabs (Table II). Among eight GBS-colonized infants, seven were born to GBS-colonized and one to non-colonized mothers. The vertical transmission rate was 15.2%.

**Table I.** Isolation Sites and Number of GBS in Mothers

| Isolation site | No of GBS (+) Mothers | % |
|---|---|---|
| Only vagina | 21 | 4.2 |
| Only rectum | 14 | 2.8 |
| Vagina and rectum | 11 | 2.2 |
| Total | 46 | 9.2 |

GBS: group B streptococci.

**Table II.** Isolation Sites and Number of GBS in Newborns

| Isolation site | No of GBS (+) Newborns | % |
|---|---|---|
| Only throat | 4 | 0.8 |
| Only umbilicus | 2 | 0.4 |
| Throat and umbilicus | 2 | 0.4 |
| Total | 8 | 1.6 |

GBS: group B streptococci.

The distribution of GBS serotypes isolated from the pregnant women and their newborn infants is shown in Table III. Neither mothers nor infants were colonized with more than one serotype of GBS. About 80% of the 54 GBS isolates were typeable.

**Table III.** Serotype Distribution of 54 GBS Isolates

| Serotype | No of GBS | % |
|---|---|---|
| Ia | 14 | 26 |
| Ib | 2 | 4 |
| II | 16 | 29 |
| III | 10 | 10 |
| IV | 1 | 2 |
| V | 0 | – |
| Non-typeale | 11 | 20 |
| Total | 54 | 100 |

GBS: group B streptococci.

Serotypes Ia, II and III were predominant among GBS isolates from both mothers and infants. Type II was the most common serotype, with a percentage of 29%, followed by type Ia and type III at 26% and 19%, respectively. Types Ib (4%) and IV (2%) were rare, and type V was absent.

Statistical analysis of factors related to GBS carriage is shown in Tables IV-VIII.

**Table IV.** Relationship Between Socioeconomic Status and GBS Carriage

| Socieconomic status | GBS-positive number (%) | GBS-negative number (%) | Total number (%) |
|---|---|---|---|
| High | 3 (6.52) | 54 (11.89) | 57 (11.4) |
| Intermediate | 32 (69.59) | 137 (30.17) | 169 (33.8) |
| Low | 11 (23.91) | 263 (57.92) | 274 (54.8) |
| Total | 46 (9.2) | 454 (91.8) | 500 (100) |

GBS: group B streptococci.

**Table V.** GBS Carriage Rates and Parity of Pregnat Women

| Parity | GBS-positive number (%) | GBS-negative number (%) | Total number (%) |
|---|---|---|---|
| 1 | 24 (52.17) | 211 (46.47) | 235 (47) |
| 2 | 10 (21.73) | 97 (21.36) | 107 (21.4) |
| 3 | 7 (15.21) | 69 (15.19) | 76 (15.2) |
| 4 and over | 5 (10.86) | 77 (16.95) | 82 (16.4) |
| Total | 46 (9.2) | 454 (90.8) | 500 (100) |

GBS: group B streptococci.

There was a statistically significant relation between GBS carriage and socioeconomic status (p=0.0001). GBS carriage was highest in the middle socioeconomic group.

**Table VI.** GBS Carriage Rates and Education Level of Mothers

| Education | GBS-positive number (%) | GBS-negative number (%) | Total number (%) |
|---|---|---|---|
| Primary school | 36 (78.26) | 335 (51.76) | 371 (74.2) |
| Secondary school | 10 (21.73) | 110 (24.22) | 120 (2.4) |
| High school | – | 9 (1.98) | 9 (1.98) |
| Total | 46 (9.2) | 454 (91.8) | 500 (100) |

GBS: group B streptococci.

**Table VII.** Distribution of GBS Carriage Among Age Groups

| Age groups | GBS-positive number (%) | GBS-negative number (%) | Total number (%) |
|---|---|---|---|
| 15-20 | 14 (30.43) | 111 (24.44) | 125 (25) |
| 21-30 | 28 (60.86) | 47 (10.35) | 75 (15) |
| 31-40 | 4 (8.69) | 295 (64.97) | 299 (59.80) |
| 40 and over | – | 1 (0.20) | 1 (0.20) |
| Total | 46 (9.2) | 454 (90.8) | 500 (100) |

GBS: group B streptococci.

**Table VIII.** Distribution of GBS Carriage and IUD Usage

| IUD usage | GBS-positive number (%) | GBS-negative number (%) | Total number (%) |
|---|---|---|---|
| IUD-positive | 8 (17.39) | 70 (15.41) | 78 (15.60) |
| IUD-negative | 38 (82.60) | 384 (84.58) | 422 (84.40) |
| Total | 46 (9.2) | 454 (91.8) | 500 (100) |

IUD: intrauterine device, GBS: group B streptococci.

There was no statistically significant difference between the groups according to parity (p=0.99).

In relation to education, GBS carriage in women who had only attended elementary school was higher than in the other groups (p<0.0001).

There was a statistically significant difference between the age groups. GBS carriage was the highest in the 21-30 age group (p<0.0001).

Intrauterine device usage had no effect on GBS carriage (p=0.62).

A total of 54 strains were examined for susceptibility to penicillin, ampicillin, tetracycline, chloramphenicol, clindomycin, ofloxacin, erythromycin, vancomycin and eftriaxone. No resistance to penicillin, ampicillin, ceftriaxone and vancomycin was found by disk diffusion. Five strains (12%) exhibited a multiple antibiotic resistance pattern.

**Table IX.** Antibiotic Susceptibility Profiles for GBS Strains Isolated from Mothers and Neonates

| Antibiotic | Susceptible % | Susceptible intermediately % | Resistant % |
|---|---|---|---|
| Penicillin | 96 | 4 | – |
| Ampicillin | 98 | 2 | – |
| Tetracycline | 4 | 3 | 91 |
| Chloramphenicol | 85 | 4 | 11 |
| Clindamycin | 87 | 4 | 9 |
| Ofloxacin | 94 | 4 | 2 |
| Erythromycin | 89 | 4 | 7 |
| Vancomycin | 100 | – | – |
| Ceftriaxone | 100 | – | – |

GBS: group B streptococci.

## Discussion

Group B streptococcus is one of the most important agents of perinatal infections all over the world regardless of race and socioeconomic conditions. The increase in morbidity and mortality along with the increase in incidences of group B streptococcal diseases make the development of preventive strategies compulsory. In the last few years, thanks especially to the studies carried out in developed countries determining maternal carriage and vertical transmission rates and serotype distribution, knowledge related to the pathogenesis and epidemiology of infections has been accumulated. Using this data, attempts have been made to improve the methods of prevention. The origin of GBS is mainly anorectal and urogenital maternal carriage. 29-70% of the newborn infants acquiring GBS

do so by vertical transmission from colonized mothers. In order to prevent vertical transmission, protocols need to be prepared and criteria should be established in identifying colonized mothers and initiating chemoprophylaxis and immunoprophylaxis.

In the previous studies, the colonization rate in Turkey has been found to be between 2.3-13.6% (16-20). A previous Turkish study of 100 pregnant women, (by Gökalp et al. 1986, found a carriage rate of 7% and vertical transmission rate of 57%[16]. Shokouhizadeh et al.[17] took endocervical and vaginal swabs from 59 pregnant woman and face and conjuctiva swabs of their babies. The carriage rate of mothers was 13.6%, while the vertical transmission rate was 12.5% Karadeniz et al.[10] screened 200 pregnant woman and their babies for GBS. The carriage rate was 8% and vertical transmission rate was 62.5%. Gökalp et al. in 1988[15] and Çarıkçı et al. in 2003[20] detected streptococcal carriage in 8. 2% and 8%, respectively. Our study had carriage rates comparable to those of previous studies and also provides important data regarding vertical transmission and serotype distribution. GBS was isolated in 46 of 500 mothers (9.2%) and in eight (1.6%) of 500 infants. Seven of eight GBS-positive newborn infants were born from colonized mothers. Only in one infant was the mode of acquisition considered to be nosocomial. In this study sampling from both vagina and rectum to determine the maternal carriage has possibly increased the isolation rate. When the samples were taken only from the vagina, the isolation rate was 4.2%, considering only from rectum it was 2.8%, but when taken from both sites it increased to 9.2%. It has been demonstrated previously that swabbing of multiple sites, especially the lower vagina and anorectum, increases the isolation rates[21,22]. Similarly, when the samples were taken from more than one site of the infant, the isolation rate was also increased. With only the throat sample, the isolation rate was 0.8%, and with only the umbilicus, it was 0.4%; when taken from both sites it reached 1.6%.

Neonatal GBS infections have a bimodal distribution according to age. During the first six days. Early onset of life, the mean age of early-onset infection is 12 hours[23]. Among eight GBS colonized infants, no early-onset infection was observed, and these infants could not be followed to determine the presence of late-onset infection. Statistical analyses of information requested from pregnant women showed that age, socio-economic status and education level of pregnant women affected the ratio, while use of IUD and parity had no relation to GBS carriage.

Data about serotype distribution in GBS isolates from colonization or invasive disease is not available in our country. The distribution of serotypes among GBS isolates from women and infants in our study is similar to that of the reports from industrialized countries[3,24]. In our studies a GBS carriage rate similar to that of other countries was detected. According to common belief, invasive GBS infections are rare in developing countries. Follow-up multiple-centered studies for newborn infants of colonized mothers should be undertaken, and the incidence of GBS diseases such as meningitis and sepsis should be detected.

Antibiotic resistance patterns of GBS isolated from pregnant women and their infants were studied. A total of 54 strains were tested for their susceptibility to certain antibiotics that have been recommended for eradication of carriage and treatment of invasive GBS diseases[2]. Using disk diffusion, we found no resistance to penicillin, ampicillin, ceftriaxone and vancomycin, while a high level of resistance to tetracycline was noted (91%). Resistance to chloramphenicol, clindamycin, erythromycin and ofloxacin was found to be 11%, 9%, 7% and 2%, respectively. The rates of resistance of GBS to tetracycline and clindamycin were similar, but resistance against chloramphenicol was higher than had been previously reported in other countries[25]. These results suggest that routine reporting of GBS susceptibilities may help the clinician in choosing effective antibiotic therapy for invasive GBS infections.

Although neonatal GBS infections are rare, because of the highly invasive serotypes, we advise GBS detection for only the high risk groups in our country.

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