



Prevalence and Antibigram of Vaginal Carriage of Group B *Streptococci* in Third Trimester of Pregnancy and Its Feto-Maternal Outcome

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Abstract

Background and Objectives: *Group-B Streptococcal (GBS) prevalence varies from place to place and this organism is responsible for serious infections in newborns such as septicemia and meningitis. This study was undertaken in order to understand the magnitude of this problem in pregnant women and its fetal outcome.*

Methods: *Two low vaginal swabs were taken from 380 antenatal women at 28 weeks and from the same cases at 37 weeks of gestation from Obstetrics & Gynecology Department, Sree Gokulam Medical College and Research Foundation, Venjaramoodu. The two swabs were immediately transported to the laboratory for processing. Out of the two low vaginal swabs collected, one swab was used for Gram's stain and the other swab was inoculated in Todd - Hewitt broth (Oxoid Ltd) containing Nalidixic acid (15 mg/L) and Gentamicin (8mg/L) and incubated at 37^oc in 5% CO₂. After 24 hrs of incubation from culture broth, subcultures were performed in 5% sheep blood agar and Macconkey agar for isolation of GBS. Identification was done based on Gram staining, colony morphology, catalase reaction, CAMP test, carbohydrate fermentation, and Hippurate hydrolysis test. Isolates were confirmed by latex agglutination test.*

Results: *Of the 380 pregnant women screened, 3.2% were colonized by GBS. GBS colonization rate was higher among pregnant women in third trimester who were ≤20 years of age and primigravidae. In our study all the strains were sensitive to Penicillin, Ceftriaxone, Linezolid, Ofloxacin and Vancomycin. Resistance was observed with Erythromycin (25%), Clindamycin (25%).*

Conclusion: *As the GBS colonization rate among pregnant women in third trimester of pregnancy is low, screening for GBS as a routine component of prenatal care is a procedure of uncertain clinical value.*

Keywords: *Group-B Streptococcus; Colonization; Prevalence.*

Introduction

Group A *Streptococci* had a place of pride as causative agent of important clinical diseases and syndrome till recently. Other members of this family are now coming to light as human

pathogens and Group B *Streptococci* is topping the list since the last few decades. Group B *Streptococci* or *Streptococcus agalactiae* is a well-recognized pathogen in veterinary medicine because of its role as causative agent of bovine

mastitis. There has been increasing interest in Group B *Streptococci* (GBS) due to their association with and recognition as pathogens of neonatal infections like sepsis and meningitis.^{1,2}

Group B *Streptococci* are present in 15-20% of pregnant women in lower genital tract³. Vaginal colonization by GBS during pregnancy is associated with life threatening neonatal infections acquired during passage through the birth canal, and it has been the most frequent cause of neonatal meningitis in infants^{4,5}. Group B *Streptococci* has been associated with low birth weight, premature delivery, premature rupture of membranes, still birth and neonatal death suggesting that it has a marked predilection for pregnant women, fetus and neonates⁶. The mother's birth canal is the principle reservoir of this infectious agent for infants who develop the more severe syndrome with early onset.⁷

Infections in newborn occurring within the first week of life are designated early onset group B *Streptococcal* (EOGBS) disease. Late onset disease develops in infants after 7 days and upto 3 months of age. The measures used to prevent EOGBS disease, however do not prevent late onset GBS disease. Infants with early onset GBS disease generally present with respiratory distress, apnoea or other signs of sepsis within the first 24-48 hours of life. The most common clinical manifestations of EOGBS are sepsis and pneumonia, less frequently meningitis. Mortality is higher among preterm infants compared with full term infants. Early onset infections are acquired vertically through exposure to GBS from the vagina of a colonized woman. Neonatal infection occurs primarily when GBS ascends from the vagina to the amniotic fluid after onset of labour or rupture of membranes, although GBS also can invade through intact membranes. GBS can be aspirated into the fetal lung, which in turn can lead to bacteremia. Infants who are exposed to the organism through this route can become infected with GBS during passage through the birth canal.

Late onset disease (7-90 days) occurs less frequently than EOGBS. Maternal obstetric complications are uncommon with late onset GBS disease. Transmission can be either horizontal (from other infected infants or health care workers) or vertical (from mother due to close proximity). The two most common clinical manifestations of late onset disease are meningitis and bacteremia. The mortality rate for late onset neonatal disease is 2-6% which is significantly lower than the rate of 10% for early onset infections. However, this rate has not declined in the Western countries despite the implementation of prophylactic measures.

In India very few studies have been carried out, mainly to study the prevalence of GBS infections. The GBS colonization rate in the vaginal flora of pregnant women varied from 0.47 to 23.3% as reported by various workers.

The factors controlling maternal GBS colonization and neonatal disease due to GBS are – age, number of previous pregnancies, phase of menstrual cycle, use of oral contraceptives. This study was undertaken in order to understand the magnitude of this problem in pregnant women and its fetal outcome.

Materials and Methods

Study Design: Descriptive study.

Study Setting: Department of Microbiology, Department of Obstetrics and Gynaecology, Sree Gokulam Medical College and Research Foundation, Venjaramoodu.

Study Period: Two years (December 2014 - November 2016).

Study Population: Antenatal cases at 28 weeks and same cases at 35-37 weeks attending the Obstetrics and Gynaecology OP of Sree Gokulam Medical College and Research Foundation.

Sample Size: 380 antenatal cases.

Inclusion Criteria: Antenatal cases at 28 weeks and same cases at 35-37 weeks attending the Obstetrics and Gynaecology OP of Sree Gokulam Medical College and Research Foundation.

Exclusion Criteria

First & second trimester antenatal cases.

Methodology**Sample collection**

Two low vaginal swabs were taken prior to the first pelvic examination from 380 antenatal women at 28 weeks and from the same cases at 35-37 weeks of gestation from Obstetrics & Gynaecology Department, Sree Gokulam Medical College and Research Foundation, Venjaramoodu. Detailed history of each case was taken, which has been mentioned in the proforma. The swabs were immediately transferred to the laboratory without delay for processing. In case of any delay appropriate transport systems (Amie's or Stuart's) were used.

Sample processing

Out of the two low vaginal swabs collected, one swab was used for Gram's stain and the other swab was inoculated in Todd-Hewitt broth (Oxoid Ltd) containing Nalidixic acid (15 mg/L) and Gentamicin (8mg/L) and incubated at 37^oc in 5 % CO₂. After 24 hrs of incubation from culture broth, subcultures were performed in 5% sheep blood agar (SBA) and MacConkey agar for isolation of GBS. The blood agar plate was incubated in a candle jar for 18 to 24 hours at 37°C and examined for the presence of β-hemolytic *Streptococci*. Broth cultures showing no visible turbidity after overnight incubation were further re-incubated and then sub cultured after 48 hours on sheep blood agar. The colony characters such as shape, size, colour, margin, consistency, elevation and zone of hemolysis, etc. were noted .

The presumptive diagnosis of GBS was based on the following:

1. **Direct smear examination (Gram staining) :**
Gram stained smear of the vaginal swab were examined for the presence of gram positive cocci in pairs, short chains or long chains.

Procedure

1. Smear was made from the swab on a clean glass slide, air dried and heat fixed.

2. It was flooded with 1% crystal violet for 1 minute and washed with water.
3. Smear was flooded with Gram's iodine for 1min and washed with water.
4. Decolourization with acetone for 3 seconds.
5. Smear was counter stained with dilute Carbol fuchsin for 30 seconds.
6. Smear was washed, air dried and examined under the oil immersion lens.

Interpretation

In 12 smears epithelial cells, few pus cells and gram positive cocci were seen in pairs, short and long chains. In the remaining smears there were only epithelial cells and normal vaginal flora.

2. **Culture on blood agar :** showed translucent to opaque, flat, glossy colony with narrow zone of beta hemolysis.

3. **Culture on Macconkey agar :** showed magenta red colonies.

4. Catalase test

The presence of catalase enzyme in the test isolate is detected using 3% hydrogen peroxide. Place one drop of H₂O₂ on a slide containing a suspension of colony Prompt effervescence indicates catalase production. All the 12 isolates were negative for catalase test.

Quality control

Positive control: *Staphylococcus aureus* ATCC 25923.

Negative control: *Enterococcus faecalis* ATCC 29212.

5. Hippurate hydrolysis test

- 1% solution of sodium hippurate is prepared and 0.4 ml volume is dispensed in a capped tube.
- The tube is inoculated heavily with the organism to be tested. The suspension should become milky.
- The tube is incubated at 37°C for 2 hours.
- 0.2 ml of ninhydrin reagent is added and further incubated for 10-15 minutes at 37°C.

Interpretation

If the test is positive, there is development of deep purple colour, which is seen in GBS. If the test is negative, there will either be no colour change or

there is development of slightly yellow-pink colour. All the isolates were positive for Hippurate hydrolysis test.

Quality control:

Positive control: *Streptococcus agalactiae*.

Negative control: *Streptococcus pyogenes*.

7. CAMP Test

- \square -hemolytic *Staphylococcus aureus* (NCTC 7428) is streaked in a straight line across the centre of 5% sheep blood agar plate.
- Known positive (group-B *Streptococcus*) and negative (group-A *Streptococcus*) controls are inoculated in a single straight line (1 cm apart from *Staphylococcus aureus* streak) at 90° angle to *Staphylococcus* streak.
- The test organism is also streaked across the plate perpendicular to the *Staphylococcus aureus* streak, leaving about 1 cm space in between the two streaks.
- The plate is incubated overnight at 37°C in candle jar or CO₂ incubator.

Interpretation

Positive: Positive readings may occur at 5-6 hours after incubation. With group B-*Streptococci* an area of increased lysis appears at the junction of two streaks indicated by an arrow head zone of hemolysis.

Negative: No enhancement of hemolysis.

Quality control:

Positive control: group B *Streptococcus*.

Negative control: group A *Streptococcus*.

8. Carbohydrate fermentation test

Fermentation of carbohydrates was studied in peptone water as the basal medium with bromocresol purple indicator and 1% sugar. The base is dispensed, sterilized by autoclaving and cooled. 1% sugar solution is prepared by dissolving 1gm sugar in 100 ml base. Adjust the ph to 7.1-7.3. Then dispense 3ml amounts and sterilize by steaming for 30 minutes. Fermentation of arabinose, mannitol, lactose, trehalose, sorbitol, sucrose, pyruvate

and raffinose were done. Inoculate two to three colonies of the test strain into each of the sugars and incubate overnight at 37°C. A colour change from violet purple to yellow indicates positive reaction.

9. Latex agglutination test for serologic grouping of β haemolytic *Streptococci*

The cell-wall grouping antigen is extracted from the GBS cell wall enzymatically, and the extract is subsequently reacted with latex beads to which group specific antibodies are bound.

Procedure

1. For extracted antigen: Use a sterilized loop to transfer well isolated GBS colonies to a small test-tube containing extraction solution and emulsify them. Incubate the emulsion at 35°C.
2. Place one drop of the antibody-coated particles on the slide.
3. Place one drop of the antigen suspension beside the antibody-coated particles.
4. Mix the two drops on the slide and spread in a square.
5. Rotate the slide by hand in a steady circular motion for one minute. Take care that the mixture does not spill outside the boundaries of the square.
6. Examine the slide macroscopically for agglutination after the time specified in the manufacturer's instructions. For best visibility hold the slide near a bright light and view against a dark background.

Interpretation

A positive result is recorded when the antigen mixed with the antibody shows agglutination, i.e. the suspension shows clumping or is granular to the point of curdling. Agglutination is best seen by tilting the slide slightly so that the fluid drains down towards the lower boundary of the square.

10. Antimicrobial sensitivity of the GBS was done by the disc diffusion technique (Kirby-Bauer disk diffusion method).

Procedure

- Fresh sub-cultures of GBS were used after overnight growth (16 hours) on blood agar plate.
- The inoculum was prepared by suspending 3-5 colonies in sterile saline to achieve a turbidity of 0.5 McFarland standard.
- A sterile cotton swab was dipped into the bacterial suspension, elevated above the liquid and rotated several times against the inside wall of the tube to remove excess of the inoculum.
- This swab was streaked evenly in three different directions on the surface of 5% sheep blood agar.
- The plate was rotated through an angle of 60° after each application.
- The plate was allowed to dry for a few minutes at room temperature with the lid closed. The antimicrobial discs was placed on

the inoculated plates using a pair of sterile forceps. 4 discs were placed on a 100 mm plate. Plates were incubated aerobically at 35⁰ c.

- After 18-24 hours of incubation, the diameter of clear zone around the disk was measured under transmitted light with measuring scale and results interpreted as susceptible, intermediate or resistant as per CLSI criteria.

Following drugs were tested:

- Penicillin (10 IU/disc).
- Erythromycin (15 µg/disc).
- Clindamycin (2 µg/disc).
- Ceftriaxone (30 µg/disc).
- Co-trimoxazole (25 µg/disc).
- Ofloxacin (5 µg/disc).
- Linezolid (30 µg/disc).
- Vancomycin (30 µg/disc).

Table-6: Antimicrobial Agents and Zone Interpretation according to CLSI Guidelines

Sl.No	Antimicrobial Agents	Disc concentration	Sensitive	Intermediate	Resistant
1	Penicillin	10 units	≥24	-	-
2	Erythromycin	15 µg	≥21	16-20	≤15
3	Clindamycin	2 µg	≥19	16-18	≤15
4	Ceftriaxone	30 µg	≥24	-	-
5	Ofloxacin	5 µg	≥16	13-15	≤12
6	Linezolid	30 µg	≥21	-	-
7	Vancomycin	30 µg	≥17	-	-

Fetomaternal Outcome

After delivery APGAR score at 1 and 5 min, birth weight, congenital anomalies, immediate complications and birth injuries, signs of asphyxia, meconium aspiration, sepsis and other associated complications were recorded. The babies were followed up in the post natal period. Neonatal morbidity and mortality were noted. Mothers were watched for third stage complications like postpartum haemorrhage and retained placenta. They were followed up in puerperal period. Vital parameters like temperature, pulse and blood pressure were frequently monitored. Women were specifically asked for foul smelling lochia and the presence of febrile morbidity. Maternal morbidity like

puerperal sepsis, urinary and respiratory tract infection and wound infection were looked for.

Both mother and baby were followed up till their stay in the hospital.

Results

A total of 380 antenatal cases were included in this study which was conducted over a period of two years from December 2014 - November 2016 at Sree Gokulam Medical College and Research Foundation, Venjaramoodu, Thiruvananthapuram.

Table-7: Occurrence of GBS in third trimester of pregnancy (28 weeks and 35-37 weeks)

Total cases studied	GBS positive mothers No (%)
380	12 (3.2%)

Out of 380 pregnant women in third trimester, 12 (3.2%) were identified as Group-B *Streptococci* positive.

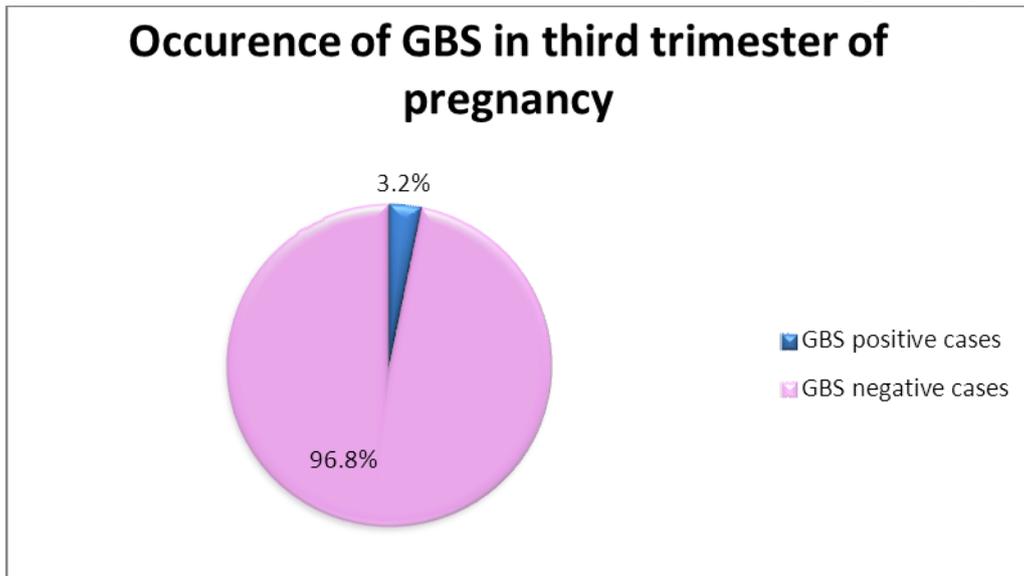


Figure- 1 : Occurrence of GBS in third trimester of pregnancy

(28 weeks and 35-37 weeks)

The occurrence of group-B *Streptococci* was studied in relation to:

1. Age
2. Number of pregnancies.

Table-8 : Occurrence of GBS in different age groups

Age group	No. of mothers	GBS negative	GBS positive	GBS positive Percent
≤ 20 years	58	53	5	8.6%
21 – 25 years	171	167	4	2.3%
26 – 30 years	107	105	2	1.9%
>30 years	44	43	1	2.3%
Total	380	368	12	3.2%

Chi square → 6.728 p-value → 0.081.NS

Most of the isolates were from the age group ≤ 20 years (8.6 %), followed by age groups 21-25yrs (2.3%) and >30yrs (2.3%). There is no association between age of mother and GBS colonization.

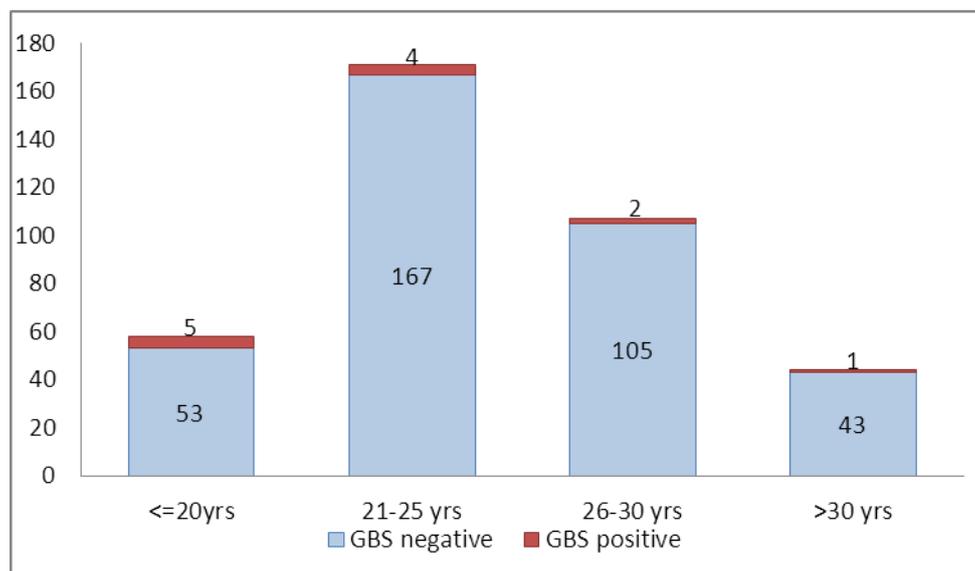


Figure-2 Occurrence of GBS in Different Age Groups

Table-9 : Relationship between GBS colonization and parity

Subject status	No. of subjects	GBS negative	GBS positive	GBS positive Percent
Primi gravidae	210	201	9	4.3%
Second gravidae	119	116	3	2.5%
Third gravidae	35	35	0	0%
Multi gravidae	16	16	0	0%
Total	380	368	12	3.2%
Chi-square:2.694, p-value 0.441 → NS				

Most of the isolates were from primi gravidae (4.3%) followed by second gravidae (2.5%). By applying Chi-square test, no significant association is observed between GBS colonization and parity.

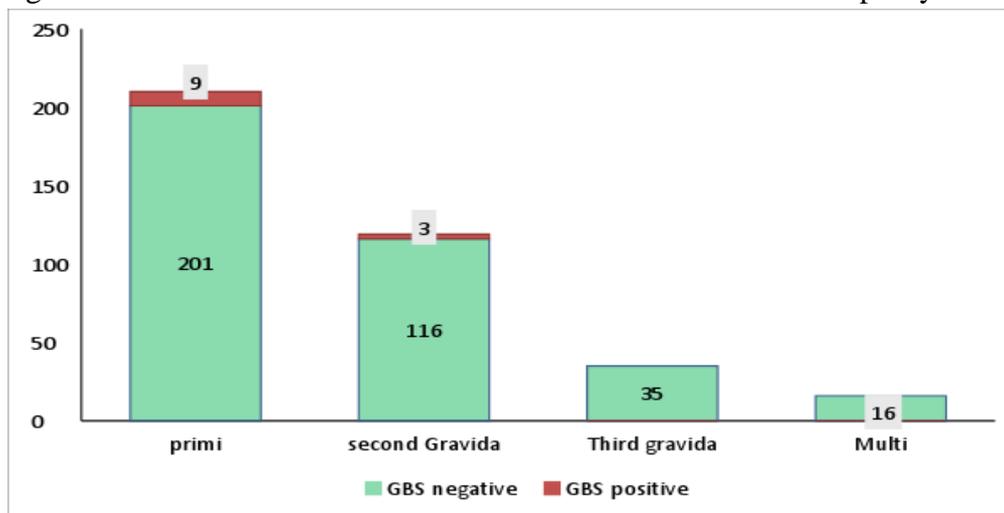


Figure-3 : Relationship between GBS Colonization and Parity

Table-10: Antibiotic Sensitivity pattern of group B *Streptococci* isolated in this study

Antibiotic	Total sensitive	GBS (%)	No. of Resistant	Percent
Penicillin	12	100.00	0	0.00
Ceftriaxone	12	100.00	0	0.00
Clindamycin	9	75.00	3	25.00
Erythromycin	9	75.00	3	25.00
Cotrimoxazole	0	0.00	12	100.00
Ofloxacin	12	100.00	0	0.00
Linezolid	12	100.00	0	0.00
Vancomycin	12	100.00	0	0.00

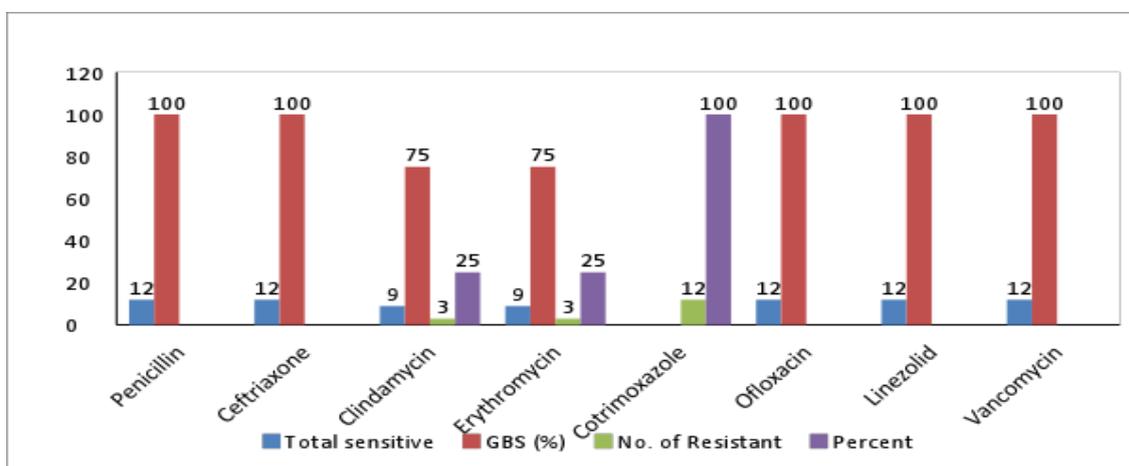


Figure-4 Antibiotic Sensitivity pattern of group B *Streptococci* isolated in this study

A total of 380 antenatal cases were screened. Only 12 cases were colonized with GBS. Among the 12 cases 2 cases presented with premature rupture of membranes (16.7%). All GBS positive cases were detected during 35-37 weeks gestation. The same cases were GBS negative at 28 weeks gestation. GBS positive cases were given prophylactic antibiotics. Neonates of the GBS positive mothers were normal except for two babies who had low birth weight (16.7%). Low birth weight maybe due to some other cause other than maternal group B colonization. There were two cases of neonatal meningitis caused by group B *Streptococci*. Antenatal mothers of the above two cases were negative during GBS screening at 28 weeks and 35 -37 weeks gestation.

There were no significant correlation between GBS colonisation and parity, age of screened antenatal mothers. The other maternal risk factors including prolonged rupture of membranes, history of intrapartum fever and low birth weight

also did not correlate with GBS colonization in pregnant women.

Discussion

Two low vaginal swabs were taken from 380 antenatal women at 28 weeks and from the same cases at 35-37 weeks of gestation from Obstetrics and Gynaecology Department, Sree Gokulam Medical College and Research Foundation, Venjaramoodu.

In our study the prevalence rate of GBS colonization in antenatal women was 3.2% at 35-37 weeks of gestation. Our statistics almost correlate with the results of Mhasker Rita et al of Bangalore who have reported a vaginal GBS colonization rate of 1.62%⁸. Kulkarni et al in Maharashtra also has observed a low rate (2.52%) of GBS colonization⁹. In a study conducted at Saudi Arabia, El Kersh et al observed a rate of 2.6% GBS vaginal colonization among 151 cases who were negative for GBS colonization in earlier visits¹⁰.

Table-11 : Low GBS colonization rates reported in different studies

Reference No	Place	Year of isolation	Sample size	Colonization rate
Kulkarni et al ⁹	India(Maharashtra)	1998-1999	317	2.52%
El Kersh et al ¹⁰	Saudi Arabia	2000-2001	151	2.6%
Mhasker Rita et al ⁸	India(Banglore)	2003-2004	741	1.62%
Present study	India(Kerala)	2014-2016	380	3.2%

Table-12 : High GBS colonization rates reported in different studies

Reference No	Place	Year of isolation	Sample size	Colonization rate
Regan et al ¹¹	USA	1984-1989	13,646	21%
Motlova et al ¹²	Czech Republic	2001-2002	586	29.3%
Nwachukwu et al ⁷⁷	Nigeria	2002-2003	200	9.0%
Orrett ⁷⁸	Trinidad	2003	201	32.9%
Elbaradie et al ⁷⁹	Egypt	2006-2007	95	17.9%

Regan et al of United States reported a high colonization rate of 21%¹¹. Similarly a very high GBS colonization rate was identified in Czech Republic by J Motlova et al (29.3%), Nwachukwu et al of Nigeria (9.0%), Orett FA in Trinidad (32.9%) and Elbaradie et al (17.9%) from Egypt in their respective studies^{12,13,14,15}. High rates of GBS colonization were detected among Saudi women (25.7%).¹⁶

Most studies in India detected only 1.5%-2.5% of GBS colonization among Indian women which almost correlate with our study. The reasons for varying results may be attributed to the fact that maternal GBS colonization varies from place to place. Other factors that may have contributed to this variation include socioeconomic factors, variation in the technique of collection of samples, local application of antibiotic cream before collection of samples. Ethnic and genetic factors

might play a role in variation of the rates of infection with GBS.

Most of our isolated strains were from primigravidae (4.3%) followed by second gravidae (2.5%). Similar findings were observed by Anthony et al (1978) that genital colonization was less frequent in women who were multigravidae¹⁷.

Yow et al (1980) reported a higher colonization rate in the primigravidae than multigravidae women which also coincides with our findings¹⁸. Vinay Hajare of Karnataka also observed a higher rate of colonization in primigravidae than multigravidae¹⁹.

In a study from the West Indies colonization rates were found to be significantly greater among multigravida women than primigravida women. Similarly in studies done by Vijayan Sharmila in Pondicherry multigravida women were more often associated with GBS colonization²⁰. However in another study involving Turkish pregnant women parity was observed to be unrelated to GBS carriage. Whereas according to Beachler and Baker (1979) no differences were found with colonized and non-colonized women with respect to age, race, marital status or parity²¹. Regan et al described GBS carriage as more common among older women and women of lower parity¹¹. In the present study most of the isolated strains were from cases belonging to age group less than or equal to 20 years (8.6%) followed by the age group of 21-25 years (2.3%) and >30 yrs(2.3%), thus in the present study, most of the GBS colonized pregnant women were from teenage and younger age group. These findings are in correlation with that of Baker et al (1977), Anthony et al (1978) and Collado et al (1981), the reasons for the predisposition of women younger than 21 years of age to vaginal colonization with GBS is less apparent^{17,22}. In a study conducted by Tupili Ramya at Tirupathi most of the isolated strains were from cases belonging to age group less than and equals to 20 years followed by the age group of 21-25 years²³. This study correlates with our study.

Our findings are in correlation with that of Vinay Hazare, et al (2012) of Karnataka, the reasons for the predisposition of women younger than 21 years of age to vaginal colonization with GBS is less apparent¹⁹. This relationship could be the result of age related development of local or humoral immunological responses that interfere with mucosal attachment and/ or persistence of GBS.

It seems that age is one of the factor for GBS colonization as observed by various workers. Among non-pregnant and pregnant women (1978) Anthony et al found that genital colonization of GBS was significantly higher in teenagers than those over 20 years of age¹⁷. Collado et al (1981) also reported a higher colonization rate in the youngest age group <20 years²² but Yow et al (1980) found no relationship between colonization and age¹⁸. The studies of GBS antibiotic prophylaxis were directed towards eradicating GBS colonization in pregnant women during the last trimester.

In each of these studies, colonization was reduced when antibiotics were administered but reemerged soon after treatment was discontinued. Only one study, that of Merenstein et al (1980), has shown a significant reduction in maternal and infant colonization rates in women treated with Penicillin²⁴.

In our study all the strains were sensitive to Penicillin, Ceftriaxone, Linezolid, Ofloxacin and Vancomycin. Resistance was observed with Erythromycin (25%), Clindamycin (25%). Currently Penicillin G (a beta lactam antibiotic) is the preferred treatment for GBS infection as it has a narrower spectrum than Ampicillin. Many studies have stated that GBS isolates were all sensitive to Penicillin and Ampicillin. In separate studies conducted by Arora et al, Kishore et al, Vinay Hazarae et al, Chen et al, Schoening T E et al in Germany all GBS isolates were sensitive to Penicillin^{19,25,26}. In contrast de Azavedo et al(2001) of Canada reported that 1 out of 178 GBS invasive strains were resistant to Penicillin²⁷. Simoes et al (2004) in his study of 52

GBS isolates found reduced sensitivity to Penicillin 8/52(15.4%), Ampicillin 9/52 (17.3%), Ciprofloxacin 14/52 (26.9%) and Nitrofurantoin 1/52 (1.9%)²⁸. In 1999, Mercer investigated 719 GBS strains that were isolated from neonates with EOGBS disease and reported that 96 (13.4%) of those strains were resistant to Ampicillin²⁹.

An alternative to the beta-lactam antibiotics is the treatment of GBS colonization/infection using antimicrobials which inhibit protein synthesis. To date many GBS strains resistant to Erythromycin and Clindamycin have been reported. In our study out of 12 GBS isolates 3 (25%) were resistant to both Erythromycin and Clindamycin. In one Australian study of 250 isolates, Stylianopoulos et al(2002) reported a 2.8% resistance to Erythromycin³⁰ while a New Zealand study of 177 isolates by Werno and Murdoch (2003) reported Erythromycin (6%) and Clindamycin (15%) resistance (Table-13).³¹ In contrast, a number of studies have been reported in USA and up to 32% of GBS isolates have been reported as being resistant to Erythromycin while upto 21% of GBS isolates were reported as being resistant to Clindamycin (Table-13)^{27,28,32,33}. Schoening T E et al reported that out of 338 GBS isolates Erythromycin resistance was 11%²⁶.

In Canada the resistance rates to Erythromycin and Clindamycin have been recorded as high as 17% and 26% respectively (Table-13)^{32,34}. Turkey, England/Wales, Portugal, Germany, South America and Greece have all reported on Erythromycin and Clindamycin resistance (Table-13)^{35,36,37,38,39,40,41}. Turkey has reported two very high rates of Erythromycin resistance at 21.2% and 22.4% (Table-13)^{35,36,40}. Andrews et al, 2000 of USA also reported a Erythromycin resistance rate of 22% among GBS isolates (Table-13).⁴² Due to increase in resistance for antimicrobials, Erythromycin and Clindamycin cannot be used as a reliable alternative treatment for women colonized with GBS who have a high risk of Penicillin anaphylaxis.

Resistance to Tetracycline has also been reported in New Zealand, Canada, UK/Wales, Portugal and Argentina and the rates range from 72.4% to 91% (Table-13).^{31,37,39} In a study done by Lakshmi et al(1998) high degree of resistance was seen with Tetracycline(87.8%) and with Kanamycin (90.9%). Studies have also found that the incidence of GBS resistant strains differs between countries.

Table-13 : GBS Resistance to Antibiotics in various studies¹¹⁰

Year	Reference	Location	Isolates	Erythro mycin%	Clinda mycin%	Tetracycl ine%
<2002	Stylianopoulos et al, 2002 ³⁰	Australia, Melbourne	250	2.8	*	*
<2002	Werno & Murdoch, 2003 ³¹	New Zealand	177	6	15	84
<2004	Simoes et al, 2004 ²⁸	USA	52	*	19	*
<2004	Andrews et al, 2000 ⁴²	USA	<182	22	7	*
2000	Sited in James, 2001 ³³	Ohio	*	15	21	*
1997	Daley and Garland, 2004 ³²	Rhode Island	*	25	6.9	*
1999	de Azavedo et al, 2001 ²⁷	California	178	32	12	*
<2004	Desjardins et al, 2004 ³⁴	Canada	338	17	26	*
1999	Daley and Garland, 2004 ³²		178	14	7	*
<2004	Figueira-Coelho et al., 2004 ³⁷	England/ Wales	353	4	*	91

<2004	Fluegge et al, 2004 ³⁸	Germany	296	10.1	5.7	*
<2003	Tsolia et al, 2003 ⁴¹	Greece	*	4.5	6	*
1999-2002	Figueira-Coelho et al, 2004 ³⁷	Portugal	252	10.7	9.9	75.4
<2003	Lopardo et al, 2003 ³⁹	South America	*	5.2	1.7	72.4
<2004	Acikgoz et al, 2004 ³⁵	Turkey	156	22.4	*	*
<2003	Arisoy et al, 2003 ³⁶ ; Radhakrishnan et al, 1995 ⁴⁰		*	21.2	9.1	*
2014-2016	Present study	India (Kerala)	12	25	25	*

Note: Information that has not been reported or tested is represented by ‘ * ’

The other maternal risk factors including prolonged rupture of membranes, history of intrapartum fever and low birth weight did not correlate with the GBS colonization in neonates. The lack of association between these potential risk factors with neonatal GBS colonization is in contrast with findings from a study done by Orrett FA et al which associated such factors with an increased risk of vertical transmission¹⁴. The lack of association with these factors can possibly be explained by the fact that number of participants in this study with such risk factors were small and this may have decreased the power of the study.

Conclusion

The present study was done at Sree Gokulam Medical College and Research Foundation. Two low vaginal swabs were taken from 380 antenatal women at 28 weeks and from the same cases at 35-37 weeks of gestation from Obstetrics & Gynaecology Department, during December 2014 - November 2016.

The following conclusions was derived from the present study.

- No GBS isolates were obtained from vaginal swabs taken at 28 weeks of gestation.
- Out of 380 pregnant women in third trimester (35-37weeks),12 (3.2%) were identified as Group-B *Streptococci* positive.
- Most of the isolates were from the age group ≤ 20 years (8.6 %), followed by age groups 21-25yrs (2.3%) and >30yrs (2.3%).
- Most of the isolates were from primi gravidae (4.3%) followed by second gravidae (2.5%).

- All GBS isolates were sensitive to Penicillin, Ceftriaxone, Vancomycin, Ofloxacin and Linezolid, 75% of isolates were sensitive to Erythromycin and Clindamycin.

The other maternal risk factors including prolonged rupture of membranes, history of intrapartum fever and low birth weight did not correlate with the GBS colonization in neonates. No significant correlation was observed between maternal age, parity and GBS colonization.

The isolation frequency of GBS among pregnant women and neonates call for further study to elucidate the extent of vertical transmission and follow up of neonates with GBS colonization in order to establish extent of disease development. In our study as the GBS colonization rate among pregnant women in third trimester of pregnancy is low, screening for GBS as a routine component of prenatal care is a procedure of uncertain clinical value.

Recommendations

- It is recommended to conduct further molecular studies to characterize GBS strains in order to further elucidate the extent of mother to child transmission (vertical transmission) by performing DNA finger printing.
- It is desirable to conduct an extensive study in order to further determine the potential risk factors for GBS colonization that have been frequently cited in the literature such as prolonged rupture of membrane, intrapartum fever, mode of delivery and low birth weight.

- Serotyping of GBS should be done to develop and implement effective vaccine for prevention of neonatal GBS disease. Serotyping is an effective epidemiological tool for studying GBS.
- Antibiotic resistant GBS may occur with more widespread use of antibiotics. Therefore surveillance of antibiotic resistance patterns among several antimicrobial classes will be important in determining optimal prophylaxis and treatment of GBS infections. Indeed the problem of antibiotic resistance is global. Therefore; health care providers worldwide should be encouraged to join public health authorities, to control the inappropriate use of antibiotics and promote responsible prescribing. This will greatly help to improve prevention and control of drug resistant organisms in communities.
- For the time-being Penicillin should be the preferred choice for treatment of GBS infections, however continuous antimicrobial surveillance is required to monitor trends of resistance.
- It would have an impact on the management of pregnant women before giving birth and their babies if these kind of studies are repeated in many health institutions. So the outcome of these studies can be used in formulating guidelines for the antenatal care.

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