A PROSPECTIVE OBSERVATIONAL STUDY OF PREVALANCE OF GROUP B STREPTOCOCCI IN ANOVAGINAL FLORA IN PREGNANT WOMEN AFTER 28 WEEKS OF GESTATION

By

DR. SHREEDEVI KORI

Dissertation submitted to the

BLDE UNIVERSITY BIJAPUR, KARNATAKA

In partial fulfillment of the requirements for the degree of

MASTER OF SURGERY

In

OBSTETRICS AND GYNAECOLOGY

Under the guidance of

DR. MANPREET KAUR J. TEHALIAM.D.

PROFESSOR

DEPARTMENT OF OBSTETRICS AND GYNAECOLOGY

B.L.D.E UNIVERSITY'S SHRI B. M. PATIL MEDICAL COLLEGE HOSPITAL & RESEARCH CENTRE, BIJAPUR, KARNATAKA

2013

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BIJAPUR.

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This work has not been submitted by me for award of any other Degree or Diploma by any other University.

Date:

Place: Bijapur.

DR. SHREEDEVI KORI
Post Graduate Student,
Department of Obstetrics and Gynaecology,
B.L.D.E.U.'s Shri B. M. Patil Medical College,
Hospital &Research Centre, Bijapur.

B.L.D.E UNIVERSITY'S
SHRI B. M. PATIL MEDICAL COLLEGE HOSPITAL & RESEARCH CENTRE,
BIJAPUR.

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I have satisfied myself that her observations noted in this dissertation are authentic and also that these conform to the standards of BLDE University, Bijapur. I have great pleasure in forwarding this dissertation to the University.

Date:

Place: Bijapur.

DR. MANPREET KAUR J. TEHALIA$_{\textbf{M.D.}}$
Professor,
Department of Obstetrics and Gynaecology,
B.L.D.E.U.'s Shri B. M. Patil Medical College,
Hospital &Research Centre, Bijapur.

B.L.D.E UNIVERSITY'S
SHRI B. M. PATIL MEDICAL COLLEGE HOSPITAL & RESEARCH CENTRE,
BIJAPUR.

CERTIFICATE BY THE CO-GUIDE

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I have satisfied myself that her observations noted in this dissertation are authentic and also that these conform to the standards of BLDE University, Bijapur. I have great pleasure in forwarding this dissertation to the University.

Date:

Place: Bijapur.

DR. PRASHANT K. PARANDEKAR$_{\textbf{M.D.}}$
Professor and Head of Department,
Department of Microbiology,
B.L.D.E.U.'s Shri B. M. Patil Medical College,
Hospital &Research Centre, Bijapur.

B.L.D.E UNIVERSITY'S
SHRI B. M. PATIL MEDICAL COLLEGE HOSPITAL & RESEARCH CENTRE,
BIJAPUR.

ENDORSEMENT BY THE HEAD OF DEPARTMENT

This is to certify that this dissertation entitled "**A PROSPECTIVE OBSERVATIONAL STUDY OF PREVALANCE OF GROUP B STREPTOCOCCI IN ANOVAGINAL FLORA IN PREGNANT WOMEN AFTER 28 WEEKS OF GESTATION"** is the bonafide work of **DR. SHREEDEVI KORI**, is in partial fulfilment of the regulation of BLDE University, Bijapur, for the award of the degree of "**Master of Surgery is OBSTETRICS AND GYNAECOLOGY"** under the guidance of **Dr. MANPREET KAUR J TEHALIA,** Professor, Department of **OBSTETRICS AND GYNAECOLOGY,** BLDE University, Bijapur. I have great pleasure in forwarding this dissertation to the BLDE University.

Date:

Place: Bijapur.

DR. P. B. JAJU$_{\textbf{M.D.}}$
Professor & Head of Department,
Department of Obstetrics and Gynaecology,
B.L.D.E.U.'s Shri B. M. Patil Medical College,
Hospital &Research Centre, Bijapur.

B.L.D.E UNIVERSITY'S
SHRI B. M. PATIL MEDICAL COLLEGE HOSPITAL & RESEARCH CENTRE,
BIJAPUR.

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Date**:** **DR. M. S. BIRADAR**$_{\textbf{M.D.}}$

Place: Bijapur. Principal,

B.L.D.E.U.'s Shri B. M. Patil Medical College,

Hospital &Research Centre, Bijapur.

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SHRI B. M. PATIL MEDICAL COLLEGE HOSPITAL & RESEARCH CENTRE,
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Date:

Place: Bijapur

DR. SHREEDEVI KORI
Post Graduate Student,
Department of Obstetrics and Gynaecology,
B.L.D.E.U.'s Shri B. M. Patil Medical College,
Hospital &Research Centre, Bijapur.

ACKNOWLEDGEMENT

On completion of this scientific document it gives me deep pleasure to acknowledge the guidance provided by my distinguished mentors.

With privilege and respect I would like to express my profound gratitude and indebtedness to my guide and esteemed teacher DR. MANPREET KAUR J. TEHALIA, Professor, Department of Obstetrics & Gynaecology, Shri B. M. Patil Medical College, Bijapur, for her constant inspiration, extensive encouragement and support, which she rendered in pursuit of my post-graduate studies and in preparing this dissertation.

I express my sincere thanks to my dear teachers Dr. (Prof.) S. V. Reddy, Dr. (Prof.) V. R. Gobbur, Dr. (Prof.) P. B. Jaju, Dr. (Prof.) S. R. Mudanur, Dr. (Prof.) G. R. Sajjan, Dr. (Prof.) S. R. Bidri, Dr. Jyoti Korbu, Dr. Deepa Patil, Dr. Girija Hanjagi, Dr. Rajashri Yaliwal, Dr. Neelamma Patil, Dr. Jayashree Sajjanar, Dr. Sumedha Katti, Dr. Shobha Shiragur, Dr. Nirmala, Dr. Suvarna Nandi , Dr. Aruna Biradar for their kind co-operation and guidance.

I am extremely thankful to my co- guide Dr. Prashant K. Parandekar, Professor and Head of the Department of Microbiology, for his constant inspiration, support and inputs in preparing this dissertation. I also thank Dr. B.V. Peerapur, Department of Microbiology for his continuous support.

I am thankful to Dr. M. S. BIRADAR, Principal, B.L.D.E.U's Shri B. M. Patil Medical College, Hospital and Research Centre, Bijapur, for permitting me to carry out this work & utilize the college resources in completion of my work.

I am deeply indebted to my parents, parents-in- law, husband Dr. Dayanand Biradar [Post graduate in surgery], sisters Dr. Vijayalaxmi and Tejaswini and my brothers Swaroop and Niroop whose constant encouragement and inspiration led me to the successful completion of my dissertation work.

I am also thankful to my fellow Post graduates, with special thanks to my friend Dr. Ashish Verma [Post graduate in surgery], for their suggestions and immense support.

My thanks to all the non-teaching staff of my department, nursing staff and all hospital staff for their co-operation in my study.

I want to also convey my gratitude to all the patients without whose co-operation, this study would not have been possible.

Last but not the least, I thank GOD and pray for his continued blessings and success.

Date: **DR. SHREEDEVI KORI**

Place: Bijapur. Post Graduate Student,

Department of Obstetrics and Gynaecology,

B.L.D.E.U.'s Shri B. M. Patil Medical College,

Hospital &Research Centre, Bijapur.

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INTRODUCTION

INTRODUCTION

Group B *Streptococcus* (GBS) is a leading cause of neonatal infection in the Western hemisphere. The recognition that maternal colonization with the organism is a key factor in the occurrence of GBS associated neonatal morbidity and mortality was a milestone in the history of perinatal health[1]. A nationwide change in health practices helped diminish mortality and morbidity associated with the disease. In India, however, the spectrum of group B streptococcal disease remains a largely under recognized problem. Puerperal sepsis has been described for centuries, and ancient Indian texts in 1500 BC have recorded that good hygiene leads to a reduction in perinatal disease[2]. In 1879 Louis Pasteur identified the streptococcus as the causative organism for puerperal sepsis[3]. Since the early 1930s when Rebecca Lancefield reported her grouping system for haemolytic streptococci, group A *Streptococcus* (*Streptococcus pyogenes*) was widely acknowledged as the major pathogen associated with puerperal sepsis[4]. GBS was initially thought to be a commensal, until 1937, when Fry reported seven cases of GBS associated puerperal fever with 3 deaths[5].

During the 1970s and 1980s, GBS emerged as a significant neonatal and maternal pathogen in the United States (US) and Western Europe with reported mortality rates of 15 to 50 per cent[6,7]. In the US, 10 to 35 per cent of pregnant women are asymptomatic carriers of GBS in the genital and gastrointestinal tract at the time of delivery[8]. The prevalence of GBS colonization during pregnancy is variable; in one study, among women who had positive GBS cultures between 26 and 28 weeks of gestation, only 65 per cent remained colonized at term, while 8 per cent of those with negative prenatal cultures were positive for GBS at term[9]. Treatment of these

colonized mothers succeeded in temporarily eradicating the organism, but most of the women were re-colonized within 6 weeks. At birth, 50 to 65 per cent of infants who are born to colonized mothers have positive GBS cultures from mucus membranes and skin (external ear canal, throat, umbilicus, anorectal sites[1,10].Approximately 98 per cent of colonized newborns remain healthy, but 1 to 2 per cent develop invasive GBS infection[7]. The overall incidence of neonatal GBS infection was approximately 2 per 1000 live births in the United States prior to the introduction of intrapartum prophylaxis[7]. Epidemiological studies in India have shown lower colonization and infection rates in general[11-15]. However on closer analysis, taking into consideration use of adequate culture techniques and microbiological media, some of the GBS colonization rates reported from India and other developing countries are similar to those reported in the United States[11]. In a study done in 507 pregnant Indian women, 12 per cent were reported to have GBS isolated from the throat and vagina, and 10 per cent had positive vaginal cultures alone[12]. Similarly, another study showed the overall carriage rate in pregnant women to be 16 per cent[13]. Although both these studies used selective broth media, culture sites did not include the anorectum and this might have lowered the yield of positive cultures. Other studies have reported colonization rates of 5 to 6 per cent, but no selective broth media were used in these cases[14,15]. Colonization rates in infants born to asymptomatic maternal carriers of GBS are 53 to 56 per cent and are consistent with rates reported in other parts of the world[13,15].

Despite significant GBS colonization rates, reports of invasive neonatal GBS disease in India are infrequent. During a 10-yr study between 1988 and 1997 in Vellore, only 10 cases of neonatal GBS infection were identified, giving an incidence of 0.17 per 1000 live births[16]. However, this number represents only the cases

occurring among deliveries in a tertiary care hospital located in a predominantly rural community. In India, where 65 per cent of women give birth at home, the true incidence of invasive GBS disease in the newborn is largely unknown[17]. In addition, blood cultures from ill neonates are not always done in many rural primary health care centres, which may contribute to the underestimation of the number of GBS cases. Preterm births and stillbirths are also usually not investigated, and thus the total burden of perinatal GBS disease remains unrecognized. The estimated incidence of neonatal GBS infection in India can be calculated from Indian epidemiological data reporting maternal and infant GBS colonization rates as 10 and 50 per cent respectively[12,13,15]. Since about 2 per cent of colonized neonates develop true infection, the attack rate of neonatal GBS infection in India may be calculated as approximately 1 per 1000 live births. Bearing in mind the above estimated attack rate and current Indian demographic data (midyear population count in the year 2001 was approximately 1027 million, and birth rate was 26 births per 1000 population per year)[18], **the projected total number of GBS infection in newborn infants in India may be as high as 26,700 cases per year**.

Pregnant women who are GBS carriers have the potential to transmit the organism to their newborn infants. There is a spectrum of maternal and fetal GBS infections ranging from asymptomatic colonization to sepsis. GBS has been implicated in adverse pregnancy outcomes, including premature rupture of membranes (PROM), preterm labor and clinical and subclinical chorioamnionitis.[19] GBS is a leading cause of morbidity and mortality among newborns. Universal screening for GBS among women at 35–37 weeks of gestation is more effective than administration of intrapartum antibiotics based on risk factors. Studies indicate that

intrapartum prophylaxis of GBS carriers and selective administration of antibiotics to newborns reduce neonatal GBS sepsis by as much as 80–95%.[20] Women with GBS colonization are at an increased risk of GBS colonization in a subsequent pregnancy. Prior GBS colonization should be considered in the algorithm to treat unknown GBS status during term labor.[21]Investigators tried to find out/identify risk factors that may influence the prevalence of GBS, like ethnicity, smoking, maternal age and number of partners[22]. The colonization rates are incoherent enough to target only high-risk women and may not be an effective strategy. The Centers for Disease Control (CDC) call for antibiotic prophylaxis in women with asymptomatic first trimester bacteriuria because this is 'a marker for heavy genital tract colonization,' and screening all other women at 35–37 weeks for vaginal and rectal colonization[23,24] and in GBS.

Hence, the present study is undertaken to study the incidence of GBS among pregnant women after 28 weeks of gestation in our population . To the best of our knowledge, this study is one among the very few researches conducted in India on GBS vaginal colonization among pregnant women.

AIMS & OBJECTIVES

AIMS AND OBJECTIVES

To estimate the prevalence of group B streptococci in pregnant women after 28 weeks of gestational age coming to OBG OPD and labour room at BLDE University's Shri B. M. Patil Medical College, Hospital and Research Centre, Bijapur.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

In 1879, Louis Pasteur identified Streptococcus as a causative organism for puerperal sepsis. Since the early 1930's when Rebecca Lancefield reported her grouping system for hemolytic streptococci, Group A Streptococcus (*Streptococcus pyogenes*) was widely acknowledged as the major pathogen associated with puerperal sepsis.[3]

Group B Streptococcus was initially thought to be a commensal, until 1937 when Fry reported several cases of group B streptococcus associated puerperal fever with three deaths.[5] During the 1970's and 1980's, Group B Streptococci (GBS) emerged as a significant neonatal and maternal pathogen in the United States and Western Europe with reported mortality rates of 15 to 50 percent.[5] In the early 1980's clinical trials demonstrated that administering antibiotics during labour to women at risk of transmitting the group B streptococcal infections to newborns could prevent invasive disease in the first week of life.

In 1981, in a prospective study of colonization with Group B Streptococci among 6706 parturients by Regan JA, Chau S, James LS[25], found an increased incidence of premature rupture of membranes and preterm delivery in patients colonized with GBS. Premature rupture of membranes occurred in 8.1% of the non-colonized population and 15.3% of the colonized population. Preterm delivery occurred in 1.8% of the non-colonized population and among 5.4% of the colonized women.

McDonald, et al [26]did a prospective study with vaginal swabs obtained from 692 women at 35-36 weeks of gestation[26]. GBS was detected in 91 (13.2% of the women). The rate of preterm labour (less than 37 weeks) was significantly higher in GBS positive women than in GBS negative women (18.7% vs. 5.5%). This association remained significant even when patients with other recognized factors predisposing to preterm labour were excluded (11.5% vs. 3.9%). The rate of premature rupture of membranes was also significantly higher in the GBS positive women (9.9% vs. 2.7%) and remained higher when patients with other recognized risk factors were excluded (6.1% vs. 1.8%). The results unequivocally showed that the pregnant women who were vaginal carriers of GBS have a significantly increased risk of premature rupture of membranes and preterm labour.

Manuel et al [27]in a study involving antenatal Spanish women found the maternal carriage rate of GBS to be 14.6%. The incidence of preterm labour was 35% in the GBS positive women and 7% in GBS negative women. The association of GBS positive status with preterm labour was significant.[27]

Katz VL et al[28] studied the maternal colonization of GBS and found it to be varying from 15 to 30% among various racial groups. Further the rate of invasive infection in newborn was found to be 1-3% per 1000 live births.[28]

Kosheleva et al[29] did a retrospective analysis of 103 pregnant women and found the effect of maternal colonization on pregnancy outcome and reported the incidence of preterm labour in GBS positive women as 21.7% and the incidence of

PROM as 13.7%. Further the perinatal mortality of babies born to GBS positive mothers was found to be 12.6%.[29]

Garland SM, Kelly N, Ugoni[30] AM concluded in a study at Royal Women's hospital that the prevalence of GBS among pregnant women was 12.9%.[30]

Dalal S, Lahiri A, Parel CC,[12] in a study regarding the carriage rate of group B Streptococci, involving 507 pregnant Indian women, reported that 12% of the women had group B Streptococci isolated from the throat and vagina, 10% had positive cultures from the vaginal sample alone.

Chaudhary U, Sabherwal U stated the carrier rate among pregnant women in India to be 16%.[13]

Mani V, Jadhav M estimated the incidence of neonatal group B Streptococcal infection in India by calculating from the Indian epidemiological data. They reported the maternal and infant Group B Streptococcal colonization rates to be 14% and 50% respectively.[15]

A cross-sectional study conducted by Bushra Yasmin Chaudhry, Naeem Akhtar, Abba Hayat Balouch[31] in 2009 at Benazir Bhutto Hospital, Rawalpindi, Pakistan showed a GBS carriage rate of 8.5% among pregnant women before delivery and an acquisition rate of 53% on the abdominal skin and 18% in the ear canals by the neonates of colonised mothers were found.[31]

Pierrette Melin ,[32]conducted a study and showed that GBS carriage rate in the vaginal and rectal flora ranges from 7 to 37%.This colonisation can be intermittent, transient or persistent. At birth, 40–60% of neonates born to a GBS carrier are colonised. Fortunately, the attack rate of EOGBS disease among colonised infants is low, with only 1–3% becoming infected.[32]

E R Wald, B Dashefsky, M Green et al[33] conducted a study in Magee Women's Hospital in Pittsburgh in 2002. One swab was cultured semi-quantitatively on 5% sheep blood agar to detect GBS. The other swab was subjected to a rapid method (25 min) for antigen detection and micronitrous acid exposure to extract the GBS antigen, followed by latex particle agglutination. A total of 464 swabs were evaluated by direct plating. Fifty-two swabs (11.2%) were found to contain GBS. Overall, the rapid method detected 21 of 52, or 40.4%, positive specimens. The sensitivity of the rapid method for identifying the most heavily colonized samples was 85.7%. This method can be used to identify maternity patients who are heavily colonized with GBS and are at high risk of delivering septic infants.[33]

A prospective study was conducted in Indira Gandhi Medical College in Pondicherry, India ,by Vijayan Sharmila, Noyal Mariya Joseph, Thirunavukkarasu Arun Babu, et al[34]. A total of 300 pregnant women were enrolled in the study. GBS strains were isolated from seven out of 300 patients, corresponding to a colonization rate of 2.3%. Of the seven patients carrying GBS, isolates were cultured only from vaginal swabs in two cases (28.6%), only from rectal swabs in two cases (28.6%) from both vaginal and rectal swabs in three cases (42.9%). Heavy colonization was present only in 42.9% (3/7) of antenatal women. None of the seven isolates were

resistant to penicillin or clindamycin, while one isolate (14.3%) was resistant to erythromycin and five isolates (71.4%) were resistant to tetracycline.

A study was carried out by Madhavi H, Vinay Hajare, Singh HKG[35] at Basaveshwar Teaching Hospital, Gulbarga from 1st Jan. 2007 to 30th June 2008. Two hundred, 3rd trimester pregnant women attending ANC clinics were included in the study. Two vaginal swabs were collected during ANC check-up in the third trimester. Smear from one swab was subjected to direct gram stain and the other was inoculated in sheep blood agar plate. Results of this study showed that the prevalence rate for GBS colonization is 7.5% .Pregnant women less than 20 years of age and primigravida were predominantly present amongst those 7.5%**.** As the age advanced & gravidity increased the prevalence of GBS colonization among third trimester pregnant women decreased.[35]

S Chua, S Arulkumaran, G Kumarsinghe et al[36] conducted a study in National University of Singapore and reported that in 326 pregnant women in whom vaginal and rectal swabs were taken, colonization rate was 14.1% in vagina and 15% in rectum.

Stephanie J Schrag, Elizabeth R Zell, Ruth Lynfield[37], conducted a study in the USA and reported the results of 5144 births, including 312 in which the newborn had early-onset group B streptococcal disease. Antenatal screening was documented for 52 percent of the mothers. The risk of early-onset disease was significantly lower among the infants of universally screened women than among those in the risk-based

screening group (adjusted relative risk, 0.46; 95% confidence interval, (0.36 to 0.60).[37]

Effects of selective intrapartum chemoprophylaxis for neonatal group B streptococcal early-onset disease were studied by Boyer KM, Gadzala CA, Kelly PD, Gotoff SP et al[9] .The effect of intrapartum ampicillin treatment on vertical transmission of group B streptococci (GBS) was examined in 575 prenatally colonized parturient women and their 580 newborn infants. Eighty women (43 receiving ampicillin) with premature labor and/or prolonged rupture of amniotic membranes were randomized. The other 495 were stratified into groups of 358 (31 receiving ampicillin) with no perinatal risk factors; 119 (28 receiving ampicillin) with premature labor and/or prolonged membrane rupture; and 23 (18 receiving ampicillin) with intrapartum fever. Ampicillin virtually eliminated vertical transmission in the treatment group with no risk factors and in both treatment groups with premature labor and/or prolonged membrane rupture. GBS colonization of neonates was detected only in women with intrapartum fever or brief (less than 1 hour) duration of treatment prior to delivery. Ampicillin treatment was associated with a highly significant reduction in maternal postpartum vaginal colonization by GBS. There were six group B streptococcal early-onset infections in infants of untreated subjects and no cases in treated subjects.[9]

Prevention of perinatal group B streptococcal disease. Revised guidelines from CDC. Schrag S, Gorwitz R, Fultz-Butts K, Schuchat[24] showed that recommendation of universal prenatal screening for vaginal and rectal GBS colonization of all pregnant women at 35-37 weeks' gestation, based on recent documentation in a large

retrospective cohort study of a strong protective effect of this culture-based screening strategy relative to the risk-based strategy.

.

Cost-effectiveness of rapid tests and other existing strategies for screening and management of early-onset group B streptococcus during labour , was a study conducted by Kaambwa B, Bryan S, Gray J, Milner P, Daniels J, Khan KS, Roberts TE [38]. It concluded that the most cost-effective strategy was the provision of routine intrapartum antibiotic prophylaxis to all women without prior screening but, given broader concerns relating to antibiotic use, this is unlikely to be acceptable. In its absence, intrapartum antibiotic prophylaxis directed by screening with enriched culture becomes cost-effective. The current strategy of risk-factor-based screening is not cost-effective compared with screening based on culture.

A study conducted by Faxelius G, Bremme K, Kvist-Christensen K, Christensen P, Ringertz et al [39]at Karolinska Hospital 1975-1986 was reviewed. GBS-septicemia was diagnosed in 40 infants within the first five days of life. The incidence was 1.24 per 1000 births. Fifty-five percent of the infants were preterm and 48% were born more than or equal to 12 hours after rupture of membranes. Prematurity and/or prolonged rupture of membranes were present in 83% of all neonates with fatal outcome. Case fatality was 22%. Deliveries by both cesarean section (31%) and vacuum extraction (26%) were increased in the mothers when compared to an overall incidence of 14 and 12% (p less than 0.01). Twenty-four (89%) of 27 mothers had low type specific IgG antibodies against the infecting GBS-serotype. Late onset GBS-septicemia was diagnosed in only two infants during the period. Seventeen mothers went through 24 subsequent pregnancies. In 11 of those,

the mothers were colonized with GBS and 10 received penicillin prophylaxis during pregnancy and/or delivery. None of the infants born after prophylaxis were colonized with GBS. Two were born prematurely and all had an uneventful course; whereas one infant delivered at 26 weeks gestation of a colonized untreated mother died of GBS-septicemia. Screening of parturients at risk and selective antibiotic prophylaxis may help to prevent early onset GBS-septicemia.

The effect of digital cervical examination on group B streptococcal culture, a study done by Knudtson EJ, Lorenz LB, Skaggs VJ, Peck JD, Goodman JR et al[40] showed the clinical prevalence of GBS was 19.5%. Discordant results were seen in 30/302 (9.9%) paired cultures (kappa = 0.68; 95% confidence interval, 0.568-0.783). An initially negative GBS culture result was positive on repeated testing in 13/243 (5.3%) pairs. Initially positive cultures were negative on repeated testing in 17/59 (28.8%) pairs. Patients with discordant results had similar characteristics as the remainder of study group. Given the observed proportion of discordant results (9.9%), the study had 80% power to detect a 5% difference between discordant pairs. [40]

Duration of intrapartum prophylaxis and concentration of penicillin G in fetal serum at delivery. In a study done by Barber EL, Zhao G, Buhimschi IA, Illuzzi. JL,[41] on 98 laboring GBS-positive women carrying singleton gestations at 37 weeks or greater were administered 5 million units of intravenous penicillin G followed by 2.5 million units every 4 hours until delivery. Umbilical cord blood samples were collected at delivery, and penicillin G levels were measured by high-performance liquid chromatography. Fetuses exposed to fewer than 4 hours of prophylaxis had higher penicillin G levels than those exposed to greater than 4 hours (P=.003). In

multivariable linear regression analysis, fetal penicillin G levels were determined by duration of exposure, time since last dose, dosage, and number of doses, but not maternal body mass index. Penicillin G levels increased linearly until 1 hour (R(2)=.40) and then decreased rapidly according to a power-decay model (R(2)=.67). All subgroups analyzed were above the minimal inhibitory concentration (MIC) for GBS (0.1 micrograms/mL)(P<.002). Individual samples were 10-179-fold above the MIC. In patients receiving maintenance dosing, penicillin G did not accumulate in the cord blood and returned to baseline after each 4-hour interval. [41]

The effect of intrapartum penicillin on vaginal group B streptococcus colony counts was found by McNanley AR, Glantz JC, Hardy DJ, Vicino D[42]. Of 50 subjects with GBS-positive antepartum cultures, 35 (70%) had positive intrapartum vaginal cultures, of which 27 received intrapartum PCN-G (pencillin G). Degree of vaginal colonization varied greatly between subjects, and counts (percentages) were not uniformly distributed. From the T(0) (time = zero) colony count standardized to 100%, fell rapidly to means +/- SE and medians of 18.2 +/- -7.5% and 0.5% at T(2) (P<.0001), 2.5 +/- 1.7% and 0.02% at T(4) (P = .006), and less than 0.2% and 0.0% at T(6 and 8) (P = 0.07 and P = 0.46, respectively).

Alvarez JR, Williams SF, Ganesh VL, Apuzzio JJ ,[43] did a retrospective study on pregnant women with PPROM from January 1, 2000, through December 31, 2005. Vaginal/rectal cultures were performed on admission and repeated daily. Patients received antibiotics until cultures were negative for 3 consecutive days. 214 were identified with PPROM; 169 of the women met the inclusion criteria. Thirty-three patients were GBS positive on admission and had negative cultures by day 3.

Neonatal sepsis occurred in 19 neonates (11.2%); 3 neonates (16%) were from mothers who tested positive for GBS on admission, and 16 neonates (84%) were from mothers who tested negative on admission. There were no cases of neonatal sepsis because of GBS.[43]

A study done by Gibbs RS, Schrag S & Schuchat,[44] GBS emerged dramatically in the 1970s as the leading cause of neonatal infection and as an important cause of maternal uterine infection. They reviewed the epidemiology, diagnosis, and therapy of GBS perinatal infection. In 1996, the first national consensus guidelines were released. Since then, there has been a 70% reduction in early-onset neonatal GBS infection, but no decrease in late-onset neonatal GBS disease.

In 2002, new national guidelines were released recommending 1) solely a screen-based prevention strategy, 2) a new algorithm for patients with penicillin allergy, and 3) more specific practices in certain clinical scenarios. Yet many clinical issues remain, including implementation of new diagnostic techniques, management of preterm rupture of membranes, use of alternative antibiotic approaches, improvement of compliance, prevention of low birth weight infants, emergence of resistant organisms, and vaccine development.[44]

Tsering Chomu Dechen, Kar Sumit, and Pal Ranabir[45] conducted an observational cross-sectional study during September 2002 to March 2004 on 524 pregnant women.

The culture positivity rate of GBS was 4.77% and coexistent organisms isolated were Candida species (36%), Staphylococcus aureus (8%) and Enterococcus species (8%). Culture positivity in the age group of 18–25 years was 5.71%, of which 5.74% were in their first pregnancy. The correlation between age group and gravida with GBS culture positivity was statistically insignificant. The culture positivity in <36 weeks of gestational age was 6.93%. This relation was statistically significant. Twenty-eight percent developed PROM. Sixty-four percent of culture positives had preterm labor.

Background

Streptococcus agalactiae, Group B streptococcus or commonly GBS, became known as an agent infecting udders of cows and was therefore given the name *Streptococcus agalactiae contagiosae.* GBS was first described as a cause of human infection in 1938, when three patients with fatal puerperal sepsis were described[5] . The bacteria remained unknown to most clinicians until the 1970s, when a dramatic increase of GBS septicaemia and meningitis in neonates was observed in different parts of the world .GBS became the most prevalent agent of serious neonatal infections and was detected in more than 40% of invasive isolates from neonates. The clinical course of invasive GBS disease in infants is often dramatic, with high morbidity, and until the middle of 1980s the case fatality was more than 50%. Research on the epidemiology and pathogenesis of GBS was initiated, and preventive actions like antibiotic prophylaxis during labour to women at risk of having a child with GBS infection were introduced in USA and Europe in the 1990s. Two different strategies to identify women at risk were recommended; the risk-factor strategy and

the screening strategy. Risk factors are prolonged rupture of membranes, premature birth, intrapartum fever, previous GBS infected infant or GBS bacteriuria detected during the current pregnancy, and GBS colonisation of the pregnant woman..

Screening for GBS colonisation is at present recommended in 35-37 weeks of gestation. However, rapid molecular methods like real-time PCR might replace the traditional culture screening, and make screening possible when the women are in labour. Even if intrapartum antibiotics have reduced early onset GBS disease it will not be 100% effective and antibiotic prophylaxis during labour has no effect on late onset GBS disease. Vaccines based on GBS antigens have been developed, and maternal vaccination is expected to prevent GBS disease in neonates, but final trials and implementation still lie some years ahead. Variations of GBS characteristics have implications for the formulation of GBS vaccines. Thus, surveillance of GBS is of importance.

Sporadic cases were reported during the next 3 decades, but this microorganism remained unknown to most clinicians until the 1970s, when a dramatic increase in the incidence of septicemia and meningitis in neonates caused by group B streptococci (GBS) was documented from geographically diverse regions[46,47,48]. Emergence of group B streptococcal infections in neonates was accompanied by an increasing number of these infections in pregnant women and non-pregnant adults. In pregnant women, infection commonly manifested as localized uterine infection or chorioamnionitis, often with bacteremia, and had an almost uniformly good outcome with antimicrobial therapy. In other adults, who typically had underlying medical conditions, infection often resulted in death. The incidence of perinatal infection

associated with GBS remained stable through the early 1990s. Case-fatality rates had declined by then, but remained substantial compared with case-fatality rates reported for other invasive bacterial infections in infants. Several notable events have occurred in recent years. Capsular type IX has been proposed, bringing the number of types causing invasive human disease .

The implementation of 2002 consensus guidelines to prevent early-onset disease in neonates through universal antenatal culture screening at 35 to 37 weeks of gestation and intrapartum antibiotic prophylaxis (IAP) has been associated with a substantial decline in the incidence of neonatal infection for the first time in 3 decades. Finally, testing of group B streptococcal vaccines in healthy adults has been achieved, offering promise that immunization to prevent maternal and infant and perhaps adult invasive group B streptococcal disease could become a reality.

THE ORGANISM:

Streptococcus agalactiae is the species designation for Streptococci belonging to Lancefield Group B. This bacterium is a facultative gram-positive diplococcus with an ultrastructure similar to that of other gram-positive cocci. Before Lancefield's classification of hemolytic streptococci in 1933[49], this microorganism was known to microbiologists by its characteristic colonial morphology, its narrow zone of B-hemolysis surrounding colonies on blood agar plates, and its double zone of hemolysis that appeared when plates were refrigerated an additional 18 hours beyond the initial incubation .Isolation from certain body sites (respiratory, genital, and gastrointestinal tracts) could be enhanced by use of broth medium containing

antimicrobial agents that inhibit growth of other bacterial species indigenous to these sites.

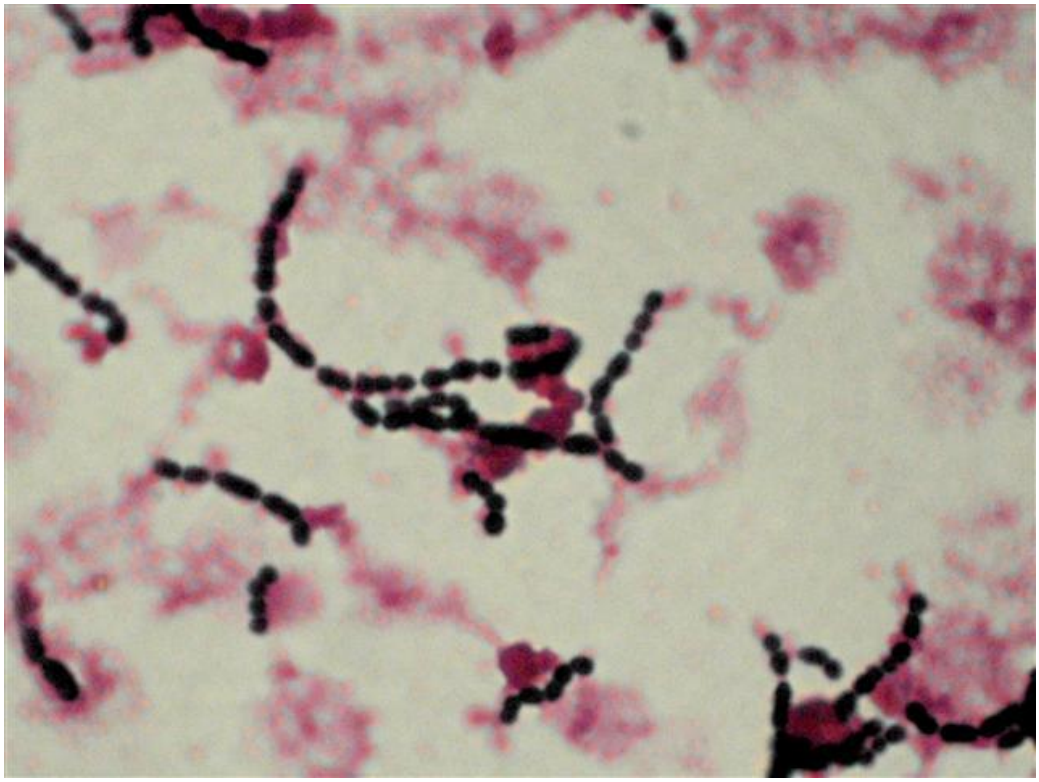


Figure 1: showing Group B Streptococci and positive zone of hemolysis on blood agar.

COLONIAL MORPHOLOGY AND IDENTIFICATION

Colonies of GBS grown on sheep blood agar medium are 3 to 4 mm in diameter, produce a narrow zone of B-hemolysis, are gray-white, and are flat and mucoid. B-hemolysis for some strains is apparent only when colonies are removed from the agar. Tests for presumptive identification include bacitracin and sulfamethoxazole-trimethoprim disk susceptibility testing (92% to 98% of strains are resistant), hydrolysis of sodium hippurate broth (99% of strains are positive), hydrolysis of bile esculin agar (99% to 100% of strains fail to react), pigment production during anaerobic growth on certain media (96% to 98% of strains produce an orange pigment), and CAMP (Christie-Atkins- Munch-Petersen) testing (98% to 100% of strains are CAMP-positive)[50]. The CAMP factor is a thermostable extracellular protein that, in the presence of the B toxin of Staphylococcus aureus, produces synergistic hemolysis when grown on sheep blood agar. Hippurate hydrolysis is an accurate method for presumptive identification of GBS, but the requirement for 24 to 48 hours of incubation limits its usefulness. GBS can be differentiated from other streptococci by a combination of the CAMP test, the bile esculin reaction, and bacitracin sensitivity testing. Biochemical micromethods identify GBS with reasonable accuracy after a 4-hour incubation period .Definitive identification of GBS requires detection of the group B–specific antigen common to all strains through use of hyperimmune grouping antiserum. Lancefield's original method required acid treatment of large volumes of broth-grown cells to extract the group B antigen from the cell wall .Supernatants were brought to neutral pH and mixed with hyperimmune rabbit antiserum prepared by immunization with the group B– variant strain (090R) (devoid of type Ia–specific antigen), and precipitins in capillary tubes were recorded. Less time-consuming serologic techniques are now

employed, but all use group-specific antiserum to identify the group B antigen in intact cells, broth culture supernatants, or cell extracts. Commercial availability and simplicity make latex agglutination–based assays the most practical and frequently used methods by hospital laboratories. Reverse transcriptase polymerase chain reaction (RT-PCR) methods have been developed more recently for grouping of clinical specimens, and PCR has been developed for genotyping of group B streptococcal isolates.

CLASSIFICATION

Lancefield defined two cell wall carbohydrate antigens employing hydrochloric acid–extracted cell supernatants and hyperimmune rabbit antisera: the group B–specific or "C" substance common to all strains and the type specific or "S" substance that allowed classification into types, initially types I, II, and III [51].Strains designated as type I were later shown to have cross-reactive and antigenically distinct polysaccharides, and the antigenically distinct type Ia and type Ib polysaccharides were defined. GBS historically designated type Ic were characterized when strains possessing type Ia capsular polysaccharide (CPS) were shown also to possess a protein antigen common to type Ib, most type II, and rarely type III strains. The type IV was identified as a new type in 1979. Antigenically distinct types, V through IX, now are characterized. Strains not expressing one of the CPS-specific antigens are designated as nontypable by serologic methods, but often can be characterized by PCR-based methods, possess increased resistance to opsonization in vitro. GBS express numerous additional surface proteins. Designation of additional a-like repetitive proteins (Alp) numerically (e.g., Alp2 and Alp3) is being considered. Most group B streptococcal strains have the gene for just one of the Alp family proteins.

Genes encoding Alp1 (also designated “epsilon”) are associated with type Ia, and genes encoding Alp3 are associated with type V strains .Some GBS contain surface proteins known as X antigens. These were first described by Pattison and coworkers [52] who introduced reagents for their detection in an attempt to classify non-typable strains further.

GROWTH REQUIREMENTS AND BACTERIAL PRODUCTS

GBS are quite homogeneous in their amino acid requirements during aerobic or anaerobic growth [53.] A glucose-rich environment enhances the number of viable GBS during stationary phase and the amount of CPS elaborated. Group B streptococcal invasiveness is enhanced by a fast growth rate and is optimal in the presence of at least 5% oxygen. Hemolysin that produces the B-hemolysis surrounding group B colonies on blood agar plates is an extracellular product of almost all strains and is active against the erythrocytes from several mammalian species. It has been isolated and characterized and is known to function as a virulence factor. Hemolysin is not detected in supernatants of broth cultures, suggesting either that it exists in a cell bound form, or that it is released by cells and rapidly inactivated.

After growth in stationary phase, GBS produce two types of pigment resembling a B-carotenoid. C5a esterase, a serine esterase, contributes to the pathogenesis of group B streptococcal disease by rapidly inactivating the neutrophil agonist C5a, preventing the accumulation of neutrophils at the site of infection .Another group of enzymes elaborated by nearly all GBS are the extracellular nucleases. Three distinct nucleases have been physically and immunologically characterized. All are maximally activated by divalent cations of calcium plus

manganese. These nucleases are immunogenic in animals, and neutralizing antibodies to them are detectable in sera from pregnant women known to be genital carriers of GBS. Their role in the pathogenesis of human infection is unknown.

GBS synthesize acylated (lipoteichoic)[54] and deacylated glycerol teichoic acids that are cell associated and can be readily extracted and purified . Strains from infants with early-onset or late-onset disease have higher levels of cell-associated and native de-acylated lipoteichoic acid, and this product seems to contribute to attachment to human cells .

EPIDEMIOLOGY AND TRANSMISSION

The relationship between GBS strains of human and bovine origin has been queried for years. There is no compelling evidence to suggest that cattle serve as a reservoir for human disease, and transmission of GBS from cows to humans is exceedingly rare .In addition, during the past decades when group B Streptococcus has been a dominant human pathogen, most of the population has lacked exposure to the two possible modes of transmission: (1) proximity to dairy cattle (direct contact) and (2) ingestion of unpasteurized milk. Application of molecular techniques to type III strains from bovine sources and strains infecting human neonates supports the assertion that these lineages are unrelated. Phylogenetic lineage determination does indicate, that some clonal complexes of invasive or colonizing strains in humans are related to “ancestral” lineages of bovine GBS.[55]

PATHOGENESIS OF NEONATAL GBS INFECTIONS AND VIRULENCE FACTORS OF GBS

GBS can reach the fetus in utero through ascending infection of the placental membranes and amniotic fluid. Alternatively, the newborn may become contaminated with the organism on passage through the birth canal. Invasive neonatal disease may be caused by both virulence factors in GBS and host factors. The GBS virulence includes factors that obstruct immunological defence mechanisms and the ability to penetrate epithelial and endothelial cellular barriers to reach the bloodstream and deeper tissues. GBS produce toxins that directly injure or disrupt host tissues, and also produce factors that provoke inflammatory pathways which may aggravate the disease [56,57].GBS colonisation of pregnant women and lack of maternal antibodies to GBS are also important factors contributing to invasive neonatal disease.

HOST FACTORS RELATED TO PATHOGENESIS

Risk Factors for Early-Onset Infection Infant and maternal factors that increase risk for early-onset group B streptococcal infection are listed below:[58,59]

1. Maternal colonization at delivery
2. High-density maternal colonization
3. Rupture of membranes before onset of labor
4. Preterm delivery <37 weeks of gestation
5. Prolonged rupture of membranes >18 hours
6. Chorioamnionitis
7. Intrapartum fever 38C (100.4 F)
8. Intrauterine monitoring
9. Maternal postpartum bacteremia

10. Multiple pregnancy
11. Group B streptococcal bacteriuria or urinary tract infection
12. Cesarean section
13. Low level of antibody to infecting CPS type
14. Previous infant with invasive group B streptococcal disease
15. Maternal race/ethnicity (African women more susceptible than American and Asian women)

Symptomatic early onset disease develops in 1% to 2% of infants born to colonized women who do not receive IAP, but this rate is considerably increased if there is premature onset of labor (before 37 weeks of gestation-15%), chorioamnionitis or interval between rupture of membranes and deliver longer than 18 hours (11%), twin pregnancy (35%) or maternal postpartum bacteremia (10%).

Maternal group B streptococcal bacteriuria and urinary tract infection are predictive of high-inoculum colonisation, which enhances infant risk for invasive infection .Heavy group B streptococcal colonization in the second trimester of pregnancy is also associated with increased risk of delivering a preterm infant with low birth weight .Among infants born to mothers with premature rupture of membranes at term gestation, maternal chorioamnionitis and colonization with GBS are strong predictors of neonatal infection .Vaginal colonization with GBS is an independent risk factor for the development of chorioamnionitis.[58]

Prolonged interval after rupture of membranes (>18hours) before delivery and preterm delivery (<37 weeks gestation) often are concomitant risk factors in neonates

with early-onset group B streptococcal infection. The estimated incidence of early-onset group B streptococcal infection is 10 times higher in preterm than in term neonates .Even with correction for preterm delivery, twin pregnancy is an independent risk factor for invasive early-onset group B streptococcal septicemia .The explanation for the increased risk in twins likely relates to genetic factors regulating host susceptibility, lack of specific antibody to the infecting strain in the mother, similar density of maternal colonization, and virulence of disease-producing strains.

ADHERANCE TO HUMAN CELLS

To establish colonisation, GBS bind efficiently to human vaginal cells, with maximal adherence at the acidic pH characteristic of vaginal mucosa. The ability of adherence to other human cells like alveolar epithelium and endothelium and brain endothelium is probably also important for the pathogenesis of neonatal sepsis[60] .Molecules that appear to play an important role in adherence are the surface proteins, C5a peptidase (a bifunctional protein, which enzymatically cleaves C5a and mediates adherence to fibronectin) and laminin-binding protein in the bacteria and in addition extra-cellular components as fibronectin in the host.

IMMUNOLOGY AND PATHOGENESIS: HOST-BACTERIAL INTERACTIONS RELATED TO PATHOGENESIS

The prevalence and severity of group B streptococcal diseases in neonates have stimulated intensive investigation to elucidate the pathogenesis of infection. The unique epidemiologic and clinical features of group B streptococcal disease pose several basic questions that provide a framework for hypothesis development and experimental testing: How does the organism colonize pregnant women and gain

access to the infant before or during delivery? Why are newborns, especially infants born prematurely, uniquely susceptible to infection? What allows GBS to evade host innate immune defenses? How do these organisms gain entry to the bloodstream and then cross the blood-brain barrier to produce meningitis? What specific GBS factors injure host tissues or induce the sepsis syndrome?. Advances in knowledge of pathogenesis have been achieved through development of cell culture systems and animal models. Refinement of molecular genetic techniques has yielded isogenic mutant strains varying solely in the production of a particular component (e.g., CPS). Such mutants are important in establishing the biologic relevance of a given trait and its requirement for virulence in vivo. The sequencing of several complete GBS genomes has provided additional context for interpretation of experimental data and comparison with other well-studied pathogens[61] .Although GBS have adapted well to asymptomatic colonization of healthy adults, they remain a potentially devastating pathogen to susceptible infants.

ASYMPTOMATIC INFECTION (COLONIZATION) IN ADULTS

Group B streptococcal infection limited to mucous membrane sites is designated as asymptomatic infection, colonization, or carriage. Comparisons of the prevalence of colonization are related to differences in ascertainment techniques. Factors that influence the accuracy of colonization detection include density of colonization, choice of bacteriologic media, body sites sampled, number of culture specimens obtained, and time interval of study. Isolation rates are higher with use of broth rather than solid agar media, with media containing substances inhibitory for normal flora (usually antimicrobials), and with selective broth rather than selective solid agar media. Among selective broth media, Todd-Hewitt broth with gentamicin

(4 to 8 mg/mL) or colistin (or polymyxin B) (10 mg/mL) and nalidixic acid (15 mg/mL) (Lim broth), with or without sheep red blood cells, has been useful for accurate detection of GBS from genital and rectal cultures.[62] Such media inhibit the growth of most gram negative enteric bacilli and other normal flora that make isolation of streptococci from these sites difficult. Use of broth media enables detection of low numbers of organisms that escape detection when inoculation of swabs is directly onto solid agar.

Isolation rates also are influenced by body sites selected for culture. Female genital culture isolation rates double with progression from the cervical os to the vulva .In addition, culture sampling of lower genital tract and rectal sites increases group B streptococcal colonization rates 10% to 15% beyond that found if a single site is cultured .The urinary tract is an important site of group B streptococcal infection, especially during pregnancy, when infection is typically manifested as asymptomatic bacteriuria. To predict accurately the likelihood of neonatal exposure to GBS at delivery, maternal culture specimens from the lower vagina and rectum (not perianal area) should be collected.

In neonates, external auditory canal cultures are more likely to yield GBS than cultures from anterior nares ,throat, umbilicus, or rectum in first 24 hours of life and isolation of organisms from the ear canal is a surrogate for the degree of contamination from amniotic fluid and vaginal secretions sequestered during the birth process. After the first 48 hours of life, throat and rectal sites are the best sources for detection of GBS, and positive cultures indicate true colonization (multiplication of organisms at mucous membrane sites), not just maternal exposure. Cultures from the

throat and rectum are the best sites for detection during childhood and until the start of sexual activity, when the genitourinary tract becomes a common site of colonization .

The prevalence of group B streptococcal colonization is influenced by the number of cultures obtained from a site and the interval of sampling. Historically, longitudinal assessment during pregnancy defined vaginal colonization patterns as chronic, transient, intermittent, or indeterminant A longitudinal cohort study of non-pregnant young women in the 1970s found that among women who were culture-negative at enrollment, almost half acquired vaginal colonization during follow-up at 3- 4 months intervals .The duration of any group B Streptococcal colonization among college students was estimated by Foxman and colleagues and is longer for women (14 weeks) than for men (9 weeks).[63] Nearly half of women vaginally colonized at delivery have had negative antenatal culture results. In a more recent longitudinal study of pregnant women, the predictive value of a positive prenatal vaginal or rectal culture from the second trimester for colonization at delivery was 67%[9]. The predictive value of a positive prenatal culture result is highest (73%) in women with vaginal and rectal colonization and lowest (60%) in women with rectal colonization only. Cultures performed 1 to 5 weeks before delivery are fairly accurate in predicting group B streptococcal colonization status at delivery in term parturients. Within this interval, the positive predictive value is 87% (95% confidence interval 83 to 92), and the negative predictive value is 96% (95% confidence interval 95 to 98). Culture specimens collected within this interval perform significantly better than specimens collected 6 or more weeks before delivery.

A higher prevalence of colonization with GBS has been found among pregnant diabetic patients than among non-diabetic controls [64].Carriage over a prolonged interval reportedly occurs more often in women who use tampons than women who do not .Colonization is more frequent among teenage women than among women 20 years of age or older and among women with three or fewer pregnancies than in women with more than three pregnancies Genital isolation rates are significantly greater in patients attending sexually transmitted disease clinics than in patients attending other outpatient facilities Ethnicity is related to colonization rates Factors that do not influence the prevalence of genital colonization in non-pregnant women include use of oral contraceptives ,marital status, presence of vaginal discharge or other gynecologic signs or symptoms ,carriage of Chlamydia trachomatis, Ureaplasma urealyticum, Trichomonas vaginalis, or Mycoplasma hominis and infection with Neisseria gonorrhea Colonization with GBS can elicit an immune response. In a group of pregnant women evaluated at the time of admission for delivery, vaginal or rectal colonization with serotype Ia, II, III, or V was associated with significantly higher serum concentrations of IgG specific for the colonizing CPS type compared with noncolonized women .Moderate concentrations of Ia, Ib, II, III, and V CPS-specific IgG also were found in association with colonization during pregnancy. Maternal colonization with type III or IV was least likely to be associated with these CPS-specific antibodies. In contrast to infection with organisms such as N. gonorrhoeae or genital mycoplasmas, genital infection with GBS is not related to genital symptoms.

GBS have been isolated from vaginal or rectal sites or both in **15% to 40%** of pregnant women[65]. These variations in colonization rates relate to intrinsic differences

in populations (age, ethnicity, parity, socioeconomic status, geographic location) and to lack of standardization in culture methods employed for ascertainment. True population differences account for some of the disparity in these reported prevalence rates. When selective broth media are used, and vaginal and rectal samples are cultured, the overall prevalence of maternal colonization with GBS by region is 12% in India and Pakistan, 19% in Asia and the Pacific Islands, 19% in sub-Saharan Africa, 22% in the Middle East and North Africa, 14% in Central and South America, and 26% in the United States[65] .The reported rates of colonization among pregnant women range from 20% to 29% in eastern Europe, 11% to 21% in western Europe, 21% to 36%in Scandinavia, and 7% to 32% in the southern part of Europe .The rate of persistence of group B streptococcal colonization in a subsequent pregnancy is higher compared with women negative for colonization in their prior pregnancy. The prevalence rates of pharyngeal colonization among pregnant and non-pregnant women and heterosexual men are similar .However, the rate approaches nearly 20% in homosexual men (who have sex with men). No definite relationship between isolation of GBS from throat cultures of adults or children and symptoms of pharyngitis has been proved ,but some investigators have suggested that these organisms can cause acute pharyngitis.

MATERNAL COLONISATION

The presence of GBS in the genital tract of the mother at delivery determines whether or not a newborn is at risk for invasive disease. Among infants born to colonized women, the risk of early-onset disease is approximately 30-fold that for infants born to women with a negative result on prenatal cultures. A direct relationship exists between the degree (inoculum size) of group B streptococcal

vaginal colonization, the risk of vertical transmission, and the likelihood of serious disease in the newborn. Consequently, a crucial step in the pathogenesis of invasive disease in the newborn caused by GBS is colonization of pregnant women. To establish colonization of the female genital tract, GBS must adhere successfully to the vaginal epithelium. Compared with other microorganisms, GBS bind very efficiently to exfoliated human vaginal cells or vaginal tissue culture cells with maximal adherence at the acidic pH characteristic of vaginal mucosa. A low-affinity interaction with epithelial cells is mediated by its amphiphilic cell wall–associated lipoteichoic acid, whereas higher affinity interactions with host cells are mediated by hydrophobic surface proteins. Soluble lipoteichoic acid competitively inhibits epithelial cell adherence and decreases vaginal colonization of pregnant mice. High-affinity protein-mediated interactions of GBS with epithelium are mediated largely through extracellular matrix components, such as fibronectin, fibrinogen, and laminin, which interact with host cell–anchored proteins such as integrins. Binding occurs to immobilized, but not soluble fibronectin, suggesting that this interaction requires close proximity of multiple fibronectin molecules and group B streptococcal adhesins. More recently, a genome-wide phage display technique revealed a fibronectin-binding property associated with the surface-anchored group B streptococcal C5a peptidase, ScpB. The dual functionality of ScpB was confirmed by decreased fibronectin binding of isogenic ScpB mutants and the direct interaction of recombinant ScpB with solid-phase fibronectin .Similar targeted bacterial Infections mutagenesis studies showed that adherence of GBS to laminin involves a protein adhesin called Lmb .Attachment to fibrinogen is mediated by repetitive motifs within the surface-anchored protein FbsA, and binding to human keratin 4 is carried out by the serine rich repeat domain protein Srr-1. More recently, GBS were revealed to express filamentous cell surface

appendages known as pili .Among eight sequenced GBS genomes, two genetic loci encoding pili were identified, although not all genomes contain both loci .One of these islands includes genes encoding PilB, an LP(x)TG motif–containing protein, that polymerizes to form a pilus backbone, along with accessory pilus proteins PilA and epithelial cell adherence was reduced in isogenic GBS mutants lacking PilA or PilC, but not mutants lacking the PilB backbone .Solution of the crystal structure of PilC reveals a specific IgG-like fold domain (N2) required for epithelial cell binding.[73]

ASCENDING AMNIOTIC INFECTION

GBS can reach the fetus in utero through ascending infection of the placental membranes and amniotic fluid. Alternatively, the newborn may become contaminated with the organism on passage through birth canal. Infection by the ascending route plays a pivotal role in early-onset disease. A direct relationship exists between the duration of membrane rupture before delivery and attack rate for early-onset disease, whereas an inverse relationship exists between the duration of membrane rupture and the age at which clinical signs of early-onset pneumonia and sepsis first appear .When the duration of membrane rupture was 18 hours or less, the attack rate was 0.7 per 1000 live births[74]; when it was more than 30 hours, the attack rate increased to 18.3 per 1000 .Histologic examination of placentaes from women with group B streptococcal chorioamnionitis showed bacterial infiltration along a choriodecidual space, implying that ascending infection may be the primary trigger in many instances of premature rupture . GBS may promote membrane rupture and premature delivery by several mechanisms. Isolated chorioamniotic membranes exposed to the organism have decreased tensile strength and elasticity and are prone to rupture .The presence

of GBS at the cervix activates the maternal decidua cell peroxidase–hydrogen peroxide– halide system, promoting oxidative damage to adjacent fetal membranes .GBS also can modify the arachidonic acid metabolism of cultured human amnion cells, favoring production of prostaglandin E2 ,which is known to stimulate the onset of labor. Stimulation of chorionic cell release of macrophage inflammatory protein-la and interleukin (IL)-8 from human chorion cells recruits inflammatory cells that may contribute to infection-associated preterm labor .GBS occasionally seem to penetrate into the amniotic cavity through intact membranes. Clinically, this mechanism of entry is suggested by anecdotal reports of neonates with fulminant early-onset infection after delivery by cesarean section and no identifiable obstetric risk factors. Migration of the organism through freshly isolated chorioamniotic membranes has been documented by scanning and transmission electron microscopy .GBS invade primary chorion cells efficiently in vitro and are capable of transcytosing through intact chorion cell monolayers without disruption of intercellular junctions They also secrete an enzyme that degrades hyaluronic acid, an important component of the extracellular matrix that is abundant in placental tissues and may facilitate amniotic invasion .

Amniotic fluid supports the proliferation of GBS[74], such that when the organism gains access to the uterine cavity a large inoculum can be delivered to the fetal lung; this results in a continuum of intrapartum (stillbirth) to early postpartum infant death. In utero infection probably accounts for the 40% to 60% of newborns with early-onset disease who have poor Apgar scores and in whom pulmonary signs develop within a few hours of birth because these infants almost invariably display clinical or histologic evidence of congenital pneumonia .Conversely, when GBS are

encountered in the immediate peripartum period or on passage through the birth canal, a lesser inoculum is delivered to the neonate. Although a small but meaningful risk of subsequent invasive disease exists, most of these newborns have asymptomatic colonization limited to mucosal surfaces and remain healthy.

TRANSMISSION OF GROUP B STREPTOCOCCI TO NEONATES

The presence of GBS in the maternal genital tract at delivery is the significant determinant of colonization and infection in the neonate. Exposure of the neonate to the organism occurs by the ascending route in utero through translocation through intact membranes, through ruptured membranes, or by contamination during passage via the birth canal. Prospective studies have indicated vertical transmission rates of 29% to 85%, with a mean rate of approximately 50% among neonates born to women from whom GBS were isolated from cultures of vagina or rectum or both at delivery. Conversely, only about 5% of infants delivered to culture-negative women become asymptomatically colonized at one or more sites during the first 48 hours of life[66].

The risk of a neonate acquiring colonization by the vertical route correlates directly with the density of colonization (inoculum size). Neonates born to heavily colonized women are more likely to acquire carriage at mucous membrane sites than neonates born to women with low colony counts of GBS in vaginal cultures at delivery [66]. Boyer and associates[57]found that rates of vertical transmission were substantially higher in women with heavy than in women with light colonization (65% versus 17%) and that colonization at multiple sites and development of early-onset disease were more likely among infants born to heavily colonized mothers. The

likelihood of colonization in a neonate born to a woman who is culture-positive at delivery is unrelated to maternal age, race, parity, or blood type or to duration of labor or method of delivery .It is unclear whether preterm or low birth weight neonates are at higher risk for colonization from maternal sources than term infants.

Most neonates exposed through their mothers to GBS have infection that is limited to surface or mucous membrane sites (colonization) that results from contamination of the oropharynx, gastric contents, or gastrointestinal tract by swallowing of infected amniotic fluid or maternal vaginal secretions. Healthy infants colonized from a maternal source show persistence of infection at mucous membrane sites for weeks[67]. The distribution of CPS types in group B streptococcal isolates from mothers is comparable to that in isolates from healthy neonates.

Other sources for group B streptococcal colonization in neonates have been established. Horizontal transmission from hospital or community sources to neonates is an important, albeit less frequently proven, mode for transmission of infection[68] .Cross-contamination from maternally infected to uninfected neonates can occur from hands of nursery personnel .In contrast to group A streptococci, which can produce epidemic disease in nurseries, GBS rarely exhibit this potential, and isolation of neonates with a positive culture result from skin, umbilical, throat, or gastric cultures is never indicated. Epidemiologic analysis suggested infant-to-infant spread by means of the hands of personnel, although acquisition from two nurses colonized with the same phage–type Ib strain was not excluded[69]. The infection control measures instituted prevented additional cases. This and other reports indicate that isolating culture-positive infants during an outbreak coupled with implementation of strict hand hygiene for infant contact significantly diminishes nosocomial acquisition.

Community sources afford potential for transmission of GBS to the neonate. Only 2 of 46 neonates culture-negative for GBS when discharged from the newborn nursery acquired mucous membrane infection at 2 months of age The mode of transmission likely is feco-oral. Whether acquired by vertical or horizontal mode, colonization of mucous membrane sites in neonates and young infants usually persists for weeks or months .

STAGES OF NEONATAL INFECTION

A) Penetration of host cellular barriers

GBS can traverse and penetrate intact placental membranes, weaken their tensile strength and promote rupture and premature delivery by several mechanisms[70]. The bacteria proliferate easily in the uterine cavity and a large inoculum can therefore be swallowed by the foetus and delivered to the foetal lung. GBS spreads from the initial pulmonary focus to the bloodstream and is circulated through other organs and tissues. An important factor in the cellular damage is β-haemolysin/cytolysin. The cytolytic, proinvasive and proinflammatory effects of GBS are partly neutralized by dipalmotyl phosphatidylcholine (DPPC), the major phospholipids constituent of human lung surfactant . This may in part explain the elevated risk of premature surfactant-deficient neonates to suffer severe GBS lung injury and invasive disease. Cellular invasion is shown to correlate with the virulence potential of GBS strains. Clinical isolates of GBS from infants with invasive GBS disease invade epithelial cells better than strains from the vaginal mucosa of asymptomatic women.

B) Direct cytotoxicity to host phagocytes and inactivation of complement

The *cylE* –encoded β-hemolysin/cytolysin toxin, which is associated with the bacterial surface membrane, produces direct cytolytic injury to macrophages and induces macrophage apoptosis. GBS also contribute to poor mobilisation of neutrophils by production of C5a peptidase, an enzyme that cleaves and inactivates human C5a, a complement component that is important in neutrophil chemotaxis[71].

C) Impairment of myocardial function

GBS directly impairs cardiomyocyte viability and function through β-hemolysin/cytolysin that possibly affects maintenance of normal calcium in intact cardiomyocytes and potentially leads to cell death. Experiments in rabbits have shown that infusion of GBS leads to lower cardiac output and decreased mean arterial pressure. This is caused by myocardial dysfunction rather than decreased vascular resistance .

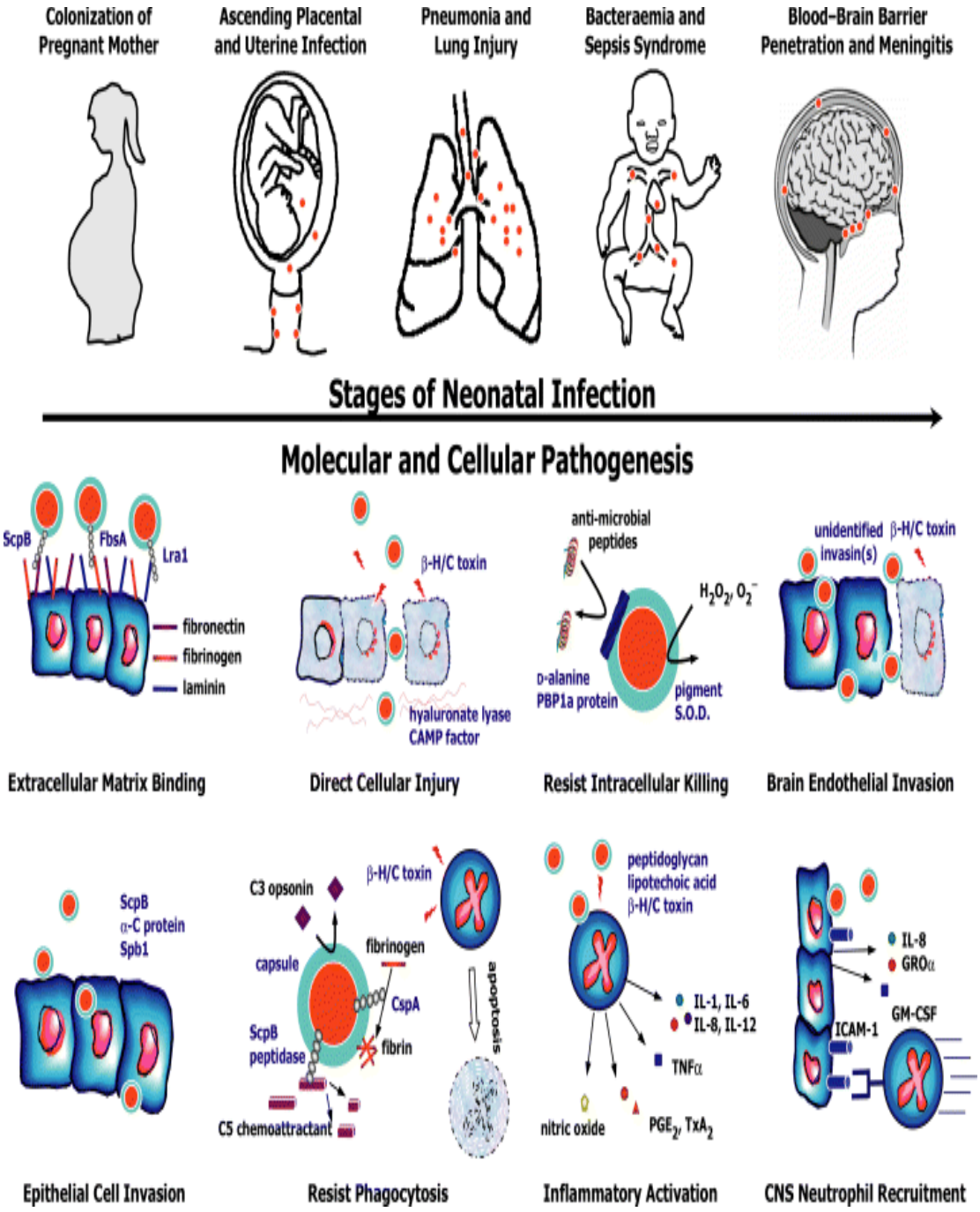


FIGURE 2: An outline of stages in the molecular and cellular pathogenesis of neonatal GBS infection (β-H/C: beta-haemolysin/cytolysin. S.O.D.: superoxide dismutase. IL: interleukin. TNFα: tumour necrosis factor-alpha. PGE2: prostaglandin E2. TxA2: thromboxane A2. GROα: growth related oncogene-alpha. ICAM-1:intercellular adhesion molecule 1. GM-CSF: granulocyte macrophage colony-stimulating factor).

ASYMPTOMATIC INFECTION IN INFANTS AND CHILDREN

Sites of colonization with GBS differ in children versus adults. In a study of 100 girls ranging in age from 2 months to 16 years, Hammerschlag and coworkers isolated GBS from lower vaginal, rectal, or pharyngeal sites, or all three, in 20% of children.[72]

PULMONARY AND BLOOD STREAM ENTRY

Early-onset group B streptococcal disease is heralded by respiratory symptoms, including tachypnea, hypoxia, cyanosis, and pulmonary hypertension One third to more than half of infants are symptomatic at birth or within 4 to 6 hours after delivery. Autopsies in fatal early-onset cases reveal that 80% have histologic evidence of lobar or multilobar pneumonia ,characterized by dense bacterial infiltration, epithelial cell damage, alveolar hemorrhage, interstitial inflammatory exudate, and hyaline membrane formation [75].When pneumonia develops in newborn primates exposed by intra-amniotic injection of GBS, bacterial density reaches 109 to 1011 organisms per gram of lung tissue. Group B streptococcal disease rarely is limited to the initial pulmonary focus, but spreads to the bloodstream and is circulated through other organs and tissues. The capacity of GBS to cause disruption of the lung epithelial and endothelial barrier evidently involves the process of intracellular invasion, direct cytolytic injury, and damage induced by the inflammatory response of the newborn host. Intracellular invasion of alveolar epithelial and pulmonary endothelial cells by GBS was first noted in newborn macaques after intra-amniotic challenge and later confirmed in human tissue culture lines derived from both cellular barriers .In vivo and in vitro electron microscopy studies show that host cytoskeletal changes are triggered that lead to endocytotic uptake of the bacterium within a

membrane-bound vacuole. Uptake requires induction of signal transduction pathways in the host cell that are mediated by Rho-family GTPases and phosphatidylinositol-3 kinase.

Cellular invasion is correlated with virulence potential. Clinical isolates of GBS from infants with bloodstream infections invade epithelial cells better than strains from the vaginal mucosa of asymptomatic women. FbsA, a group B streptococcal fibrinogen binding protein, Lmb, which mediates laminin binding and ScpB, which interacts with fibronectin, each play a role in promoting efficient epithelial or endothelial cell invasion. Another GBS surface protein, Spb1, was identified by subtractive hybridization to play a specific role in serotype III GBS invasion of epithelial cells. In addition, surface-anchored a C protein specifically interacts with host cell glycosaminoglycan on the epithelial cell surface to promote group B streptococcal internalization. By contrast, CPS decreases intracellular invasion, presumably through steric interference of certain receptor ligand interactions.

The cellular damage seems to result largely from the actions of B-hemolysin/cytolysin. Pore-forming toxin lyses lung epithelial and endothelial cells and compromises their barrier function. At subcytolytic doses, it promotes intracellular invasion and triggers the release of IL-8, the principal chemoattractant for human neutrophils. The cytolytic, proinvasive, and proinflammatory effects of group B streptococcal B-hemolysin/cytolysin all are neutralized by dipalmitoyl phosphatidylcholine, the major phospholipid constituent of human lung surfactant. This finding may partly explain the increased risk in premature, surfactant-deficient

neonates for severe lung injury and invasive disease from group B streptococcal infection. Treatment with exogenous surfactant reduces histologic evidence of lung inflammation, improves lung compliance, and mitigates bacterial growth in preterm rabbits infected with GBS .Clinical studies exploring the effect of surfactant administration on human infants with group B streptococcal sepsis also suggest a beneficial effect.

BLOOD BRAIN BARRIER PENETRATION AND MENINGITIS

The pathophysiology of group B streptococcal meningitis varies according to age at onset. In early-onset disease, autopsy studies show little or no evidence of leptomeningeal inflammation, despite the presence of abundant bacteria, vascular thrombosis, and parenchymal hemorrhage [76]. By contrast, infants with late-onset disease usually have diffuse purulent arachnoiditis with prominent involvement of the base of the brain .Similar age related differences in central nervous system (CNS) pathology are evident in the infant rat model of invasive disease. These histopathologic differences reflect underdevelopment of the host immunologic response in the immediate neonatal period, with a higher proportion of deaths resulting from overwhelming septicemia. To produce meningitis, GBS must penetrate human brain microvascular endothelial cells, the single-cell layer constituting the blood-brain barrier. Inflammation of individual brain vessels can lead to focal lesions, whereas diffuse alterations of cerebral blood flow could cause generalized hypoxic ischemic injury and cerebral edema. Arteriolar dysfunction is associated with the presence of oxygen free radicals thought to be a by-product of the phagocytic killing by infiltrating neutrophils.

INVASIVE NEONATAL INFECTIONS:

Definition of neonatal sepsis

Neonatal sepsis refers traditionally to sepsis in newborn babies during the first month of life. However, increased survival of immature and premature babies has resulted in a large group of infants with a high susceptibility to infections for a long time after birth, and the inclusion period for neonatal sepsis and meningitis often covers the whole hospital period. Neonatal sepsis may be classified according to the time of onset of the disease; early onset disease (EOD) and late onset disease (LOD). This distinction has clinical relevance as EOD is mainly due to bacteria acquired before and during delivery, and LOD to bacteria acquired after delivery (from nosocomial or community sources). Unfortunately, there is no consensus as to what age limits apply, making it difficult to compare studies where cases are grouped into EOD and LOD without further details. In most literature on GBS, EOD is 0-6 days and LOD 7-90 days after birth.

Sepsis, SIRS

The terms “sepsis” or “septicaemia” are traditionally used for isolation of bacteria in blood in combination with clinical symptoms. The term SIRS (systemic inflammatory response syndrome) was originally proposed to describe the non-specific inflammatory process occurring in adults after trauma, infection, burns, pancreatitis and other diseases. The criteria for use in adults have later been modified for use in children and infants, and include a core temperature >38.5 ºC or <36 ºC, tachycardia, increased respiratory rate and an elevated or depressed leukocyte count .Sepsis may be defined as SIRS in the presence of or as a result of suspected or proven infection[78]. Severe sepsis is defined as sepsis plus one of the following;

cardiovascular organ dysfunction or acute respiratory distress syndrome or two or more other organ dysfunctions. Septic shock is defined as sepsis and cardiovascular organ dysfunction .

Aetiology and predominant pathogens of neonatal sepsis

In developing countries, there appears to be a wide variety of bacteria causing EOD and LOD. In most studies, Gram-negative organisms are predominant. Among Gram-negative organisms, *Klebsiella spp.*, *Escherichia coli*, *Pseudomonas spp.* and *Salmonella spp.* are the most often reported. Among Gram-positive bacteria, *Staphylococcus aureus,* Coagulase negative staphylococci (CoNS), *Streptococcus pneumoniae and Streptococcus pyogenes* are the most often reported species .This variation may be true, but important confounders may include different definitions of EOD and LOD, different inclusion criteria for studies (including population sampled), inability to culture certain organisms, small numbers, and/or short periods of surveillance. The latter may be particularly important, as surveillance may be occurring during, or indeed may have been initiated because of, an outbreak of a specific pathogen and may not therefore be representative. Organisms responsible for neonatal infections in developed countries have changed in the last decade . While *S. pyogenes* and *S. pneumoniae* constituted half of the cases at Yale from 1933 to 1943, no cases caused by these bacteria were detected in the period 1989-2003 .Following the introduction of sulfonamides and penicillin, Gram-negative bacteria, and in particular *E. coli,* became predominant in neonatal infections. From the 1970s ,GBS emerged as the predominant microbe, especially in the first 24 hours after birth. In the last twenty years ,Gram-positive organisms have dominated both EOD and LOD in term infants, while *E. coli* have been more common in premature infants.

MUCOSAL IMMUNE RESPONSE

Genital colonization with GBS may elicit specific antibody responses in cervical secretions. Women with group B streptococcal type Ia, II, or III rectal or cervical colonization have markedly elevated levels of IgA and IgG to the colonizing serotype in their cervical secretions compared with cervical secretions from noncolonized women. Elevated amounts of IgA and IgG to the protein antigen R4 also have been found in women colonized with type III strains (most type III strains contain R4 antigen) compared with noncolonized women. These findings suggest that a mucosal immune response occurs in response to colonization with GBS. Induction of mucosal antibodies to surface group B streptococcal polysaccharide or protein antigens may prevent genital colonization, diminishing vertical transmission of infection from mothers to infants.

COMPLEMENT AND ANTIGENS

Shigeoka and colleagues[77] showed that specific antibody was required and that the classical complement pathway maximized opsonization of types I, II, and III GBS. Capsule-specific antibodies also facilitated alternative complement pathway–mediated opsonization and phagocytosis of type III GBS. The relationship between antibody concentration and the rate constant of killing of type III strains was found to be linear and determined, at least in part, by the number of antibody molecules bound per organism. β-hemolysin/cytolysin toxin, which is associated with the bacterial surface membrane, produces direct cytolytic injury to macrophages and induces macrophage apoptosis .GBS also contribute to poor mobilisation of neutrophils by production of

C5a peptidase, an enzyme that cleaves and inactivates human C5a, a complement component that is important in neutrophil chemotaxis .

IMPACT OF NEONATAL SEPSIS

In developing countries the neonatal mortality rate ranges from 17 to 68 per 1000 live births[79] in the first 28 days, and one third of these deaths are caused by infections. Some recent studies have shown a declining incidence of EOD in infants born after 37 weeks gestational age and also a declining incidence of invasive GBS disease. Infections caused by CoNS have increased, especially LOD. The decline of EOD may be related to improved perinatal care of infants at risk and increased use of antibiotic prophylaxis in pregnant women and neonates. On the other side, the improved neonatal care has also led to increased survival of immunocompromised immature and premature neonates susceptible to late onset infections like CoNS. CoNS are considered as opportunistic pathogens with increased virulence in immunocompromised patients, and they are also associated with the use of central venous catheters for patients with severe underlying conditions.

INVASIVE GBS DISEASE IN INFANTS

EARLY ONSET GBS DISEASE (EOD)

EOD (age at onset 0-6 days) almost always manifests itself within 24 hours of birth (median age 8 hours in 90% of cases, 5% appears during 24-48 hours).In premature infants, onset of symptoms is often within 6 hours of birth. GBS colonisation in pregnant women is the single most important risk factor for early onset newborn disease due to vertical transmission and colonisation of the infant during

delivery. The most common manifestations of EOD are septicaemia, pneumonia and meningitis. Irrespective of the site of involvement, respiratory signs (apnoea, grunting, tachypnea or cyanosis) are the clinical findings in more than 80% of neonates, and they can be difficult to oxygenate. A differential diagnosis of GBS sepsis is RDS (Respiratory distress syndrome). Also, radiographically, features consistent with and indistinguishable from those of hyaline membrane disease are present in more than one half of neonates with GBS and pulmonary infection. Treatment with surfactant improves gas exchange in a majority of these infants, although the response is slower than in non-infected infants .Other associated signs include lethargy, poor feeding, hypothermia or fever, abdominal distension, pallor, tachycardia and jaundice. Hypotension is an initial finding in approximately 25%. Infant with foetal asphyxia related to GBS infection in uteri may have shock and respiratory failure at delivery. Meningitis is seen in 5-10% of neonates with EOD, but most of them present with the same symptoms as those without meningitis.

LATE ONSET DISEASE (LOD)

LOD affects the infant from 7 days to 90 days of age. Nosocomial infection of premature infants in neonatal intensive care units (NICU) and transmission of virulent GBS strains from mother to infant via skin or breast milk might explain some of the cases. However, most infants with LOD have no known risk factors and an uneventful early neonatal history, and in most of these infants the mechanisms of infection are not revealed .LOD often presents with hypothermia or hyperthermia, hyperglycaemia or irritability. Grunting respiration and apnoea are less frequent initial findings than in EOD[80]. Meningitis is a frequent clinical manifestation, occurring in estimatedly 35-50% of cases .

LATE LATE-ONSET INFECTION

Infections in infants older than 89 days of age can account for 20% of cases of late-onset disease The terms very late onset, late late-onset, and beyond early infancy have been applied to disease in these infants. Most of these infants have a gestational age of less than 35 weeks. The need for prolonged hospitalization and the immature host status in these infants probably contributes to infection beyond the interval for term neonates. Bacteremia without a focus is a common presentation. Occasionally, a focus for infection, such as the CNS, intravascular catheter, or soft tissues, is identified .In the outpatient setting, infants older than 89 days of age are likely to have a temperature greater than 39 C (>102.2 F) and a white blood cell count exceeding 15,000/mm3 .A viral infection can precede the onset of bacteremia .When there are no other apparent risk factors for late late-onset infection in a term infant, immunodeficiency including HIV infection should be considered .

INCIDENCE OF INFECTION IN NEONATES AND PARTURIENTS

Two clinical syndromes occur among young infants with group B streptococcal disease that are epidemiologically distinct and relate to age at onset. Historically, the attack rates for the first of these syndromes, designated early-onset because it occurs within the first 6 days of life (mean onset 12 to 18 hours), ranged from 0.7 to 3.7 per 1000 live births. The attack rates for late onset infection (mean onset 7 to 89 days of age) ranged from 0.5 to 1.8 per 1000 live births. Multistate active surveillance that identified cases of invasive disease in a population of 10.1 million in 1990 reported an incidence of 1.6 and 0.3 per 1000 live births for early-onset and late onset disease[59] .There has been a dramatic decline in the incidence of early-onset disease with implementation of universal antenatal culture screening and

use of intrapartum antibiotic prophylaxis(IAP). From 1993-1998, when risk based and culture-based methods were in use, incidence of early-onset disease declined by 65%, from 1.7 to 0.6 per 1000 live births .Comparison of the two approaches showed the superiority of the culture-based approach .The incidence of early-onset disease has declined an additional 27% in association with implementation in 2002 of revised consensus guidelines advocating a culture-based approach for identifying organism and for prevention of early-onset disease to a rate of 0.34 per 1000 live births in 2007 [60] . Culture based approach is taking vaginal and rectal cultures between 35-37 weeks of gestational age and processing them for identification of GBS.

Infants born prematurely constituted 23% of the total infants with early-onset disease and 52% of the total with late-onset disease. The importance of group B Streptococcus as a common pathogen for the perinatal period relates to the pregnant woman as well as her infant. Postpartum endometritis occurs with a frequency of approximately 2%, and clinically diagnosed intra-amniotic infection occurs in 2.9% of women vaginally colonized with GBS at the time of delivery. The risk of intra-amniotic infection is greater in women with heavy colonization. Implementation of intrapartum chemoprophylaxis has been associated with a significant decline in the incidence of invasive Group B Streptococcal Infections disease in pregnant women, from 0.29 per 1000 live births in 1993 to 0.23 per 1000 live births in 1998 and a further decline to 0.12 per 1000 live births in 1999-2005. Half of these infections were associated with infection of the upper genital tract, placenta, or amniotic sac and resulted in fetal death. Among the other infections, bacteremia without a focus (31%), endometritis without fetal death (8%), and chorioamnionitis without fetal death (4%) were the most common manifestations.

ANTIMICROBIAL THERAPY OF GBS DISEASE IN NEONATES

Most invasive GBS strains have been, and still are susceptible to penicillin G. Most strains are also susceptible to ampicillin, semisynthetic penicillins, vancomycin, linezolid, trimethoprim, sulfamethoxazole and first, second and third generation cephalosporins .Resistance to erythromycin and clindamycin has increased during the last decades .In several studies, most GBS strains show resistance to tetracyclines, metronidazole and aminoglycosides .However, if aminoglycosides are combined with penicillin, synergestic effect is observed. Despite their uniform susceptibility to penicillin G, GBS require higher concentrations for growth inhibition in vitro than are required for strains belonging to group A streptococci .Although some studies indicate that 6-7 days therapy might be sufficient for uncomplicated bacteraemia, recommended duration of treatment of GBS infections has been 10-14 days for bacteraemia without focus or with soft tissue infection, 2 to 3 weeks for meningitis or bacterial arthritis and 3 to 4 weeks for osteomyelitis.

SEPTIC ARTHRITIS AND OSTEOMYELITIS

The mean age at diagnosis of osteomyelitis (31 days) is greater than that for septic arthritis (20 days). The mean duration of symptoms is shorter for septic arthritis than for osteomyelitis (1.5 days versus 9 days). In some infants with osteomyelitis, failure to move the involved extremity since hospital discharge after birth, or shortly thereafter, may be noted; this lack of movement can persist for 4 weeks before the diagnosis is made .Decreased motion of the involved extremity and evidence of pain with manipulation, such as lifting or diaper changing, are common signs of bone infection. Warmth or erythema can occur occasionally ;a history of fever is reported in only 20% of infants. The paucity of signs suggesting infection and the finding of

pseudoparalysis have led to an initial diagnosis of Erbs palsy and to assessment for possible child abuse .Osteomyelitis have had concomitant infection in the shoulder joint, isolated septic arthritis of the shoulder joint has not been reported. Group B streptococcal bone and joint infections have a good prognosis.

CELLULITIS OR ADENITIS

The manifestation of late-onset group B streptococcal infection seen as facial cellulitis ,submandibular cellulitis and cellulitis/adenitis syndrome has been reported. Presenting signs include poor feeding; irritability; fever; and unilateral facial, preauricular, or submandibular swelling, usually, but not always, accompanied by erythema. The mean age at onset is 5 weeks (range 2 to 11 weeks), and in contrast to all other expressions of late-onset infection, there is a striking male predominance (72%). The most common sites are the submandibular and parotid, and enlarged adjacent nodes become palpable within 2 days after onset of the soft tissue infection. Less common sites of involvement with cellulitis are the face, preauricular or inguinal areas, scrotum, anterior neck region, and prepatellar spaces .In one patient, cellulitis of the neck occurred in association with an infected thyroglossal duct cyst. Bacteremia almost always is detected in these infants (92%), and cultures of soft tissue or lymph node aspirates have yielded GBS in 83% of the infants in whom this procedure was performed. These infants usually are not seriously ill, few have associated meningitis, and recovery within a few days of initiation of appropriate antimicrobial therapy is the rule. Fulminant and fatal facial cellulitis has been described in a 7-hour-old neonate ,however, and associated meningitis has been described in two infants

MATERNAL INFECTIONS

In 1938, Fry described three fatal cases of endocarditis in postpartum women. This was the initial insight that group B Streptococcus was a human pathogen and could cause puerperal infection. Postpartum infections including septic abortion, bacteremia, chorioamnionitis, endometritis, pneumonia, and septic arthritis were recorded sporadically thereafter, but group B streptococcal infections in postpartum women, as in neonates, were uncommonly reported before 1970 .The dramatic increase in incidence of neonatal infections in the 1970s was paralleled by an increased incidence of infections in pregnant women. Before the institution of IAP in the 1990s, GBS accounted for 10% to 20% of blood culture isolates from febrile women in obstetric services These women had a clinical picture characterized by fever, malaise, uterine tenderness with normal lochia, and occasionally chills. Faro,[81] described 40 women with group B streptococcal endometritis and parametritis among 3106 women giving birth over a 12 month interval, an incidence of 1.3 per 1000 deliveries. GBS were isolated from the endometrium in pure culture in one third of cases or in addition to other organisms in the remainder; one third of the women had concomitant bacteremia. In most, signs of infection developed within the first 24 hours after cesarean section. Clinical features included chills,tachycardia, abdominal distention, and exquisite uterine, parametrial, or adnexal tenderness. Higher fever correlated with risk for concomitant bacteremia. Recovery was uniform after administration of appropriate antimicrobial agents. Six infants born to these women developed group B streptococcal septicemia, however, and infection was fatal in three. The observation that maternal febrile morbidity could serve as an early clue to bacteremic neonatal infection is important, and infants of such women should be carefully evaluated.

The contemporary incidence of invasive disease in pregnant women is 0.12 per 1000 live births[82] .This incidence has declined significantly in association with implementation of IAP to prevent early-onset neonatal disease. Half of the 409 pregnancy-associated disease cases identified in the United States from 1999- 2005 by an active population-based surveillance system were associated with infection of the upper genital tract, placenta, or amniotic sac and resulted in fetal death. Among the remainder, manifestations of disease included bacteremia without a focus (31%), endometritis without fetal death (8%), chorioamnionitis without fetal death (4%), pneumonia (2%), and puerperal sepsis (2%). Isolates in pregnancy-associated infections were obtained from blood in 52% of women and from the placenta, amniotic fluid, or conceptus in most of the remainder. When pregnancy outcome was known, most of the women (61%) had a spontaneous abortion or stillborn infant, 5% had infants who developed clinical infection, 4% had induced abortions, and 30% had infants who remained clinically well. Most obstetric patients with group B streptococcal infection, even in the presence of bacteremia, show a rapid response after initiation of antimicrobial therapy. Potentially fatal complications can occur, however, including meningitis, ventriculo-peritoneal shunt infection ,abdominal abscess, endocarditis, vertebral osteomyelitis ,epidural abscess ,or necrotizing fasciitis. Group B streptococcal bacteriuria during pregnancy is a risk factor for intrauterine or neonatal infection. Asymptomatic bacteriuria, cystitis, or pyelonephritis occurs in 6% to 8% of women during pregnancy. In women with asymptomatic bacteriuria, approximately 20% are caused by GBS Group B streptococcal bacteriuria is a marker for heavy vaginal colonization, so the finding of bacteriuria indicates enhanced risk for maternal and neonatal infections .In the series reported by Moller and associates[83] that predated IAP, a cohort of 68 women with

asymptomatic group B streptococcal bacteriuria had significantly increased risk of preterm delivery compared with nonbacteriuric controls. Stillbirth because of congenital group B streptococcal infection can occur even in the current era, and a woman with any quantity of group B streptococcal bacteriuria during pregnancy should receive IAP.

DIAGNOSIS, ISOLATION AND IDENTIFICATION OF THE ORGANISM

Methods for detection of GBS colonisation in pregnant women

Culture[84]

"Gold standard" for GBS screening is culture performed at 35-37 weeks gestation from swabs collected from both the vagina and the rectum. The use of selective media (agar plates and broth) for culture supplemented by antibiotics like colistin (10 μg/ml) or nalidixic acid (15 μg/ml) are recommended .The selective agar plates may be examined after 24 hours while the inoculated selective, enrichment broth is incubated for 18-24 hours and then subcultured onto sheep blood agar. If GBS is not identified after the incubation of 18-24 hours, the blood agar plate should be reincubated and examined at 48 hours to identify suspected organisms. Suspected colonies may be tested using slide agglutination tests for specific identification .Studies have shown that the use of standard direct blood agar plating rather than selective, enrichment medium leads to false negative culture results in as many as 50% of pregnant women colonised by GBS .The culture taken at 35-37 weeks of gestation, may not accurately predict genital tract colonisation during labour because colonisation may be transient and colonisation may occur after the time of screening.

Studies have shown sensitivities of a positive test (the ability to predict vaginal colonisation at time of labour) in week 35-37 from 54% to 91% .

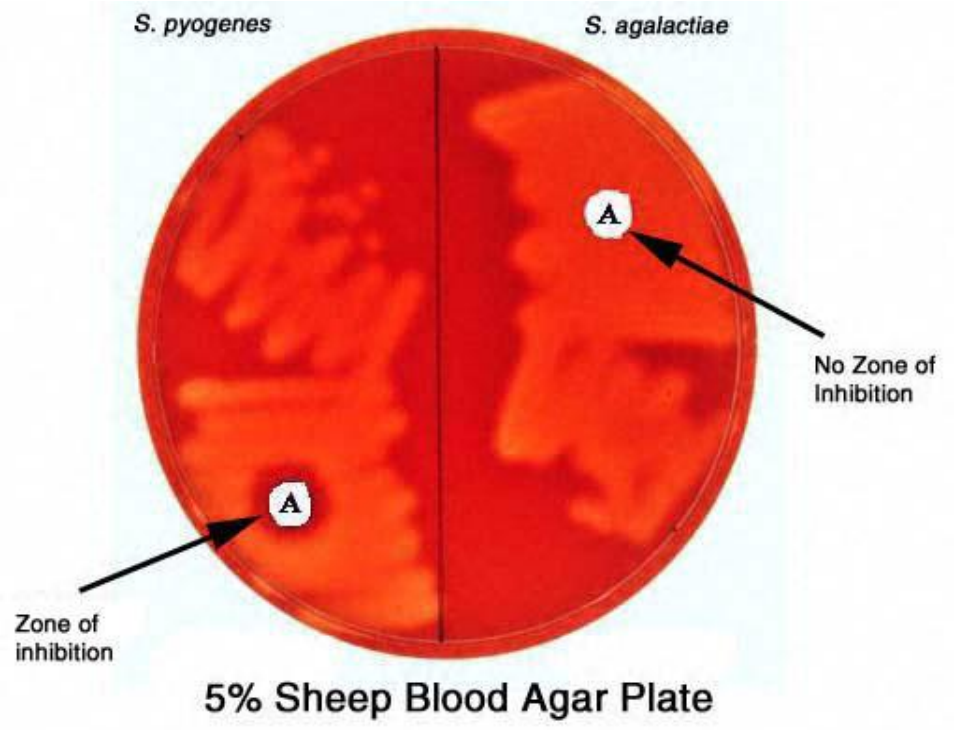


Figure 3: Growth of GBS on 5% sheep blood agar

Antigen tests [85]

GBS strains can also be identified by the production of group B Lancefield antigen Consequently, many latex agglutination tests and imunoassays that detect this antigen for GBS identification have been developed for rapid detection of GBS colonisations without previous culture. However, even if the specificity has been high (98-100%), the overall sensitivity of these commercially available immunological assays has been low and not sufficiently accurate for routine use in the intrapartum detection of women colonized with GBS .

Figure 4: Showing latex agglutination kit

DNA hybridisation[86]

Probe hybridisation for GBS targets specific43 broth with a sensitivity of 94.7-100% and specificity of 96.9-99.5% compared with culture[86]. The sensitivity is much lower when incubation is shorter. Thus, available probe hybridization methods are suitable for GBS identification from overnight cultures in selective enrichment broth, but are poorly sensitive for direct detection and identification of GBS from recto vaginal swabs obtained from pregnant women during labour.

Polymerase chain reaction (PCR)[87]

Identification of GBS can be made by detecting a part of the DNA; the genetic target, which is unique for GBS. DNA extraction from GBS is dependent on lysis of the bacteria which sometimes may be challenging due to the robust polysaccharide capsule and cell wall of GBS. The PCR starts with the denaturation step where double-stranded target DNA is denatured (melted) into single stranded DNA by increasing the temperature to approximately 95ºC. The temperature is then lowered to approximately 55-58ºC; this permits the annealing of the specific PCR primers to the single stranded target DNA. Finally, for efficient synthesis of DNA copies, the temperature is adjusted to be optimal for the DNA polymerase activity (extension), normally 72ºC. To amplify target DNA the cycles through these temperatures are repeated several times (25 to 40 depending on the application).

DIFFERENTIAL DIAGNOSIS

The clinical features in neonates with early-onset group B streptococcal infection mimic the features in infants with sepsis caused by other etiologic agents and by some noninfectious illnesses. Radiographic findings of pneumonia are present

in some neonates with early-onset group B streptococcal sepsis. Neonates with early-onset group B streptococcal pneumonia can have apnea and shock within the first 24 hours of life, a 1-minute Apgar score of 5 or less, and an unusually rapid progression of pulmonary disease .Infection also should be considered in neonates with persistent fetal circulation associated with respiratory distress, neutropenia, and systemic hypotension .The differential diagnosis for late-onset group B streptococcal infection depends on the clinical presentation. For infants with meningitis, the characteristic CSF Gram stain findings can provide a presumptive diagnosis. When this method is inconclusive usually in the setting of partial treatment, other organisms, including viruses, E. coli, Neisseria meningitidis, S. pneumoniae, and nontypable Haemophilus influenzae, must be considered. Fever usually is a presenting feature in term infants, and empirical therapy with broad-spectrum antibiotics customarily is employed until results of cultures permit a specific diagnosis of bacteremia or focal infection are available. The paucity of signs characteristic of group B streptococcal osteomyelitis and the history that signs have been present since birth have caused confusion with Erb's palsy and neuromuscular disorders. The characteristic bony lesion,tenderness of the extremity when a careful examination is performed, and isolation of the organism from blood, bone, or joint fluid usually provide a definitive diagnosis .Finally, the lengthy list of uncommon manifestations of infection between 1 week and 3 months of age and beyond indicates that GBS should be suspected as an etiologic agent, regardless of site of infection, for infants in this age group.

TREATMENT

GBS have been a frequent cause of infection in neonates for 4 decades, resulting in increased awareness of associated risk factors and need for prompt and aggressive therapy. Despite striking declines, however, death and disability from these infections still occur. In addition, relapses or reinfections, although uncommon, occur in the face of suboptimal therapy. These facts should prompt efforts to develop improved treatment modalities.

IN VITRO SUSCEPTIBILITY

Uniform susceptibility of GBS to penicillin G has continued for more than 50 years of usage.[75] More recently, reduced susceptibility of certain strains of GBS to penicillin and other b-lactam antibiotics has been documented and experimentally traced to point mutations in penicillin-binding proteins (e.g., PbP2x) reminiscent of first-step mutations in the evolution of pneumococcal penicillin resistance decades ago. The clinical implications of this finding are as yet unclear. Efforts should be continued, however, to monitor clinical isolates for mutations that would suggest the evolution of penicillin resistant strains. In vitro susceptibility of GBS to ampicillin,semisynthetic penicillins, vancomycin, teicoplanin, linezolid, quinopristin/dalfopristin, gatifloxacin, levofloxacin and first-generation, second-generation (excluding cefoxitin), and third-generation cephalosporins also is the rule, although the degree of in vitro activity varies .Ceftriaxone is the most active of the cephalosporins in vitro. Imipenem and meropenem are highly active .Resistance to quinolones can occur through mutations in the gyrase and topoisomerase IV genes, usually in patients who have received prior quinolone therapy .Resistance to erythromycin and clindamycin is increasing. Contemporary data from multiple studies

indicate that 20% to 30% of isolates are erythromycin-resistant, and 10% to 20% are resistant to clindamycin [88].Rates of resistance in colonizing isolates can be 40% for erythromycin and clindamycin. These high rates of resistance are reported from geographically diverse regions .

The percentage of tetracycline-resistant strains is 75% to nearly 90%. Resistance of GBS to bacitracin, nalidixic acid, trimethoprim-sulfamethoxazole, metronidazole, and aminoglycosides is uniform. Despite resistance of most group B streptococcal strains to aminoglycosides, synergy often is observed when an aminoglycoside (especially gentamicin) and penicillin or ampicillin are used in combination .The best combination theoretically to accelerate the killing of GBS in vivo is penicillin or ampicillin plus gentamicin. Therapeutic concentrations of gentamicin in the serum are not required to achieve synergy. By contrast, the rapid and predictable bactericidal effect of penicillin or ampicillin on GBS in vitro is ablated by the addition of Rifampicin.Although in vivo data are lacking, the in vitro antagonism of rifampin when combined with penicillins suggests that they should not be employed concurrently in the treatment of proven or suspected group B streptococcal disease. Among the newer B-lactam antibiotics reputed to attain high concentrations of drug in the CSF, only cefotaxime, ceftriaxone, meropenem, and imipenem achieve minimal bactericidal concentrations (MBCs) comparable with MBCs of penicillin G and ampicillin (0.01 to 0.4 mg/mL) ,and limited data suggest that their efficacy is equivalent to that of penicillin G. Despite their uniform susceptibility to penicillin G, GBS require higher concentrations for growth inhibition in vitro than strains belonging to group A. The minimal inhibitory concentration (MIC) of penicillin G to GBS is 4-fold to 10-fold greater than the MIC for group A

strains (range 0.003 to 0.4 mg/mL). This observation, combined with the observation indicating the significant influence of inoculum size on in vitro susceptibility to penicillin G, may have clinical relevance . When the inoculum of group B Streptococcus is reduced from 10^5 to 10^4 colony-forming units (CFU)/mL, a twofold lower concentration of penicillin G is sufficient to inhibit in vitro growth. Similarly, if the inoculum is increased from 10^4 to 10^7 CFU/mL, the MBC of ampicillin is increased from 0.06 to 3.9 mg/ml. Such in vitro observations may have in vivo correlates because some infants with group B streptococcal meningitis have CSF bacterial concentrations of 10^7 to 10^8 CFU/Ml[89] .

ANTIMICROBIAL THERAPY

Penicillin G is the drug of choice for treatment of group B streptococcal infections. The recommended dosage for treatment of meningitis is high because of the following(1) relatively high MIC of penicillin G for GBS (median 0.06 mg/mL) with respect to attainable levels of this drug in the CSF (2) the high inoculum in the CSF of some infants (3) reports of relapse in infants with meningitis treated for 14 days with 200,000 U/kg/day of penicillin G and (4) the safety of high doses of penicillin G in the newborn. To ensure rapid bactericidal effects, particularly in the CSF, we recommend penicillin G (450,000 to 500,000 U/kg/day) or ampicillin (300 to 400 mg/kg/ day) for the treatment of meningitis.

There is no evidence to suggest increased risk for adverse reactions at these higher doses even in premature infants. In the usual clinical setting, antimicrobial therapy is initiated before definitive identification of the organism. Initial therapy should include ampicillin and an aminoglycoside appropriate for the treatment of

early-onset neonatal pathogens including GBS. We continue combination therapy until the isolate has been identified as GBS and, in patients with meningitis, until a CSF specimen obtained 24 to 48 hours into therapy is sterile. Kim suggests that MIC and MBC determinations be considered in the following settings[90]: (1) a poor bacteriologic response to antimicrobial therapy, (2) relapse or recurrence of infection without a discernible cause, and (3) infections manifested as meningitis or endocarditis. If tolerance is shown, therapeutic choices include using penicillin G or ampicillin alone or employing cefotaxime. No data are available to indicate the better of these choices . For an infant with late-onset disease in whom CSF reveals gram-positive cocci in pairs or short chains, initial therapy should include ampicillin and gentamicin or cefotaxime, rather than penicillin G alone, because (1) GBS are a frequent cause of meningitis in infants 1 to 8 weeks of age, and combination therapy can improve efficacy early in the course of infection, and (2) Listeria monocytogenes can be confused by CSF Gram stain with group B Streptococcus, and ampicillin and gentamicin are synergistic in vitro against most strains of Listeria. If pneumococcal meningitis is a consideration, cefotaxime and vancomycin would be a reasonable empirical regimen pending culture confirmation. Because group B streptococcal meningitis is uncommon beyond 8 weeks of age, no change is suggested from the use of conventional agents as the initial treatment of meningitis in term infants older than 2 months. For preterm infants remaining hospitalized from birth, empirical therapy can include vancomycin and an aminoglycoside. If meningitis is suspected, ampicillin or cefotaxime should be included in the regimen because vancomycin achieves low CSF concentrations and has a substantially higher MBC against GBS and L. monocytogenes than ampicillin. When the diagnosis of group B streptococcal infection is confirmed, and CSF for patients with meningitis obtained 24 to 48 hours

into therapy is sterile, treatment can be completed with penicillin G monotherapy. Good outcomes have been achieved when parenteral therapy is given for 10 days for bacteremia without a focus or with most soft tissue infections, 2 to 3 weeks for meningitis or pyarthrosis, and 3 to 4 weeks for osteomyelitis or endocarditis .Limited evidence suggests that a 7-day course of therapy can suffice for uncomplicated bacteremia, but additional data would be required to support a change in current recommendations. Infants with septic arthritis should receive at least 2 weeks of parenteral therapy; infants with bone involvement require 3 to 4 weeks of therapy to optimize an uncomplicated outcome. Drainage of the suppurative focus is an adjunct to antibiotic therapy. In infants with septic arthritis excluding the hip or shoulder, one-time needle aspiration of the involved joint usually achieves adequate drainage. With hip or shoulder involvement, immediate open drainage is warranted. For most infants with osteomyelitis, some type of closed or open drainage procedure is required for diagnosis because blood cultures typically are sterile. These procedures must be performed before or early in the course of therapy to ensure successful isolation of the infecting organism..

SUPPORTIVE MANAGEMENT

Prompt, vigorous, and careful supportive care is important to the successful outcome of most group B streptococcal infections. When early-onset disease is accompanied by respiratory distress, the need for ventilatory assistance should be anticipated before onset of apnea. Early treatment of shock, often not suspected during its initial phase, when systolic pressure is maintained by peripheral vasoconstriction, is crucial. Persistent metabolic acidosis and reasonably normal color are characteristic of this early phase. In patients with meningitis, effective seizure

control is required to achieve proper oxygenation, to decrease metabolic demands, prevent additional cerebral edema, and optimize cerebral blood flow. Monitoring of urine output and attention to electrolyte balance and osmolality are needed to detect and manage the early complications of meningitis, such as inappropriate secretion of antidiuretic hormone and increased intracranial pressure. Such intense and careful supportive management requires treatment in an intensive care unit of a tertiary care facility. Survival was not improved significantly with use of ECMO when all infants or only hypotensive infants were compared.

PREVENTION

Theoretically, early-onset and late-onset group B streptococcal disease could be prevented if susceptible hosts were not exposed to the microorganism or if exposure occurred in the setting of protective immunity. Several approaches to prevention have been advocated; conceptually, these are directed at eliminating exposure or enhancing host resistance by chemoprophylaxis or immunoprophylaxis. Both strategies have limitations with respect to implementation, but could be targeted for the prevention of maternal and neonatal infections and are theoretically achievable.

CHEMOPROPHYLAXIS

Historical Precedents

Chemoprophylaxis was suggested as a means to prevent early-onset group B streptococcal infection by Franciosi and coworkers in 1973[46]. Because maternal genital colonization was recognized to expose infants to the organism, oral penicillin treatment for colonized women was subsequently proposed. Carriers of GBS identified by third-trimester vaginal culture received a course of an oral antimicrobial. Approximately 20% to 30% remained colonized after treatment, and in most of these women, GBS were isolated from vaginal cultures at delivery .Reacquisition from colonized sexual partners was suggested as an explanation for these high failure rates, but failure rates remained high when colonized pregnant women and their spouses received concurrent treatment with penicillin by the oral or the parenteral route .One explanation cited for failure of this approach was the difficulty in eradicating a constituent of the normal bowel flora[91].

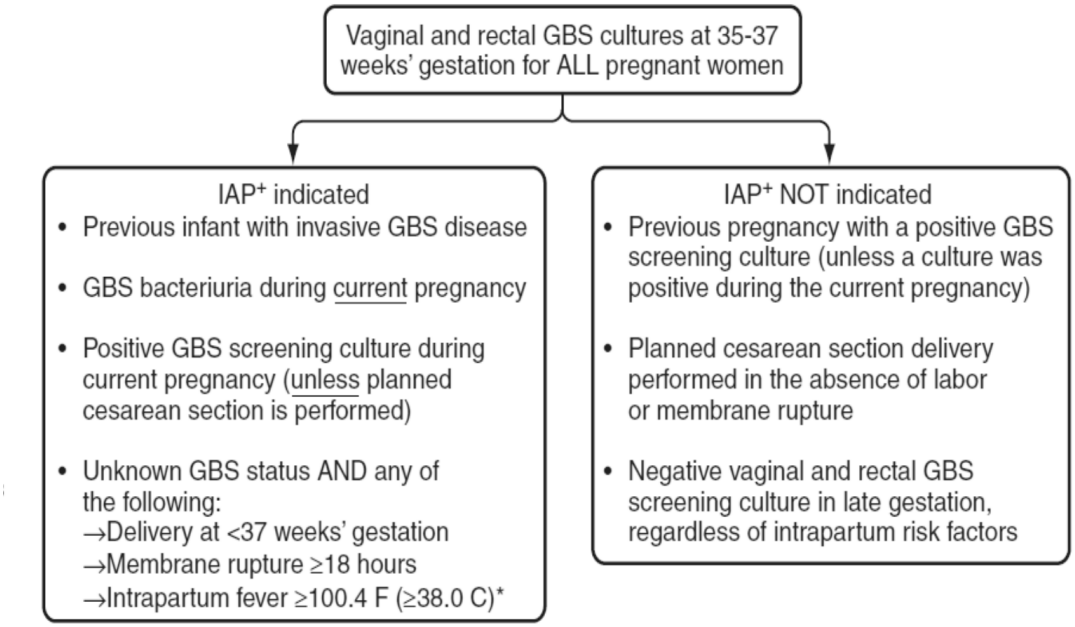
Yow and colleagues[92] gave intravenous ampicillin at hospital admission to 34 women in labor and vaginally colonized with GBS and successfully interrupted vertical transmission of colonization in all. Boyer and Gotoff provided in 1986 the first documentation that IAP could prevent invasive early-onset neonatal infection[9]. Women colonized with GBS who had risk factors for early-onset infection were randomly assigned to receive routine labor and delivery care or intrapartum ampicillin intravenously until delivery. Group B streptococcal sepsis developed in 5 of 79 neonates in the routine care group, 1 of whom died, whereas 85 infants born to women in the ampicillin treatment group remained well. Intrapartum ampicillin

prophylaxis for group B streptococcal carriers also resulted in reduced maternal morbidity These data established the efficacy of IAP for prevention of early-onset neonatal disease and reduction of group B Streptococcus–associated febrile maternal morbidity. The cost-effectiveness of this approach subsequently was validated. In 1992, the American College of Obstetricians and Gynecologists (ACOG) and the American Academy of Pediatrics (AAP) published separate documents regarding maternal IAP for the prevention of early-onset group B streptococcal infection. The ACOG technical bulletin was educational, whereas the AAP guidelines were directive. The AAP guidelines specified that if culture screening was performed antenatally, specimens should be obtained from lower vaginal and rectal sites, and culture positive women with one or more risk factors and group B streptococcal colonization should be given intrapartum intravenous penicillin G or ampicillin. The ACOG proposed that culture screening could be avoided by providing treatment for all women with risk factors. Neither the AAP nor ACOG approach was implemented widely, and invasive disease rates remained unacceptably high. There are difficulties inherent to ascertainment of group B streptococcal colonization status rapidly even when assays can be processed 24 hours a day. Latex particle agglutination and enzyme immunoassays for detection of group B streptococcal antigen in cervical or lower vaginal swab specimens are not sufficiently sensitive to determine colonization status accurately at hospital admission, especially for women with a low density of organisms .

An optical immunoassay (Strep B OIA test; Biostar, Boulder, CO) was considerably more sensitive than earlier assays for detecting light (13% to 67%) and heavy (42% to 100%) or overall (81%) colonization and has outperformed enzyme

immunoassays in direct comparisons Assays using a DNA hybridization methodology have shown variable sensitivity .Bergeron and colleagues described a fluorogenic RT-PCR technique for rapid identification of women colonized with GBS at admission for delivery. The sensitivity of RT-PCR and of conventional PCR was 97%, the negative predictive value was 99%, and the specificity and positive predictive value were 100%. Results were available from RT-PCR in 45 minutes; by comparison, conventional PCR required 100 minutes, and conventional cultures required 36 hours minimum. Field testing of commercially available assays such as the Xpert GBS Assay (Cepheid, Sunnyvale, CA) that uses automated RT-PCR technology and IDI-StrepB (Infectio Diagnostic, Quebec, Canada) that uses a PCR assay to amplify group B streptococcal target has been conducted .The performance of RT-PCR and optical immunoassay is sufficiently robust for use in point-of-care settings .A cost-benefit analysis suggests that widespread implementation would afford benefit over the current culture-based strategy, but, to date, these newer methods should be considered as adjunctive tests to antenatal culture-based methods for detection of GBS .

INTRAPARTUM ANTIBIOTIC PROPHYLAXIS



IAP+ = Intrapartum antibiotic prophylaxis.
*If chorioamnionitis is suspected, broad-spectrum antibiotic therapy that includes an agent known to be active against GBS should replace GBS IAP.

Revised recommendation for culture-based screening for maternal colonization with group B streptococci(GBS) and administration of intrapartum antibiotic prophylaxis (IAP). (Adapted from Centers for Disease Control and Prevention .Prevention of perinatal group B streptococcal disease: revised guidelines from CDC. MMWR Morb Mortal Wkly Rep 51[RR-11]:1-22, 2002.)

Screening strategy

Rectovaginal specimens from pregnant women with gestational age 35-37 are cultured to detect GBS colonisation and IAP is recommended to colonised women. Screening strategy and indications for intrapartum antibiotic prophylaxis as recommended by CDC, 2002.

Vaginal and rectal GBS screening cultures at 35-37 weeks gestation for all pregnant women (unless the woman had GBS bacteriuria during the current pregnancy or a previous infant with invasive GBS disease)

Intrapartum prophylaxis indicated if:

- Previous infant with GBS disease
- GBS bacteriuria during the current pregnancy
- Positive GBS screening culture during current pregnancy (unless a planned caesarean delivery, in the absence of labour, or amniotic membrane rupture, is performed)
- Unknown GBS status and any of the following:
    - Delivery at <37 weeks gestation
    - Amniotic membrane rupure >18 hours
    - Intrapartum temperature >38ºC

Intrapartum prophylaxis not indicated if:

- Planned caesarean delivery performed in the absence of labour or amniotic membrane rupture (Regardless of maternal GBS culture status).
- Negative vaginal and rectal GBS screening culture in late gestation during the current pregnancy, regardless of intrapartum risk factors.

The current era of IAP dates from 1996, when consensus recommendations for the prevention of early-onset group B streptococcal disease were endorsed by the CDC, AAP, and ACOG. These recommendations indicated that obstetric care providers and hospitals should adopt a culture-based or a risk-based policy to identify women to receive IAP. The culture-based approach employed lower vaginal and rectal cultures obtained at 35 to 37 weeks of gestation to identify candidates for IAP. The risk-based strategy identified IAP recipients by factors known to increase the likelihood of neonatal group B streptococcal disease: labor onset or membrane rupture

before 37 weeks of gestation, intrapartum fever greater than or equal to 38# C ("100.4# F), or rupture of membranes 18 or more hours before delivery. In both strategies, women with group B streptococcal bacteriuria or previous delivery of an infant with group B streptococcal disease were to receive IAP. By 2002, Culture-based screening more often resulted in administration of IAP for at least 4 hours before delivery. The 2002 revised CDC guidelines recommending a universal culture-based approach to prevention of perinatal group B streptococcal disease are endorsed by the AAP and the ACOG. Currently, all pregnant women should be screened in each pregnancy for group B streptococcal carriage at 35 to 37 weeks of gestation. The risk-based approach is an acceptable alternative only in circumstances in which the culture has not been performed or results are unavailable before delivery. Culture specimens should be obtained from the lower vagina and the rectum using the same or two different swabs. These swabs should be placed in a non-nutritive transport medium, transferred to and incubated overnight in a selective broth medium, and subcultured onto 5% sheep blood agar medium or colistin–nalidixic acid medium for isolation of GBS. At the time of labor or rupture of membranes, IAP should be given to all pregnant women identified antenatally as carriers of GBS.

REGIMENS FOR INTRAPARTUM ANTIBIOTIC PROPHYLAXIS AS RECOMMENDED BY CDC:

Recommended: Penicillin G IV 5 MU initially and 2.5MU every 4 hours until delivery.

Alternative: Ampicillin IV 2 g IV loading dose and then 1g every 4 hours or 2g every 6 hours until delivery

If penicillin allergic:

- Patients not at high risk for anaphylaxis: Cefazolin IV 2g IV and then 1g IV every 8 hours until delivery
- Patients at high risk for anaphylaxis and GBS susceptible to clindamycin and erythromycin.
- Clindamycin 900 mg IV, every 68hours until delivery or Erythromycin 500mg IV every 6 hours until delivery.
- GBS resistant to clindamycin or susceptibility unknown: Vancomycin 1g IV every 12 hours until delivery.

Group B streptococcal bacteriuria during the current pregnancy or prior delivery of an infant with invasive group B streptococcal disease always is an indication for IAP, so antenatal screening is unnecessary for these women. If culture results are unknown at the onset of labor or rupture of membranes, the risk factors listed above in figure should be used to determine the need to institute IAP. Women who present with preterm labor before antenatal group B streptococcal screening should have cultures obtained and IAP initiated. If labor ceases and cultures are negative, IAP is discontinued, and antenatal screening is performed at 35 to 37 weeks of gestation. If labor ceases and cultures are positive, some experts recommend oral amoxicillin for another 5 to 7 days. Planned cesarean section before rupture of membranes and onset of labor constitute exceptions to the need for IAP for women colonized with GBS. These women are at extremely low risk for having an infant with early-onset disease. Culture-negative women who are delivered at 37 weeks of gestation or later need not receive IAP routinely, even when a risk factor is present. Therapeutic use of broad spectrum antibiotics in labor should be employed as is

appropriate for maternal indications, such as intra-amniotic infection. The recommended maternal intrapartum chemoprophylaxis regimen consists of penicillin G (5 million U initially and 2.5 million U every 4 hours thereafter until delivery).Penicillin or ampicillin given 4 or more hours before delivery reliably prevents vertical transmission and early-onset disease. Ampicillin administered as a 2-g intravenous loading dose and then 1 g every 4 hours until delivery is an alternative to penicillin .The rationale for the high initial dose of the B-lactam antibiotic relates to the desired drug concentrations needed in the amniotic and vaginal fluids (peak approximately 3 hours after completion of the initial dose) to reduce substantially the number of GBS at either site. IAP "failures" typically occur when penicillin or ampicillin has been initiated 2 or less hours before delivery; clindamycin has been given without susceptibility testing, and clindamycin- resistant early-onset group B streptococcal neonatal sepsis ensued; or appropriate IAP is given in the setting of clinically apparent or silent intra-amniotic infection. Prophylaxis for penicillin-allergic women must take into account increasing resistance among GBS to erythromycin and clindamycin. Women not at high risk for anaphylaxis (e.g., a rash without anaphylaxis or respiratory compromise) should receive cefazolin, 2 g intravenously as an initial dose and then 1 g every 8 hours until delivery. Cefazolin has pharmacokinetics similar to penicillin with respect to peak concentrations in serum and amniotic fluid of pregnant women. Women whose group B streptococcal isolates are tested and found to be clindamycin susceptible and who are at high risk for anaphylaxis with penicillin can receive clindamycin at a dose of 900 mg every 8 hours. If susceptibility testing is unavailable or the results are unknown, or when isolates are resistant to clindamycin, vancomycin, 1 g intravenously every 12 hours until delivery, is an alternative for women with serious penicillin hypersensitivity

reactions. Neither the pharmacokinetics of vancomycin in amniotic or vaginal fluids nor its efficacy in preventing early-onset disease has been investigated. The risk of anaphylaxis from administration of penicillin is low. Estimates range from 4 events per 10,000 to 4 per 100,000 patients. Anaphylaxis associated with administration of a B-lactam antibiotic as IAP for the prevention of early-onset group B streptococcal infection has been reported, but is rare .Most pregnant women reporting a penicillin allergy that is not anaphylaxis have negative skin test on hypersensitivity testing and are able to receive IAP with penicillin. A fetal demise in association with new-onset penicillin allergy during IAP has been reported in a woman with rheumatoid arthritis [93].No adult fatalities in association with IAP are reported, and the risk of a fatal event is low because the antimicrobials are administered in a hospital setting where medical intervention is readily available.

RESIDUAL PROBLEMS IN DETECTION

Numerous residual problems, barriers to implementation, and missed opportunities must be overcome to achieve maximal benefit from IAP .Procedural issues, such as (1) suboptimal culture processing and collection of cultures earlier than 5 weeks before delivery, constitute one set of problems. (2) Laboratories may not adhere to recommended methods for isolation of GBS, a problem that remains despite the 2002 consensus recommendations which is a culture based approach and one that results in colonized women delivering infants with early-onset disease. (3) Even optimal antenatal culture methods miss some women who are colonized at delivery, exposing their neonate to GBS and resulting in colonization or illness. (4) Another problem is that women who are not screened adequately more often are medically underserved; women in their teens, blacks, and Hispanics are more likely than whites

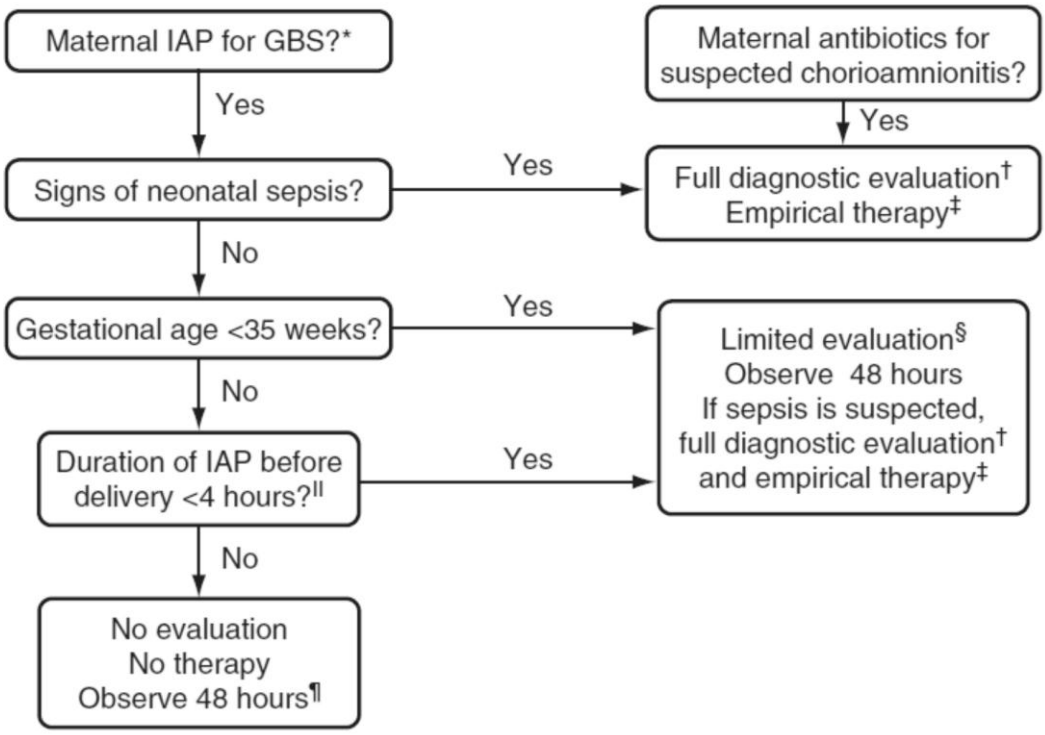
to receive inadequate prenatal care and prenatal testing, and are less likely to receive recommended prevention interventions. (5) Problems surround lack of recommended IAP in certain women who deliver before antenatal screening occurs (i.e., 35 to 37 weeks of gestation). These women should have vaginal and rectal cultures performed and routinely receive IAP, but this recommendation is the one least commonly implemented. Whether this is because delivery ensues too quickly to administer IAP, or the recommendation is unclear to obstetric providers, or both, is unknown. (6) Also, adherence to guidelines in penicillin allergic women is suboptimal, and cefazolin as the appropriate IAP for women with a non-serious penicillin allergy is administered uncommonly. (7) Reliance on clindamycin as the alternative agent in women without serious penicillin allergy results in inadequate IAP in at least 20% of patients when antimicrobial susceptibility testing of colonizing isolates is not performed antenatally. (8) A final issue is a need for increased awareness of perinatal group B streptococcal infection. In one report, only 47% of women younger than 50 years of age reported having heard of group B Streptococcus .Women with a high school education or less, with low household income, or reporting black, Asian/Pacific Islander, or "other" races had lower awareness than that noted in other women. Efforts to raise awareness should target women from groups that traditionally are medically underserved. Hospital infection control teams can contribute to these efforts by spearheading educational efforts toward effective implementation among hospital obstetric staff and laboratory personnel .

Impact of intrapartum antibiotic prophylaxis on neonatal sepsis

The efficacy of IAP in preventing early-onset group B streptococcal infection has been shown in numerous observational studies and in countries other than the

United States when guidelines have been implemented .The impact of increased use of IAP on the occurrence of sepsis caused by organisms other than GBS is a subject of ongoing evaluation. Concern exists that neonatal sepsis caused by organisms other than group B Streptococcus is increasing while group B streptococcal sepsis is decreasing and that the organisms causing non–group B streptococcal sepsis are likely to be ampicillin resistant. Surveillance trends are insufficient to establish a relationship between IAP for group B Streptococcus and E. coli sepsis risk, but single hospital–reported increases in E. coli sepsis that have occurred in preterm and very low birth weight infants are of concern .A significant increase in the rate of early-onset sepsis caused by E. coli has been observed in multicenter studies, but only infants of very low birth weight (<1500 g birth weight) were evaluated .In a multisite surveillance of trends in incidence and antimicrobial resistance of early-onset sepsis, stable rates of sepsis caused by other organisms were found, but an increase in ampicillin- resistant E. coli was observed among preterm but not term infants .A relationship between neonatal death caused by ampicillin-resistant E. coli and prolonged antepartum exposure to ampicillin was noted by Terrone and colleagues. In another report, the frequency with which ampicillin-resistant Enterobacteriaceae were isolated was similar after exposure to ampicillin or penicillin .Repeat cultures 6 weeks postpartum revealed no increase in antibiotic resistance in either GBS or E. coli from women who had received IAP .Ongoing population based surveillance is required to monitor these trends and to identify possible reasons for the increase in ampicillin-resistant E. coli infections in preterm neonates, in particular, the use of antenatal antimicrobial agents other than IAP.

Management of neonates born to mothers receiving intrapartum antimicrobial prophylaxis:



* If no maternal IAP for GBS was administered despite an indication of being present, data are insufficient on which to recommend a single management strategy.

† Includes complete blood cell (CBC) count and differential, blood culture, and chest radiograph if respiratory abnormalities are present. When signs of sepsis are present, a lumbar puncture, if feasible, should be performed.

‡ Duration of therapy varies depending on results of blood culture, cerebrospinal fluid findings, if obtained, and clinical course of the infant. If laboratory results and clinical course do not indicate bacterial infection, duration may be as short as 48 hours.

§ CBC with differential and blood culture.

II Applies only to penicillin, ampicillin, or cefazolin and assumes recommended dosing regimens.

¶ A healthy-appearing infant who was 38 weeks' gestation at delivery and whose mother received 4 hours of IAP before delivery may be discharged home after 24 hours IF other discharge criteria have been met and a person able to comply fully with instructions for home observation will be present. If any one of these conditions is not met, the infant should be observed in the hospital for at least 48 hours and until criteria for discharge are achieved.

Recommended management of newborn infants exposed to maternal intrapartum antibiotic prophylaxis (IAP) for group streptococcal (GBS) infection. (Adapted from Centers for Disease Control and Prevention. Prevention of perinatal group B streptococcal disease: revised guidelines from CDC. MMWR Morb Mortal Wkly Rep 51[RR-11]:1-22, 2002.)

VACCINES

Maternal antibody deficiency to GBS is associated with increased neonatal susceptibility to invasive GBS disease [94].Immunization of women during or before pregnancy could prevent peripartum maternal disease and protect infants from perinatally acquired infection by transplacental transfer of protective IgG antibodies .The group B antigen, which is common to all strains, does not seem to be important for specific immunity to GBS infection. Maternal antibodies against the group B specific antigen do not protect against neonatal infection. However, serotype-specific antibodies to GBS capsular polysaccharide (CPS), have been shown to cross the placenta, promote opsonophagocytosis and killing of GBS [95].Early studies showed low immunogenicity in response to the polysaccharide capsule of GBS alone ,but by combining the GBS polysaccharide with tetanus toxoid, an excellent immune response could be produced .Also several of the surface protein antigens induce protective immunity in animal models .Vaccine trials have shown that if surface proteins are conjugated to CPS, they enhance the immunogenicity of the CPS .Alternative approaches to vaccines are based on surface proteins of GBS, on the recognition of immunogenic pili that extend from the surface of the bacterium ,and on fusion proteins . A successful GBS vaccine could reduce mucosal bacterial colonization and produce both humoral and mucosal immunity, and is expected to prevent more cases of neonatal disease than the current strategies with IAP. Effectiveness and safety would be an essential part of a licensing strategy. The prime obstacle to the development and testing of a GBS vaccine is probably the spectre of the liability associated with vaccine delivery in pregnant women[96]. Concerns for the safety of the mothers and fetuses require exhaustive and costly evaluation of candidate vaccines and the issue of liability is both serious and complex. Potential

challenges other than medico-legal issues include lack of protection passed to infants born prematurely, the unknown effects on neonates' immune responses and regulatory issues. However, trials of vaccine efficacy and safety are required for licensing of the vaccines. Such efficacy trials are likely to use substitute outcomes based on serological markers of a protective immune response, since trials to assess neonatal infection would need to be extremely large. Extensive post-marketing surveillance for effectiveness and safety would be an essential part of a licensing strategy. In order to successfully proceed in this field of maternal immunisation, it is necessary to define the actual risk, so that studies can be appropriately designed to demonstrate safety. been shown to be suitable to identify GBS from 18h to 24h cultures in selective enrichment46 Studies of concerns that would be associated with GBS vaccination during pregnancy from the perspectives of pregnant women and health care providers have been performed .Given all the factors involved in deciding whether to accept a vaccine or not, it appeared that being well informed about GBS was the most important factor. For any vaccine to be implemented, effective strategies for building public and individual trust are critical. These strategies need to be weighed against the pros and cons of the current IAP strategy as well as vaccination.

MATERIALS & METHODS

MATERIAL AND METHODS

Source and data

All pregnant women after 28 weeks of gestational age who came to OBGY OPD and labour room at BLDE University's Shri B.M. Patil Medical College, Hospital and Research Centre, Bijapur from Oct 2009 to June 2011. It was a prospective observational study.

Exclusion criteria:

Pregnant women with h/o:

1. per vaginal examination,
2. per rectal examination before taking swabs, and
3. h/o antibiotic therapy before 48 hours.

Method of collection of sample

The study required collection of lower vaginal swab (LVS) and rectal swab taken for GBS culture from each recruited subject. The LVS was obtained by inserting a sterile swab 2cms in to the lower entrance of vagina and sides of vagina to be swabbed. The rectal swab was obtained by inserting a swab in to rectum past external sphincter. Each cotton tipped swab was placed in Stuart's medium. The swab collected was transferred within 24 hours into Todd-Hewitt broth and incubated for 16 to 24 hours. The identity was confirmed by HI-Strep TM latex agglutination kit test.

Method of study:

Collection of data from cases included collection of swabs from anovaginal flora, transport and culture on appropriate media, and then the results were presented using descriptive statistics like bar chart, pie chart and frequency tables etc.

Sample size:

Incidence of GBS colonisation is around 45%[1].

Applying formula $n=4pq/l^2$, p=0.45, q=1-p, and keeping allowable error of 20%.

Estimate of sample size would be 122.

Statistical analysis used in this study:

1) Z test (Proportion test)
2) Students t test

OBSERVATIONS & RESULTS

OBSERVATIONS AND RESULTS

During the study period i.e. from October 2010 to May 2012, a total of 123 pregnant women were included in the study.

In this study, primarily the prevalence of Group B Streptococci in anovaginal flora in pregnant women after 28 weeks of gestation is studied. However, during the study, observations were also made regarding the age, height, weight, obstetric score, gestational age at delivery, mode of delivery, complications in baby and maternal morbidity and mortality.

Following are the observations made during this study, shown in both tabular and graphical form.

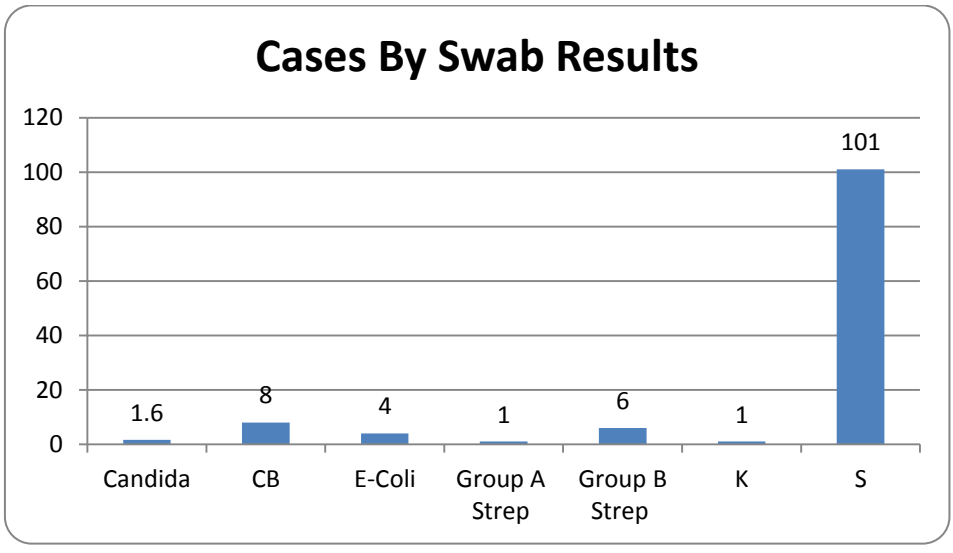
Distribution of cases by swab results:

Out of 123 pregnant women, 6 patients were tested positive for Group B Streptococci, which accounts for 4.9%, which is a very high prevalence rate. The prevalence of other organisms is given below in Table 1.

Table 1:Distribution of Cases by swab results

Swab Results	**Numbers**	**%**
Candida	2	1.6
Citrobacter(CB)	8	6.5
E-coli	4	3.3
Group A Strep.	1	0.8
Group B strep.	**6**	**4.9**
Kliebsiella(K)	1	0.8
Sterile(S)	101	82.1
Total	123	100

Figure 1



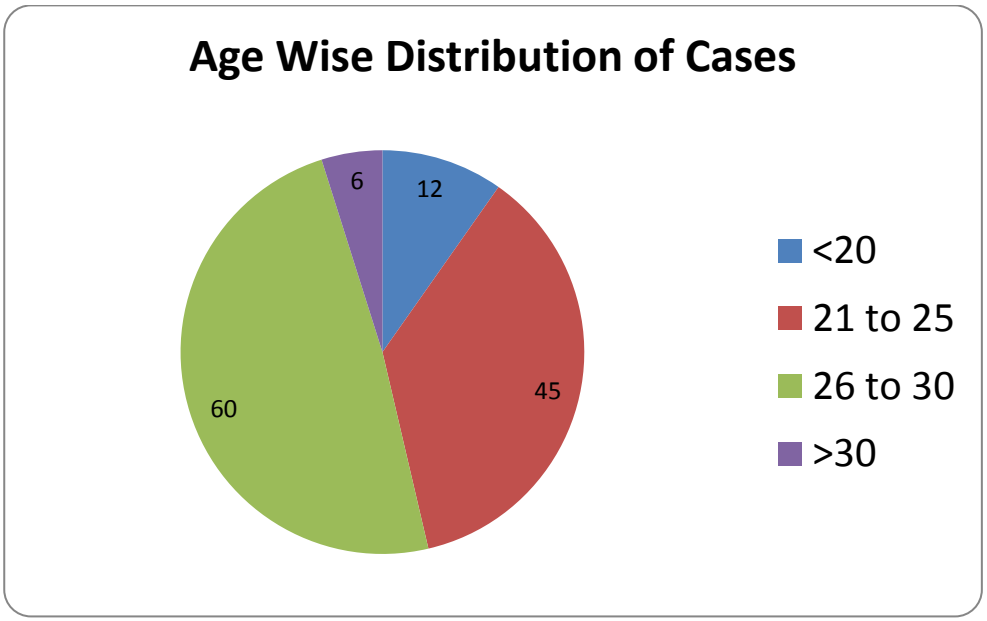
Age wise distribution of cases including both GBS positive and GBS negative

Age wise distribution of cases tested (both positive and negative) shows that12 (9.7%) women were below 20 years of age group, 45(36.5%) were in 21-25 years age group,60(48.8%) were between 26-30 years and 6(4.8%) were beyond 30 years of age group.The maximum incidence 48.8% was between 26-30 years.

Table 2: Age wise distribution of cases including both GBS positive and negative

Age(years)	No.	Percentage	Cumulative frequency	CF %
<20	12	9.7	12	9.7
21-25	45	36.5	57	46.2
26-30	60	48.8	117	95.2
>30	06	4.8	123	100
TOTAL	**123**	**100**		

Figure 2



Age wise distribution of cases separately in GBS positive and GBS negative

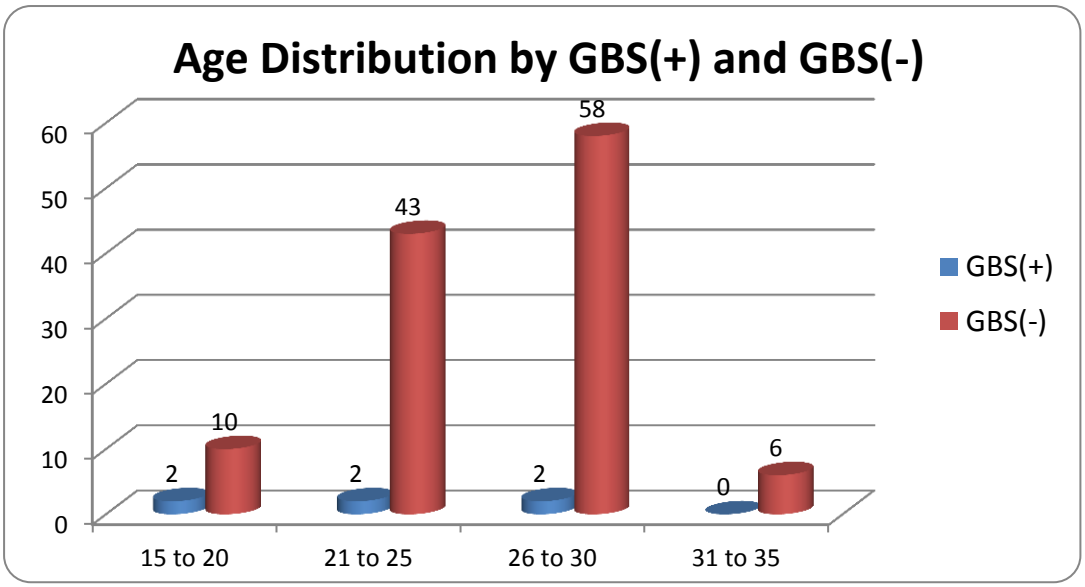
In GBS positive group, 2(33.3%) were below 20 years ,2(33.3%) were between 21-25 years, 2(33.3%) were between 26-30 years and none above 30 years, whereas 49.57% women were in age group of 26-30 years in GBS negative group and else women above and below this range.

Table3: Age Distribution by GBS(+) and GBS(-)

Sl. No.	Age(years)	GBS(+) n=6	GBS(-) n=117	Total n=123	Chi square	P value
1	<20	2(33.33%)	10(8.54%)	12		
2	21-25	2(33.33%)	43(36.75)	45		
3	26-30	2(33.33%)	58(49.57%)	60	4.15	<0.025
4	31-35	0	6(5.1%)	6		
	Total	**6**	**117**	**123**		

Chi square calculated > tabulated at 1 degree freedom and p value is less than 0.025 which is significant.

Figure 3



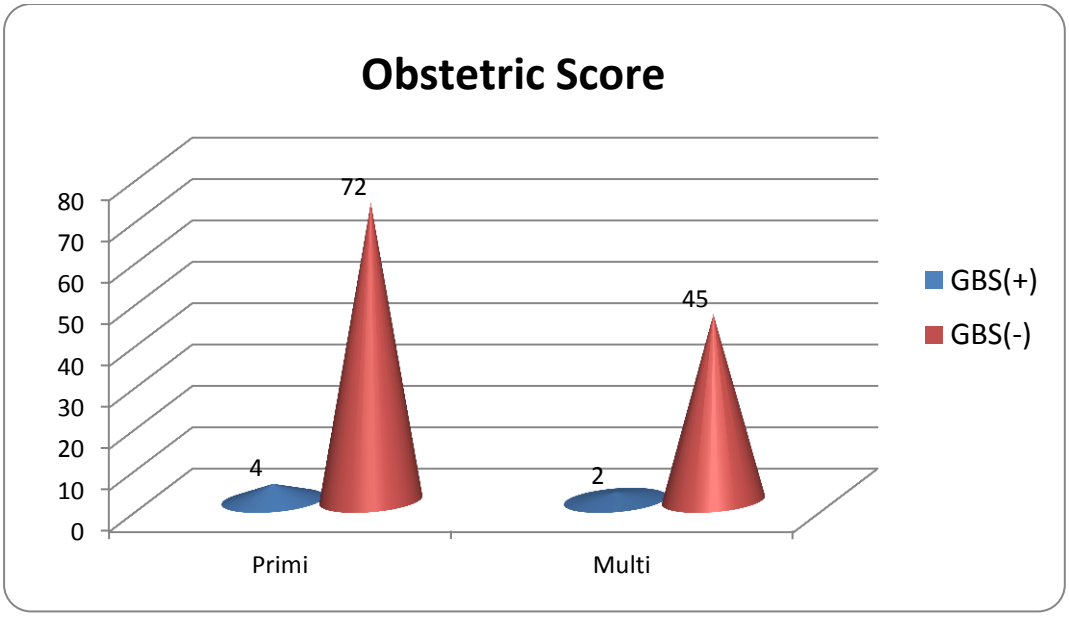
Distribution of cases according to parity

In our study maximum number of cases i.e. 4 (66.66%) were primigravida and other 2(33.33%) were multigravida in GBS positive group and in GBS negative group 72(61.5%) were primigravida and other 45 (38.46%) were multigravida.

Table 4: Obstetric Score

	GBS(+) n=6	**GBS(-) n=117**	**Total**
Primi	4(66.66%)	72(61.53)	76
Multi	2(33.33%)	45(38.46%)	47
Total	**6**	**117**	**123**

Figure 4



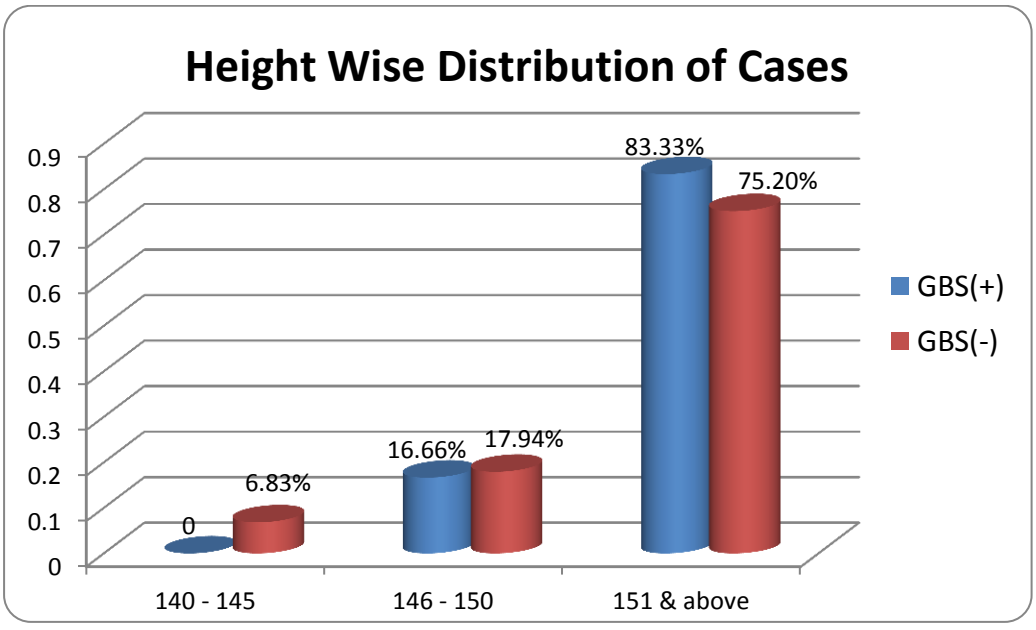
Height wise distribution of cases

5 women i.e. 83.33% were above 151cms of height in GBS positive group and 88 women i.e. 75.2% were above 151cms in GBS negative group which accounts to a maximum in our study. Other patients were below this height in both groups as shown in table below.

Table 5: Height of Pregnant Women

Height(cms)	GBS(+)n=6	GBS(-)n=117	Total
140-145	0	8(6.83%)	8
146-150	1(16.66%)	21(17.94%)	22
151 & above	5(83.33%)	88(75.2%)	93
Total	**6**	**117**	**123**

Figure 5



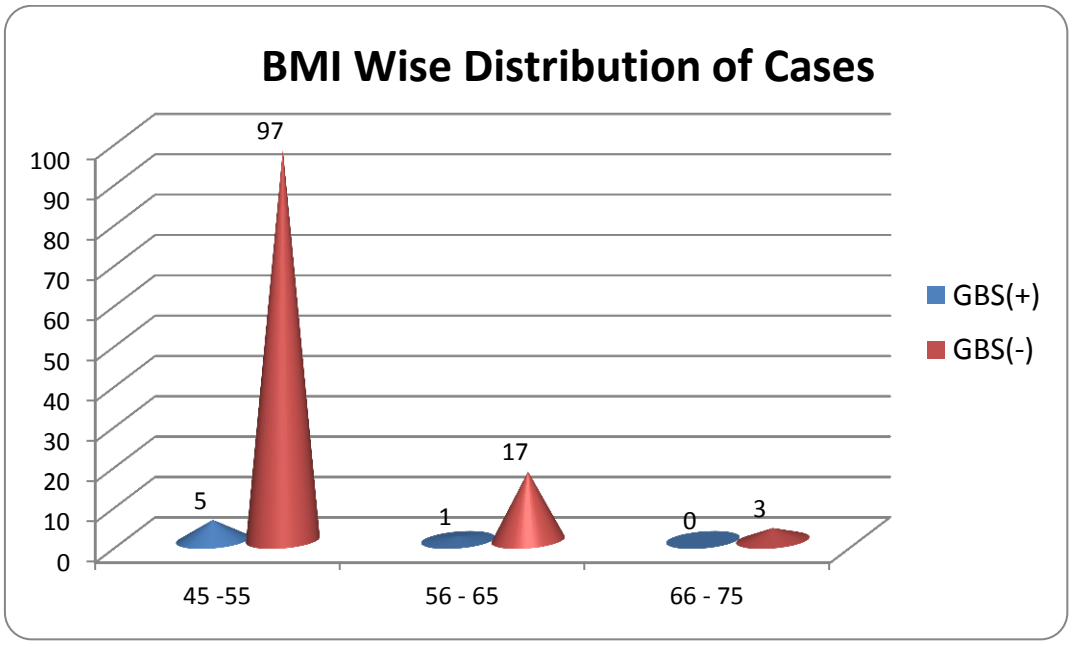
BMI wise distribution of cases

In our study 5(83.33%) women in GBS positive group, and 97(82.09%) in GBS negative women were in BMI group of 18.5-24.9 i.e. normal BMI. Others are above this BMI group as shown below in table. Increased BMI had no relation to GBS positivity in our study.

Table 6: BMI of Pregnant Women

BMI(kg/m2)	GBS(+)n=6	GBS(-)n=117	Total
18.5-24.9	5 (83.33%)	97 (82.09%)	102
25.0-29.9	1 (16.66%)	17 (14.5%)	18
30.0-34.9	0	3 (2.5%)	3
Total	**6**	**117**	**123**

Figure 6



Distribution of cases as per gestational age at which the swabs were collected

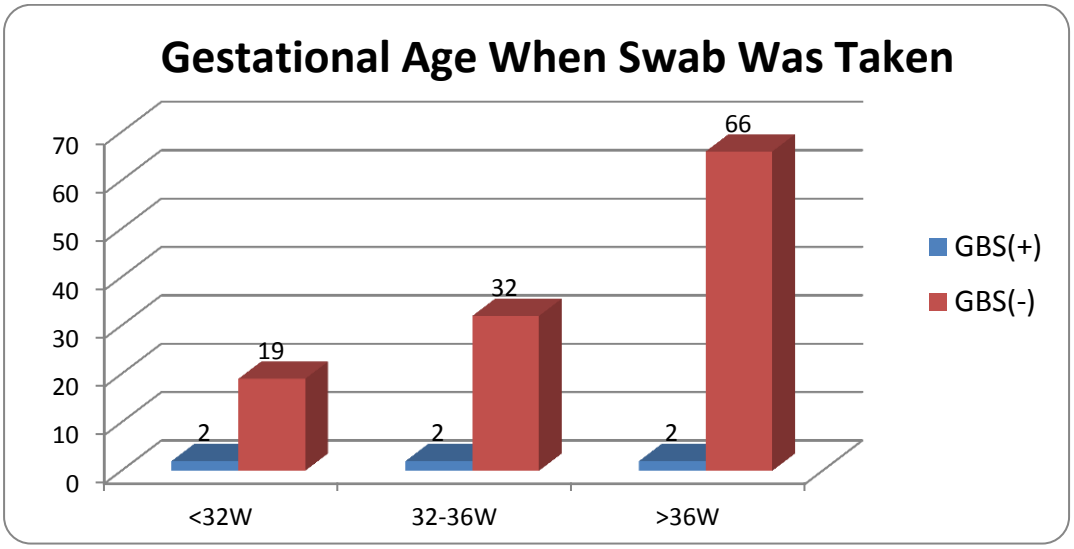
In our study 2 (33.33%) swabs were from women above 36 weeks of gestation, 2 (33.33%) swabs were from women between 32-36 weeks of gestation and other 2 (33.33%) swabs were taken <32 weeks of gestation in GBS positive group where as in GBS negative group in 66 (56.4%) of women swabs collected were above 36 weeks of gestation and others were below it as shown below.

Table7: Gestational age (GA) at which swab was taken

Sl. No	GA Period	GBS(+)n=6	GBS(-)n=117	Chi square	P value
1	<32W	2(33.33%)	19(16.2%)		
2	32-36W	2(33.33%)	32(27.35%)		
3	>36	2(33.33%)	66(56.4%)	5.62	< 0.01
	Total	**6**	**117**		

Since chi square calculated is more than tabulated at 1 degree freedom with p value being less than p<0.01 , the association is highly significant.

Figure 7



Distribution of cases according to gestational age at delivery

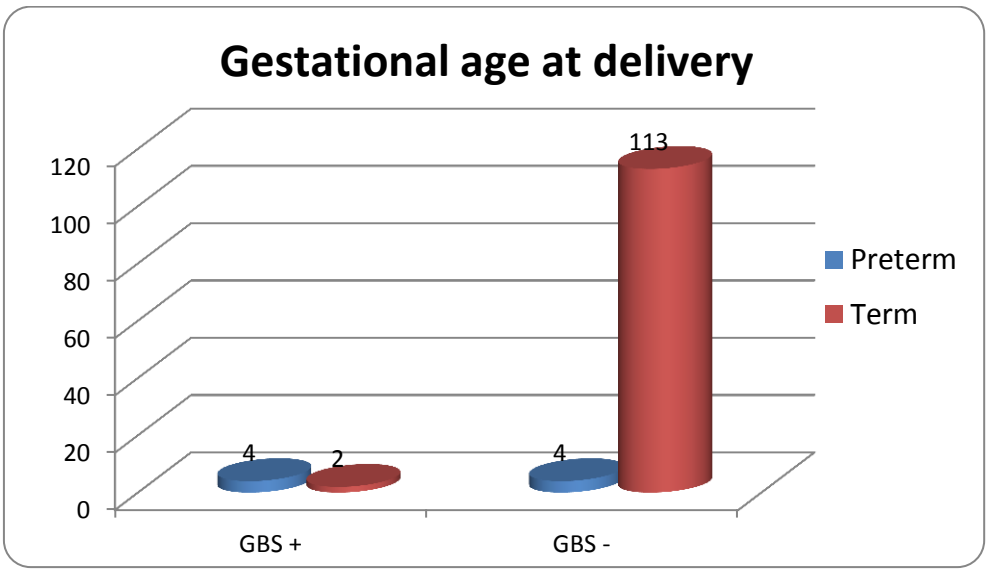
Preterm delivery rate in GBS positive group was 66.66% as compared to only 3.4% in GBS negative group which is statistically significant.In GBS positive group 16.6% preterm delivery were in <32 weeks gestation,33.33% in <34 weeks and 16.6% were in <36 weeks gestation.

Table 8: Gestational age at delivery

		GBS(+) n=6	GBS(-) n=117	Total	Chi square	P value
Preterm	<32weeks	1(16.66%)	1(0.85%)	8	37.54	<0.0001
	<34weeks	2(33.33%)	2(1.70%)			
	<36weeks	1(16.66%)	1(0.85%)			
Term		2(33.33%)	113(96.5%)	115		
Total		**6**	**117**	**123**		

Chi-Square = 37.54 and P value <0.0001 at 1 degree of freedom. Hence preterm delivery rate is highly associated with GBS (+) group.

Figure 8



Preterm delivery rate without premature rupture of membranes (PROM)

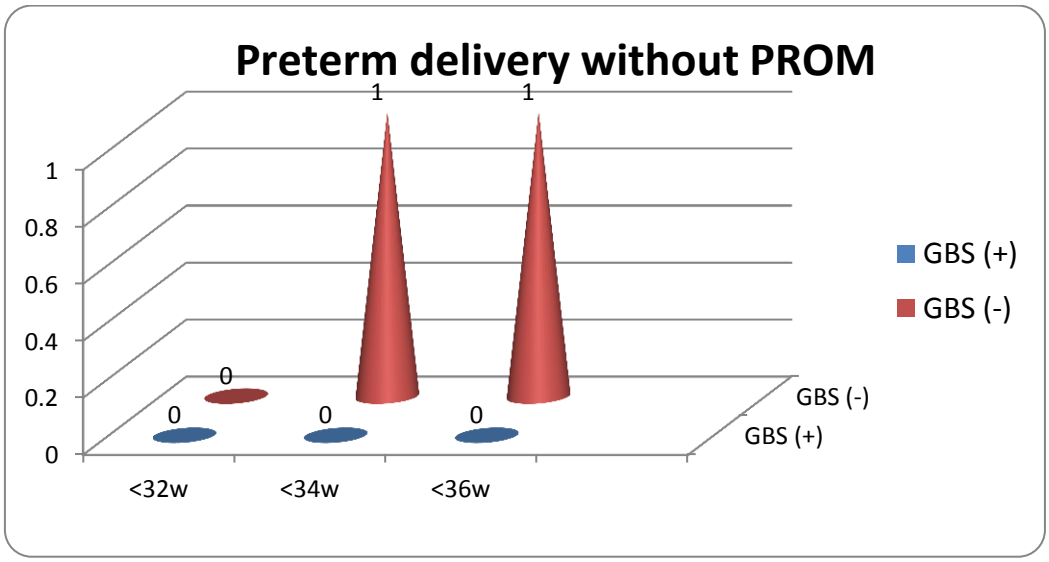
In GBS positive women there was 0% incidence of preterm delivery when not associated with PROM where as in GBS negative women 1(0.8%) baby delivered each in <34 weeks of gestation and < 36 weeks respectively. This denotes that PROM is strongly associated with GBS positive status leading to preterm labour.

Incidence of only preterm labour in GBS positive is 0/6 i.e. 0%.Preterm labour in GBS negative is 2/117 i.e.1.6%.

Table 9: Preterm delivery rate without PROM

Preterm (GA) without PROM	GBS (+) n=4	GBS (-) n=4
<32weeks	0	0
<34weeks	0	1(0.8%)
<36weeks	0	1(0.8%)

Figure 9



Preterm delivery rate with premature rupture of membranes (PROM)

All the preterm deliveries which occurred in GBS positive women where associated with PROM which signifies that GBS positive status is associated with PROM and preterm labour and only 0.8% babies in GBS negative delivered <32weeks and <34 weeks of gestational age (GA).

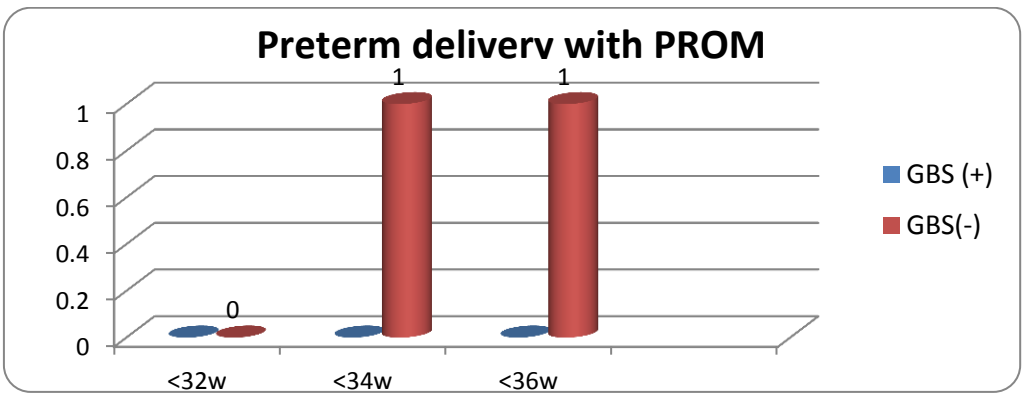
PROM in GBS positive is 4/6 i.e. 66.66% and in GBS negative 2/117 i.e.1.6%.PROM with established PTL in GBS positive is in 4/6 i.e. 66.66% and in GBS negative its 2/117 i.e. 1.6%.

Table 10: Preterm delivery rate with PROM

GA at PROM	PROM with PTL in GBS (+) n=6	PROM with PTL in GBS (-)n=117	Chi square	P value
<32weeks	1(16.66%)	1(0.8%)		
<34weeks	2(33.33%)	1(0.8%)	0.375	0.5423
<36weeks	1(16.66%)	0		

Chi square calculated is 0.375 and p value is 0.5432 at 1 degree freedom, hence the association is insignificant

Figure 10



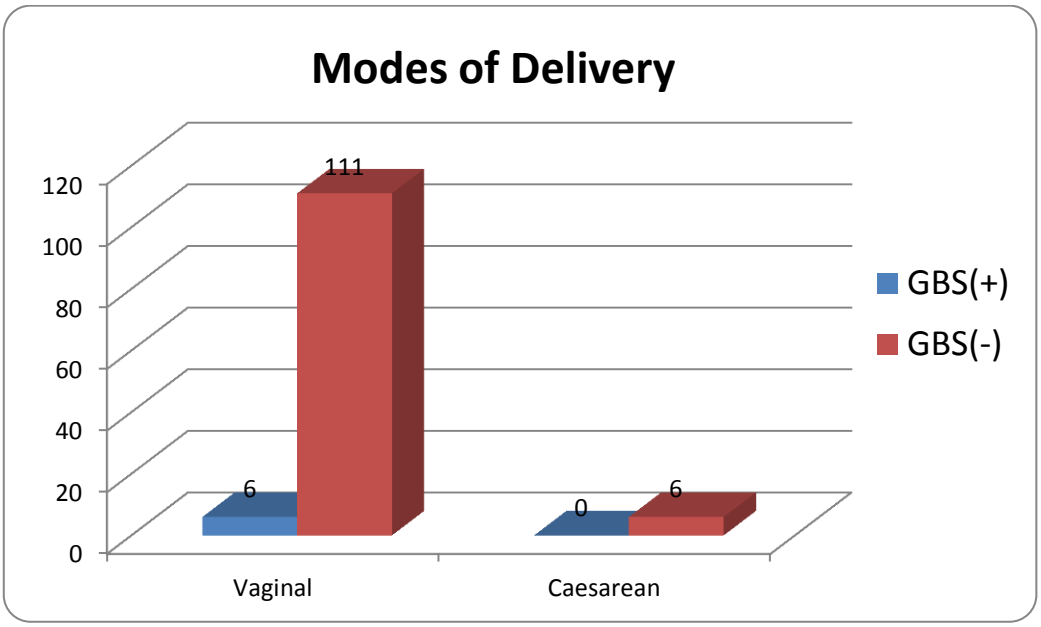
Distribution of cases by mode of delivery

All deliveries in GBS positive i.e. 6 (100%) cases were by vaginal route. In GBS negative 111(94.82%) were by vaginal route and other 6 (5.1%) were by caesarean section.

Table 11: Mode of delivery

	GBS(+)n=6	**GBS(-)n=117**	**Total**
Vaginal	6(100%)	111(94.82)	117
caesarean	0 (0%)	6 (5.1%)	6
Total	**6**	**117**	**123**

Figure 11



Distribution of cases by APGAR of baby

In GBS positive women 4(66.6%) of babies had abnormal APGAR at birth which is very high compared to 20.5% in GBS negative group.

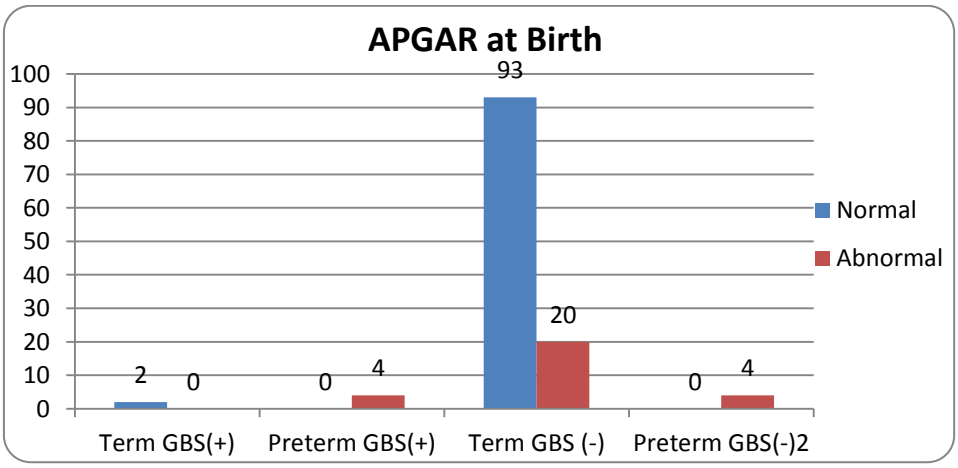
In GBS positive women, 4 (66.66%) of babies delivered by preterm delivery had abnormal apgar at birth whereas only 4 (3.4%) of preterm babies in GBS negative women had abnormal apgar at birth.

In GBS positive women 2/4 with PV leak had >18 hours leak and both babies of these women had abnormal apgar i.e. 50% but no history of prolonged rupture of membranes was found in any women in GBS negative women

Table12: APGAR at Birth

	GBS positive n=6		GBS negative n=117	
	Term	**Preterm**	**Term**	**Preterm**
Normal	2(33.33%)	0%	93(79.5%)	0%
Abnormal	0%	4(66.66%)	20(17.09%)	4 (3.4%)

Figure 12



Distribution of cases by maternal mortality

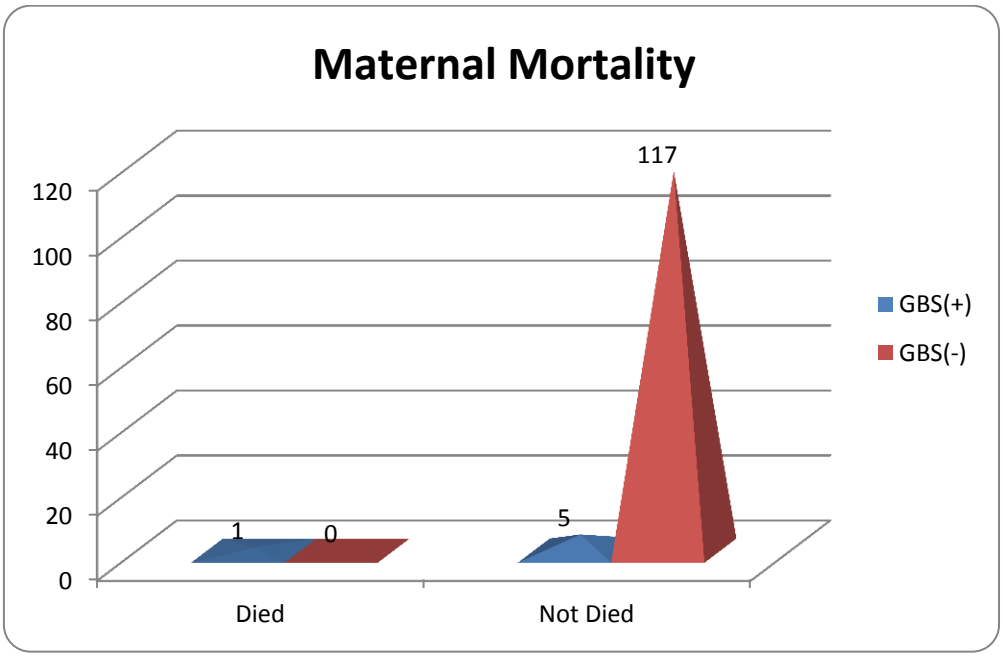
Maternal mortality rate is 16.67% (1/6) in GBS positive group which is highly significant when compared to no deaths in GBS negative group.

Table-14 Maternal Mortality

Survival Status	GBS(+) n=6	GBS(-) n=117	Total	Chi square	P value
Died	1(16.67%)	0%	1	4.42	<0.05
Not died	5(83.33%)	117(100%)	122		
Total	6	117	123		

As calculated Chi square is more than tabulated and p value < 0.05, GBS positivity is highly associated with maternal mortality.

Figure 14



Distribution of cases by complications in baby

In our study 4 babies out of 6 in GBS positive group i.e. 66.66% were admitted to NICU in view neonatal jaundice and birth asphyxia as compared to only 4 out of 117(3.41%) in GBS negative group for the same period of gestation.

4 preterm babies in GBS positive received antibiotics for 5-7 days where as only 2 out of 4 preterm babies in GBS negative received antibiotics in NICU.

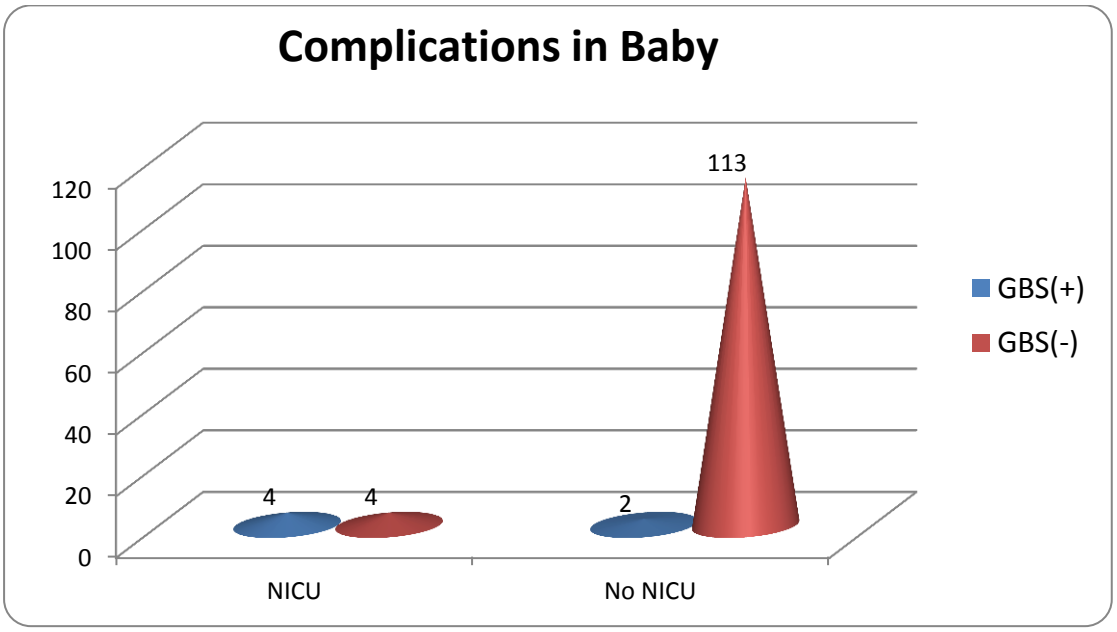
Antenatal steroids were given in 3/6 (50%) in GBS positive women and 3/117 (2.56%) in GBS negative women but still the complications related to respiratory system were higher in GBS positive compared to GBS negative women. One baby among 4 in GBS positive women admitted to NICU was on ventilator and died accounting for 33.33% mortality.no babies in GBS negative women were on ventilator.

Table 13: Complications in baby and NICU admission of babies

Admission		**GBS(+)n=6**	**GBS(-) n=117**	**Total**	**Chi square**	**P value**
NICU	Jaundice	2(33.33%)	2(1.7%)	8	49.20	<0.0001
	Birth asphyxia	2(33.33%)	2(1.7%)			
No NICU		2(33.33%)	113(96.58%)	116		
Total		6	117	123		

Chi square calculated is more than tabulated at 1 degree of freedom and p<0.0001, hence GBS positivity is associated with NICU admissions.

Figure 13



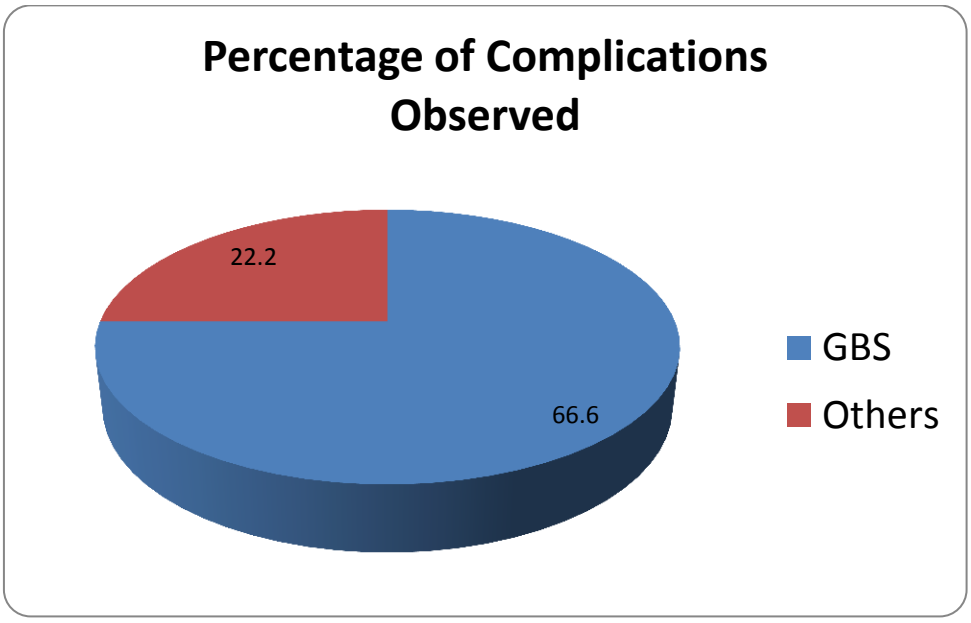
Overall complications seen in this study

The overall complications are very significantly high in GBS positive group compared to GBS negative group.

Table 15: Statistical Analysis for test of significance of complications between GBS & Others

SWAB Results	% of Complications Observed	Z-Value	P-Value
GBS	66.6	2.139	<0.041
Others	22.2		

Figure 15



There is a significant difference between prevalence of complications between GBS and others. It means complications are more prevalent in case of GBS than others.

ANALYSIS & DISCUSSSION

ANALYSIS AND DISCUSSION

CULTURE POSITIVITY:

In this study, the culture positivity rate of GBS was 4.9% (Table 1) which is comparable to the study done by Tsering Chomu Dechen, Kar Sumit, et al. [45]

The culture positivity found in our study is very high compared to the study done by Vijaya Sharmila, et al [34]where prevalence is only 2.3% .But when compared to studies done by Dalal et al[12]and Chaudhary et al[13] the prevalence is low in our study and this can be attributed to low sample size in study or a geographical distribution of cases.

AGE DISTRIBUTION

In our study culture positivity was 66.6% in age group of less than 20 years in GBS positive group which is similar to study done by Madhavi H, Vinay Hazare, et al[35]. As age advanced, there was no change in the prevalence of GBS colonization. Previous study showed an age < 20years as high risk for GBS positivity[35]. However, the same result was not reflected in our study with an equal distribution of cases in all the age groups studied (Table 3).

OBSTETRIC SCORE

In our study , maximum number of women i.e. 66.66% were primigravida in GBS positive and this result is comparable with that of study done by Tsering Chow Dechen, Kar Sumit, et al[45].

As the parity increased the prevalence of GBS colonization in pregnant women who were screened, decreased and this result is comparable to study done by Madhavi H, Vinay Hazare, et al[35].

In GBS negative women 61.5% were primigravida and 38.46% were multigravida in our study.

RELATION OF HEIGHT AND WEIGHT TO GBS POSITIVITY

We could not find any correlation between GBS positivity and height and BMI of patients (Table 4 & 5).

INCIDENCE OF PREMATURE RUPTURE OF MEMBRANES AND PRETERM LABOUR

The incidence of premature rupture of membranes in our study is 66.66% in GBS positive women which is very high and statistically significant when compared to only 1.6% in GBS negative women which is very less . These results are comparable to study done by Tsering Chow Dechen et al[45]

Incidence of only preterm labour without PROM in GBS positive is 0/6 i.e. 0%.Preterm labour in GBS negative is 2/117 i.e.1.6%. PROM in GBS positive is 4/6 i.e. 66.66% and in GBS negative 2/117 i.e.1.6%.PROM with established PTL in GBS positive is in 4/6 i.e. 66.66% and in GBS negative its 2/117 i.e. 1.6% .

But these results are not comparable to studies done by Regan JA, Chau S,et al[25] and McDonald et al[26] where the rate of PROM is 15.3% and 9.9% respectively.

COMPLICATIONS IN BABY

In our study 66.6% of babies delivered were admitted to NICU in view of jaundice and birth asphyxia in GBS positive group which is statistically significant compared to only 3.4% babies admitted to NICU in GBS negative group with a p value < 0.0001 .Out of 4 NICU admissions in GBS positive group 2(50%) were due to jaundice and other 2 (50%) due to birth asphyxia.

One baby died due to complications of severe birth asphyxia and this accounts for 25% perinatal mortality in which is statistically highly significant.

MATERNAL MORTALITY

In our study, 1 mother died due to puerperal sepsis due to GBS which accounts for 16.67% which is statistically significant with a p value < 0.05, compared to no maternal mortality in GBS negative group. This maternal mortality rate due to GBS is very significant and prompt early detection of GBS in antenatal women and proper treatment reduces this maternal mortality which is a burning issue in developing countries till date.

SUMMARY

SUMMARY

This study was conducted from October 2010 to May 2012, where a total of 123 patients were included in the study

- Out of 123 pregnant women, 6 patients were tested positive for Group B Streptococci, which accounts for 4.9%, which is a very high prevalence rate.
- In GBS positive group, 2(33.3%) were below 20 years ,2(33.3%) were between 21-25 years, 2(33.3%) were between 26-30 years and none above 30 years, whereas 49.57% women were in age group of 26-30 years in GBS negative group and else women above and below this range.
- In GBS positive 66.66% were primigravida and in GBS negative women 61.5% were primigravida and 38.46% were multigravida in our study
- There was no relation of and BMI to GBS positivity
- The incidence of premature rupture of membranes in our study is 66.66% in GBS positive women compared to only 1.6% in GBS negative women
- Incidence of only preterm labour without PROM in GBS positive is 0/6 i.e. 0% and in GBS negative is 2/117 i.e.1.6%.
- The preterm babies of GBS positive women had low apgars compared to preterm in GBS negative women.
- In our study 66.66% of babies born to GBS positive mothers were admitted to NICU in view of complications when compared to only 3.4% in GBS negative group. With p value of <0.0001.
- In our study, 1 mother died due to puerperal sepsis due to GBS which accounts for 16.67% which is statistically significant with a p value < 0.05, compared to no maternal mortality in GBS negative group

CONCLUSION

CONCLUSION

The analysis of our results show that the antenatal women are at high risk for GBS colonization and both maternal and neonatal complications are very high in GBS positive women compared to GBS negative women. Group B Streptococci continues to be poorly understood by pregnant women who try to understand and weigh up its risks and implications so as to make the best decision about screening. This study is one of the few studies done on prevalence of GBS colonization in pregnant women done in India.

This study highlighted that every women is at risk of GBS colonization and women of child bearing age of 20-30 years are at greater risk of GBS colonization. Neonates born to GBS colonized mothers are at higher risk to get colonized and develop neonatal complications. The rate of premature rupture of membranes and preterm labour are significantly high in GBS colonized mothers leading to increased neonatal as well as maternal morbidity and mortality. In our study ,as well as studies done previously by Regan et al [25]and Mc Donald et al[26] ,the incidence of PROM with preterm labour is very high (66.66%) in GBS positive women. This increases the neonatal morbidity due to prematurity and accompanying complications. Moreover , in our study the apgar scores(66.66%),NICU stay(66.66%) , and neonatal complications (66.66% & p value of <0.0001) are more severe in babies of GBS positive mothers. **The preterm infants of GBS positive women are more prone to exhibit low apgars and had signs of asphyxia which are severe compared to corresponding gestational ages but whose mothers are GBS negative**.Hence pulmonary affection is more in babies of GBS positive mothers.

Screening provides the clinician with maternal GBS colonization results, which provides with a basis for early maternal preventable treatment options. However, GBS screening is an expensive and time consuming procedure.

Our study results prompt us to recommend that GBS screen should be done at 28-30 weeks of gestation too, to prevent complications of preterm labour and PPROM with accompanying severe neonatal problems. As far as the cost effectiveness of double screening is concerned , i.e. one at 28-30 weeks and another at 35-37 weeks, this hurdle may be overcome by giving an empirical anti GBS treatment in all mothers who were previously GBS positive in labour at term. Chemoprophylaxis helps in prevention of serious neonatal infection caused by GBS. Sticking to the current recommendation of GBS screening at 35-37 weeks , we will miss GBS positivity at earlier gestation and an opportunity to prevent the serious complications of preterm labour and PPROM.

Now to conclude, all expectant mothers and fathers have a basic human right to give birth to a healthy neonate and hence as a part of antenatal program, India should adopt and implement the recommended GBS screening based protocol for all expectant mothers during late gestation.This will significantly reduce both maternal and neonatal complications..

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BIBLIOGRAPHY

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ANNEXURE I

INFORMED CONSENT

ANNEXURE I

SAMPLE INFORMED CONSENT FORM

B.L.D.E.A.U.'s SHRI B. M. PATIL MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTER, BIJAPUR – 586103, KARNATAKA

TITLE OF THE PROJECT—	A prospective observational study of prevalence of group B streptococci in anovaginal flora in pregnant women after 28 weeks of gestation.
PRINCIPAL INVESTIGATOR—	DR. SHREEDEVI KORI
P.G. GUIDE NAME —	DR. MANPREET KAUR J. TEHALIA Professor in Department of OBGY.

Purpose of research:-

I have been explained about the reason for doing this study and selecting me as a subject for this study. To have also been given free choice for either being included or not in the study.

This study is to estimate the prevalence rate of GBS in anovaginal flora in pregnant women.

Procedure:-

I have been explained that I will be part of study my history and physical findings will be taken from me and have been explained about the need for undergoing certain investigations like anovaginal culture and GBS rapid latex kit test.

Risks and discomforts

I understand that I may experience some pain or discomfort while examining or asking questions and I understand that this can be decreased by providing suitable privacy and there is no detrimental effect to me or my child.

Benefits

I understand that my participation in the study will have both direct and indirect benefit to me .This study will help to know the prevalence rate of GBS in anovaginal flora , whether I am positive for it and suitable treatment will also be prescribed , if so this will benefit me and my baby both directly and indirectly ,as this infection can have serious effects on my and my baby's health

Confidentiality

I understand that the medical information produced by this study will become a part of hospital records and will be subject to confidentiality and privacy regulation of BLDE University Shri. B.M Patil Medical College. Information of sensitive personal nature will not be part of medical record, but will be stored in the investigation research file and identified only by a code number. The code key connecting names to numbers will be kept in a secured location.

If the data are used for publication in the medical literature or for teaching purpose no name will be used.

I understand that the relevant designated authority is permitted to have an access to my medical record and to the data produced by the study for audit purpose. However, they are required to maintain confidentiality.

Study subject consent statement:

I confirm that Dr. Shreedevi Kori has explained to me the purpose of research, the study procedure, that I will undergo and the possible discomforts as well as benefits that I may experience in my own language. I have been explained all the above in detail in my own language and I understand the same. Therefore I agree to give consent to participate as a subject in this research project.

_____ _____

(participant) Date

_____ _____

(witness to signature) Date

ANNEXURE II

CASE

PROFORMA

ANNEXURE II

PROFORMA FOR CASE TAKING

Name-

Age- Occupation-

IP. No.- Socio Economic Status-

Address- Date of Admission-

Phone No.- Time of Admission-

Chief Complaints-

History of Present Pregnancy-

Antenatal History

- Booked/Unbooked
- Immunised/Unimmunised

Gestational age as per last Menstrual period-

Past History-

Family History-

Personal History-

Obstetric History

- Married Life
- Obstetric Score
- Last Child Birth
- Last Abortion

Previous Pregnancy details:

Details of previous pregnancies	I	II	III1	IV	V	Present Pregnancies
Duration of Pregnancy						
Spontaneous/Induced Abortion						
Booked/Unbooked						
Chorioamniotis PTL PROM PPROM IUGR						
Mode of Delivery						
Home Delivery						
Hospital Normal / Instrumental						
LSCS						
Elective						
Emergency						
Indication						
Live Birth/ Still Birth						
Sex of the Baby						
Wt. of the Baby						
Neonatal Outcome Birth Weight APGAR One Minute Five Minute						
Neonatal Admission Yes/No						
Neonatal Morbidity - Sepsis, Pneumania, Severe Respiratory distress, LBW/ Others						

Menstrual History: PaMC :
LMP :
EDD :

General Physical Examination

Build and Nourishment :
Height : Pulse :
Weight : BP :
Breast : Temp :
Thyroid : RR :
Spine :

Pallor / Icterus / Cyanosis / Clubbing / Edema / Lymphadenopathy

Systemic Examination

CVS :
RS :
PER ABDOMEN : Corresponding
Any Abnormal Finding

Per Speculum Examination : Normal
Abnormal

Per Vaginal Examination : Not Done
If Done Normal
Abnormal

Puerperal Complication : PPH
Pyrexia
Sepsis
Sub Involution
Prolonged Hospital Stay

CTG – Cardiotocogram
IVD – Instrumental Vaginal Deliveries
LSCS – Lower Segment Caesarean Section
NICU – Neonatal Intensive Care Unit
LBW – Low Birth Weight

ANNEXURE III

KEY TO MASTER CHART & MASTER CHART

ANNEXURE III

KEY TO MASTER CHART

GA	**Gestational Age**
OBS SCORE	**Obstetrics Score**
P	**Primi Gravida**
FTND	**Full Term Normal Delivery**
LSCS	**Lower Segment Caesarean Section**
HT	**Height**
WT	**Weight**
BMI	**Body Mass Index**
NICU	**Neonatal Intensive Care Unit**
CB	**Citrobacter**
S	**Sterile**
K	**Klebsiella**
E.COLI	**Escherichia coli**
PROM	**Premature Rupture of Membrane**
LB	**Live Birth**

MASTER CHART

SL. No.	AGE	GA<32W /32-36W />36W SWAB TAKEN	OBS SCORE/GA AT DELIVERY	PREVIOUS PREG DETAILS-GA/mode of delivery /SEX/ WEIGHT OF BABY	HT in cm	WT/BMI	Present pregnancy-mode/sex/wt/ BABY(NICU/ NO)	COMPLI-CATIONS IN BABY	PUERPERIUM	SWAB RESULT
1	31	32-36W	P/38W	NO	154	58/24.45	VD/F/2.5/NO	NO	NORMAL	CB
2	22	32-36W	P/38W	NO	148	54/24.65	VD/M/2.6/NO	NO	NORMAL	S
3	28	32-36W	G2P1L1 /37W	38/FTND/LB/M/2.6	150	54/24.0	VD/M/2.8/NO	NO	NORMAL	S
4	27	32-36W	G3P2L2 /37W	38/39/FTND/LB F/M/2.6/2.5	154	50/21.08	VD/F/2.5/NO	NO	NORMAL	K
5	22	32-36W	P/38W	NO	160	58/22.65	VD/F/2.6/NO	NO	NORMAL	E.COLI
6	20	32-36W	P/38W	NO	160	60/23.43	VD/F/2.5/NO	NO	NORMAL	S
7	22	<32W	P/38W	NO	149	50/22.52	VD/M/2.5/NO	NO	NORMAL	CB
8	22	32-36W	G2P1L1 /38W	39/FTND/LB/M/2.5	154	49/20.65	VD/F/2.6/NO	NO	NORMAL	CB
9	26	32-36 W	G2P1L1/38W	38/FTND/LB/F/2.7	160	58/22.65	VD/F/2.5/NO	NO	NORMAL	CB
10	19	<32W	P/37W	NO	154	48/20.23	VD/M/2.5/NO	NO	NORMAL	S
11	30	<32W	G2P1L1 /38W	39/FTND/LB/F/2.5	160	58/22.65	VD/F/2.5/NO	NO	NORMAL	S
12	21	32-36W	P/38W	NO	158	54/21.63	VD/M/2.5/NO	NO	NORMAL	CB
13	32	>36W	G3P2L2/39W	38/39/FTND/LB /M/M/2.6/2.6	141	67/33.70	VD/M/2.5/NO	NO	NORMAL	S
14	20	32-36W	P/38W	NO	148	53/23.37	VD/M/2.4/NO	NO	NORMAL	S
15	25	>36W	G2P1L1 /38W	30/FTND/LB/F/2.5	152	54/23.37	VD/M/2.6/NO	NO	NORMAL	S
16	20	<32W	G2P1L1/38W	38/FTND/LB/M/2.8	158	50/20.02	VD/F/2.8/NO	NO	NORMAL	S
17	24	>36W	G2P1L1/39W	38/FTND/LB/M/2.8	158	50/20.02	LSCS/M/2.5/NO	NO	NORMAL	S
18	23	>36W	G2P1L1/39W	38/EMG LSCS/LB/M/3.0	142	42/20.82	VD/F/2.2/NO	NO	NORMAL	S
19	21	32-36W	P/38W	NO	151	52/22.80	VD/F/2.5/NO	NO	NORMAL	S
20	20	<32W	P/37W	NO	156	52/21.36	VD/M/2.5/NO	NO	NORMAL	E.COLI
21	24	<32W	P/38W	NO	158	56/22.43	VD/F/2.5/NO	NO	NORMAL	E.COLI
22	26	32-36W	P/37W	NO	149	48/21.62	VD/F/2.5/NO	NO	NORMAL	S
23	24	32-36W	P/38W	NO	152	56/24.23	VD/F/2.5/NO	NO	NORMAL	CB
24	24	>36W	P/37W	NO	156	54/22.18	VD/F/2.6/NO	NO	NORMAL	S
25	22	32-36W	G4P1L1A2/37W	38/FTND/LB/F/2.6	158	54/21.63	VD/F/2.8/NO	NO	NORMAL	S
26	28	<32w	P/38W	NO	156	50/20.50	VD/F/2.5/NO	NO	NORMAL	S
27	22	>36W	G2P1L1/37W	40/EMG LSCS/LB/F/3.0	150	56/24.88	VD/F/2.5/NO	NO	NORMAL	S
28	22	32-36W	G4P1L1A2/37W	38/FTND/LB/F/3.0	150	45/20	VD/F/2.6/NO	NO	NORMAL	S
29	20	>36W	G2P1L1/37W	40/FTND/LB/M/3.5	160	54/21.08	VD/F/2.5/NO	NO	NORMAL	S
30	30	>36W	G3P2L2/38W	BOTH LSCS/LB/F /F/2.6/2.6	145	48/222.82	LSCS/F/2.5/NO	NO	NORMAL	CANDIDA
31	25	>36W	G2P1D1 /38W	35/LSCS/LB/2.5	160	58/22,65	LSCS/F/2.6/N0	NO	NORMAL	S
32	22	>36W	P/40W	NO	150	4/21.33	VD/F/2.6/NO	NO	NORMAL	S
33	20	32-36W	P/36W	NO	160	58/22.65	VD/F/2.6/NO	NO	NORMAL	S
34	28	>36W	G4P3L3/40W	ALL FT/FTND /LB/ALL F	150	54/24	VD/F/2.5/NO	NO	NORMAL	S

35	29	>36W	G2A1/37W	38/EMG LSCS/LB/M/2.5	140	56/28.57	LSCS/F/3.0/NO	NO	NORMAL	S
36	22	32-36W	G2A1/38W	NO	142	50/24.79	VD/F/2.5/NO	NO	NORMAL	S
37	24	>36W	G3P3L2 /38W	FT/ALL ND/LB/M/M/2.5	150	54/24.0	VD/F/3.7/NO	NO	NORMAL	S
38	21	>36W	G2P1L1/38W	FT/EMG LSCS/LB/M	146	50/28.45	LSCS/F/2.4/N0	NO	NORMAL	S
39	22	>36W	P/38W	NO	154	50/21.08	VD/F/2.5/NO	NO	NORMAL	S
40	22	>36W	P WITH LEAK/33W	NO	157	58/23.53	VD/F/1.9/NICU	YES	ABNORMAL	S
41	35	>36W	P/38W	NO	158	48/19.22	VD/F/2.2/NO	NO	NORMAL	S
42	22	<32W	P/38W	NO	150	56/24.88	VD/F/2.4/NO	NO	NORMAL	S
43	23	>36W	G2P1L1/33W	39/LB/M/2.8	146	66/30.96	VD/F/1.8/NICU	YES	ABNORMAL	S
44	20	>36W	P/37W	NO	156	58/23.83	VD/F/2.5/NO	NO	NORMAL	S
45	**20**	**>36W**	**G2P1L1 WITH LEAK/32W**	**38/FTND/F/LB/2.6**	**156**	**50/20.54**	**VD/M/1.9/ NICU**	**SEPSIS & DIED**	**ABNORMAL**	**GROUP B STREP**
46	22	>36W	P/37W	NO	154	50/21.08	VD/M/2.6/NO	NO	NORMAL	S
47	28	32-36W	P/38W	NO	154	50/21.08	VD/M/2.6/NO	NO	NORMAL	S
48	26	>36W	P/37W	NO	154	60/25.29	VD/F/2.5/NO	NO	NORMAL	S
49	22	>36W	G2A1/37W	NO	148	48/21.91	VD/F/2.5/NO	NO	NORMAL	S
50	24	>36W	P/37W	NO	150	58/25.71	VD/F/2.5/NO	NO	NORMAL	S
51	26	>36W	G2P1L1/39W	39/FTND/LB/M/2.8	150	60/26.66	VD/F/2.6/NO	NO	NORMAL	S
52	22	>36W	G2P1L1/40W	38/FTND/LB/F/2.6	148	50/22.82	VD/F/2.6/NO	NO	NORMAL	S
53	28	>36W	P/37W	NO	160	54/21.09	VD/F/2.6/NO	NO	NORMAL	S
54	22	>36W	P/38W	NO	158	54/22.83	VD/F/2.6/NO	NO	NORMAL	CB
55	24	>36W	P/37W	NO	148	50/22.82	VD/F/2.8/NO	NO	NORMAL	S
56	20	<32W	P/36W	NO	158	50/20.02	VD/F/2.7/NO	NO	NORMAL	S
57	27	<32W	G4P1L1A2/38W	38/FTND/LB/2.5	156	50/20.54	VD/F/2.6/NO	NO	NORMAL	S
58	**20**	**32-36W**	**P WITH LEAK/35W**	**NO**	**160**	**58/22.65**	**VD/F/2.2/NICU**	**YES**	**ABNORMAL**	**GROUP B STREP**
59	25	32-36W	P/38W	NO	158	56/22.43	VD/F/2.6/NO	NO	NORMAL	S
60	28	>36W	P/38W	NO	158	56/22.43	VD/M/2.6/NO	NO	NORMAL	S
61	21	>36W	P/38W	NO	160	62/24.21	VD/M/2.6/NO	NO	NORMAL	S
62	25	>36W	G2P1L1/38W	39/FTND/LB/F/2.8	160	56/21.87	VD/F/2.6/NO	NO	NORMAL	S
63	25	>36W	G2P1L1/38W	39/FTND/LB/F/3.0	168	54/19.85	VD/F/2.4/NO	NO	NORMAL	S
64	21	>36W	P/37W	NO	170	58/20.02	VD/F/2.6/NO	NO	NORMAL	S
65	21	>36W	P/38W	NO	168	68/24.11	VD/F/2.6/NO	NO	NORMAL	S
66	22	>36W	P/37W	NO	165	65/23.80	VD/F/2.5/NO	NO	NORMAL	S
67	27	>36w	G2P1L1/37W	38/FTND/LB/N/2.8	160	68/26.50	VD/F/2.5/NO	NO	NORMAL	S
68	30	<32W	P/37W	NO	160	54/21.09	VD/F/2.6/NO	NO	NORMAL	S
69	28	32-36W	P/38W	NO	158	54/21.68	LSCS/M/2.5/NO	NO	NORMAL	S
70	26	<32W	p/38W	NO	158	58/23.29	VD/F/2.2/NO	NO	NORMAL	S
71	20	>36W	p/38W	NO	158	58/23.29	VD/F/2.2/NO	NO	NORMAL	S
72	26	>36W	P/38W	NO	154	59/25.65	VD/F/2.5/NO	NO	NORMAL	S
73	27	>36W	G2P1L1/38W	38/FTND/LB/F/3.0	156	60/25	VD /M/2.8/NO	NO	NORMAL	S
74	28	>36W	P/37W	NO	156	50/20.80	VD/F/2.5/NO	NO	NORMAL	S
75	27	32-36W	P/38W	NO	155	58/24.16	VD/F/2.6/NO	NO	NORMAL	S

76	26	>36W	P/37W	NO	154	58/24.16	VD/F/2.8/NO	NO	NORMAL	S
77	29	>36W	P/38W	NO	154	58/24.16	VD/M/2.7/NO	NO	NORMAL	CB
78	28	>36W	P/38W	NO	156	59/24.27	VD/M/2.5/NO	NO	NORMAL	S
79	28	>36W	G3P2L2	38/39/FTND/LB/M/F	160	60/23.43	VD/M/2.6/NO	NO	NORMAL	S
80	25	>36W	P/37W	NO	154	57/24.78	VD/F/2.8/NO	NO	NORMAL	S
81	29	<32W	p/38W	NO	156	53/23.43	VD/M/2.7/NO	NO	NORMAL	ECOLI
82	**24**	**>36W**	**G2P1L1 WITH PROM/35+2D**	**39/FTND/LB/N/2.8**	**154**	**60/22.64**	**VD/M/2.1/ NICU**	**YES**	**ABNORMAL**	**GROUP B STREP**
83	24	>36W	G2A1/40W	NO	160	60/23.43	VD/F/2.8/NO	NO	ABNORMAL	S
84	30	>36W	G2P1L1	40/FTND/LB/M/2.4	140	40/20.40	VD/F/2.8/NO	NO	NORMAL	S
85	30	>36W	G2P1L1	40/FTND/LB/M/2.4	160	60/22.64	VD/M/2.7/NO	NO	NORMAL	S
86	26	>36W	P/38W	NO	160	60/22.64	VD/M/2.8/NO	NO	ABNORMAL	CANDIDA
87	30	<32W	G2P1L1/38W	40/FTND/LB/F/3.2	164	56/20.89	VD/M/2.9/NO	NO	ABNORMAL	S
88	27	32-36W	G3P2L2/39W	38/42/FTND/LB/M/M/2.5/2.5	154	70/29.50	VD/F/2.8/NO	NO	NORMAL	S
89	28	<32W	G2P1L1/40W	38/LSCS/LB/F/2.8	158	6/24.080	VD/M/2.7/NO	NO	ABNORMAL	S
90	22	<32W	P/35W	NO	160	54/21.09	VD/F/1.9/NICU	YES	ABNORMAL	S
91	26	>36W	P/39W	NO	152	58/25.20	VD/M/3.0/NO	NO	NORMAL	S
92	26	32-36W	G2P1L1/37W	40/FTND/LB/F/3.0	154	60/26.08	VD/F/3.1/NO	NO	NORMAL	S
93	27	>36W	P/38W	NO	154	62/26.16	VD/M/2.6/NO	NO	ABNORMAL	S
94	29	>36W	P/40W	NO	154	58/24.17	VD/M/2.6/NO	NO	ABNORMAL	GPOUP A STREP
95	25	>36W	G3P2L2/39W	40/38/FTND/LB/M/F/3.0/3.3	156	64/26.33	VD/M/3.0/NO	NO	NORMAL	S
96	27	32-36W	P/39W	NO	152	65/28.26	VD/F/2.8/NO	NO	NORMAL	S
97	25	>36W	P/38W	NO	152	60/26.08	VD/F/2.8/NO	NO	ABNORMAL	S
98	28	>36W	P/40W	NO	158	64/26.60	VD/F/2.8/NO	NO	ABNORMAL	S
99	26	>36W	G2P1L1/39W	40/FTND/LB/M/3.2	160	58/22.65	VD/M/2.9/NO	NO	ABNORMAL	S
100	26	>36W	G2P1L1/40W	38/FTND/LB/F/2.6	156	58/22.86	VD/F/2.5/NO	NO	NORMAL	S
101	24	32-36W	P/37W	NO	160	58/22.65	VD/M/2.5/NO	NO	ABNORMAL	S
102	24	>36W	P/38W	NO	162	60/22.90	VD/M/2.5/NO	NO	NORMAL	S
103	30	<32W	p/38W	NO	160	58/22.60	VD/M/2.6/NO	NO	ABNORMAL	S
104	31	>36W	P/37W	NO	158	56/22.48	VD/F/2.5/NO	NO	NORMAL	S
105	28	<32W	P/40W	NO	156	54/22.22	VD/M/2.6/NO	NO	ABNORMAL	S
106	29	32-36W	P/37W	NO	156	54/22.22	VD/M/2.5/NO	NO	NORMAL	S
107	30	32-36W	G2P1L1/38W	37/FTND/LB/M/2.7	160	58/22.65	VD/F/2.6/NO	NO	ABNORMAL	S
108	28	>36W	G2P1L1/38W	39/FTND/LB/F/2.8	156	58/23.86	VD/M/2.6/NO	NO	NORMAL	S
109	27	32-36W	P/37W	NO	156	58/23.86	VD/F/2.5/NO	NO	ABNORMAL	S
110	26	>36W	G3P2L1/40W	38/40/FTND/LB/M/F/2.8/2.6	162	59/20.20	VD/M/2.6/NO	NO	ABNORMAL	S
111	27	32-36W	p/38W	NO	158	56/22.48	VD/M/2.6/NO	NO	NORMAL	S
112	28	>36W	P/38W	NO	156	56/20.04	VD/F/2.8/NO	NO	ABNORMAL	S
113	29	32-36W	G2P1L1 WITH LEAK/<32W	39/FTND/LB/F/2.6	158	58/23.29	VD/M/1.7/NICU	YES	ABNORMAL	S
114	**30**	**<32W**	**P/40W**	**NO**	**156**	**56/20.04**	**VD/F/2.5/NO**	**NO**	**NORMAL**	**GROUP B STREP**
115	31	>36W	P/40W	NO	148	56/25.50	VD/M/2.5/NO	NO	ABNORMAL	S

116	32	>36W	P/37W	NO	146	54/25.35	VD/F/2.8/NO	NO	NORMAL	S
117	30	>36W	G2P1L1/38W	38/FTND/LB/M/2.4	144	48/22.96	VD/M/2.5/NO	NO	ABNORMAL	S
118	29	32-36W	p/38W	NO	150	48/21.33	VD/M/2.5/NO	NO	NORMAL	S
119	30	>36W	P/40W	NO	144	50/24.15	VD/F/2.6/NO	NO	ABNORMAL	S
120	**26**	**<32W**	**P WITH LEAK/34W**	**NO**	**152**	**54/25.35**	**VD/F/2.0/NICU**	**YES**	**ABNORMAL**	**GROUP B STREP**
121	27	<32W	P/38W	NO	160	59/23.04	VD/M/2.6/NO	NO	ABNORMAL	S
122	27	32-36W	P/37W	NO	160	59/23.04	VD/M/2.7/NO	NO	NORMAL	S
123	**27**	**32-36W**	**P WITH /37W**	**NO**	**148**	**63/28.76**	**VD /M/2.8/NO**	**NO**	**NORMAL**	**GROUP B STREP**