# Incidence and risk factors for group B *Streptococcus* colonisation in pregnant women experiencing preterm labour and neonatal outcomes

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**Abstract.** Group B *Streptococcus* (GBS), also known as *Streptococcus agalactiae*, is the normal flora present in the female urogenital tract and rectum. Globally, it is estimated that 15 to 40% of pregnant women are colonised by GBS. Its chief clinical significance is that it is an acknowledged risk factor for the early onset of neonatal sepsis (even when colonisation is asymptomatic), as it can be transferred during delivery to neonates from their colonised mothers, and can cause sepsis and meningitis in newborns. The present analytical cross-sectional study included 100 women experiencing preterm labour. Data were collected from the January 1, 2023 to November 1, 2023. Preterm birth was defined as birth at a gestational age from 28 to 36 weeks + 6 days. The exclusion criteria were women pregnant with a foetus diagnosed with congenital anomalies, with premature rupture of membranes, or women with fever, an elevated white blood cell count or any features suggestive of chorioamnionitis. Prior to delivery, a vaginal and rectal swab sample was obtained during a vaginal examination and was sent to the laboratory for the culture of GBS. All mothers received intrapartum antibiotic prophylaxis. The prevalence of GBS colonisation was found to be 8%. As regards the risk factors associated with maternal GBS colonisation, a statistically significant association was detected between GBS colonisation and both diabetes and a higher pre-pregnancy body mass index. As regards the outcomes, a positive GBS colonisation was significantly associated with a lower gestational age at birth and poor neonatal survival. Although the present study found that vertical transmission may be controlled by the proper administration of intrapartum antibiotic prophylaxis, babies of mothers with maternal colonisation had poor outcomes (5/8, 62.5% did not survive), as GBS colonisation was significantly associated with a lower gestational age (which was significantly associated with neonatal mortality). Moreover, the present study effectively consolidated the association between maternal group B colonisation and both diabetes mellitus and obesity. Therefore, women with diabetes mellitus and obesity require special attention for the early detection and early management of GBS colonisation.

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## Introduction

Group B *Streptococcus* (GBS) is a β-haemolytic, Gram-positive bacteria coccus that asymptomatically colonises the lower genital and gastrointestinal tracts (1). Due to the ability of GBS to adhere and infiltrate the placental chorionic villi, vaginal colonisation during gestation poses a risk for ascending infection and neonatal transmission upon delivery (2). In the absence of preventative measures, GBS will be passed vertically to the newborn via contaminated bodily and amniotic fluids before or during delivery in ~50% of colonised mothers, resulting in neonatal illnesses, such as neonatal meningitis, pneumonia and neonatal sepsis (3-5).

The third trimester screening of pregnant women for this organism, along with subsequent antibiotic prophylaxis for maternal colonisation, has significantly decreased the prevalence of early-onset neonatal illness from 1.7 cases per 1,000 live births in the early 1990s to 0.22 cases per 1,000 live births in 2017 (6).

Thus, all mothers who test positive for GBS during routine screening at 35 to 37 weeks of pregnancy should receive intrapartum antibiotic prophylaxis (IAP). The American College of Obstetrics and Gynecology also recommends intrapartum antibiotic prophylaxis for pregnant women who have a history of GBS bacteriuria at any point during the current or previous pregnancy. In the case that the GBS status of a pregnant woman is unknown, the American College of Obstetrics and Gynecology recommends IAP in the event that specific risk factors are present. These include 'preterm labour before 37 weeks, rupture of membranes for 18 hours or more, a maternal fever of 100.4˚F or higher during labour, or a positive rapid GBS nucleic acid amplification test during childbirth' (7).

Preterm birth accounts for 500,000 neonatal deaths annually globally, including 44% of all mortality among children <5 years of age. The predominant cause of premature deliveries is microbial infections, with 10% attributable to GBS (2). The

vaginal colonisation with GBS is a well-acknowledged risk factor for preterm birth. The bacteria induce inflammation, histopathological chorioamnionitis and preterm premature rupture of membranes, leading to preterm birth, either by ascending infection or by generating membrane vesicles (8).

The present study aimed to assess the prevalence of GBS colonisation among women experiencing preterm labour, and to identify and analyse the maternal risk factors associated with GBS colonisation. The present study also aimed to investigate potential neonatal implications associated with this infection.

## Patients and methods

*Location of study and study design*. The present study was conducted at the Baghdad Teaching Hospital, Medical city, Baghdad, Iraq. Data were collected from January 1, 2023 to November 1, 2023. The present study used an analytical cross-sectional design. Informed consent was obtained from all participants prior to data collection. Verbal consent was obtained from all participants prior to data collection. An official letter of approval (no. 338) was obtained from the Scientific Committee of the Scientific Council of Obstetrics and Gynecology-Iraqi Board for Health Specializations, in accordance with the Declaration of Helsinki.

*Data and sample collection*. A total of 100 pregnant women with established preterm labour (gestational age, 28 to 36 weeks + 6 days) were included in the present study. Preterm labour was diagnosed based on documented uterine contractions and cervical dilation exceeding 4 cm. All deliveries occurred prior to 37 weeks of gestation. The gestational age was calculated depending on the last menstrual period (LMP) of the mother and the first trimester ultrasound scan. A full history and thorough general and obstetrical examinations were performed for all women.

Basic sociodemographic and obstetric data were collected [age, parity, gestational age, past medical history, body mass index (BMI) and birth weight]. A total of 10 $cm^3$ of blood were collected from the pregnant women for general analyses: Complete blood count, renal function test, liver function test, thyroid function test, blood group and Rh factor. A vaginal and rectal swab sample were also obtained during a vaginal examination.

As the infection prevention and control settings in the hospital were not deemed as being of a high standard (in the hospital, due to the overcrowding of the wards, and the close proximity between patients made it difficult to maintain a safe distance between patients, increasing the risk of airborne and contact-based infections), the consultant gynaecologist decided to administer antibiotic therapy to all mothers with preterm labour (IV ceftriaxone vial 1 gm in a single dose, and in the case of allergy, vancomycin vial at 1 gm).

In the laboratory (private sector), using the VITEK-2 machine, the following procedures were performed: i) Inoculation and incubation: The specimen was inoculated with enrichment broth (nutrient) for 24 h and then transferred into the appropriate culture media (Blood agar, MacConkey agar or Sebouraud dextrose agar) (BioMérieux) to subculture, at the appropriate temperature and conditions for GBS growth (typically 35-37˚C for 24 h). ii) Isolation: The colony was examined for growth on the culture plates. GBS colonies typically appear as small, greyish-white colonies, with a surrounding zone of beta-haemolysis, on the blood agar. iii) Identification: A sterile loop was used to select a single colony and transfer it to the appropriate VITEK-2 identification card, designed for the *Streptococcus* species. The VITEK-2 system (BioMérieux) provided a profile of the organism based on its characteristics after (6-8 h), and generated a report that included the identification of the *Streptococcus* species, including GBS. The result was received after 3 days.

Following delivery, all neonates were followed-up for 1 week as follows: i) Those who required admission to the Neonatal Care Unit had blood cultures obtained, in the event that their admission lasted >3 days. ii) Those who were discharged from the Neonatal Care Unit of the hospital, had their follow-up performed by phone, for up to 1 week.

*Inclusion and exclusion criteria*. The inclusion criteria were women with singleton pregnancies, presenting with preterm

Table I. Basic characteristics of the study sample.

| Basic characteristics | Frequency | Percentage |
|---|---|---|
| Age | | |
| <20 years | 1 | 1.0 |
| 20-29 years | 48 | 48.0 |
| 30-39years | 41 | 41.0 |
| ≥40 years | 10 | 10.0 |
| Residence | | |
| Urban | 83 | 83.0 |
| Rural | 17 | 17.0 |
| Estimated pre-pregnancy BMI | | |
| Normal weight (estimated BMI, 18.5-25.0 kg/$m^2$) | 71 | 71.0 |
| Overweight or obese (estimated BMI, >25.0 kg/$m^2$) | 29 | 29.0 |
| Smoking status | | |
| Yes | 0 | 0.0 |
| No | 100 | 100.0 |

BMI, body mass index.

Table II. Clinical characteristics of the study sample.

| Clinical characteristic | Frequency | Percentage |
|---|---|---|
| Chief complaint | | |
| Abdominal pain | 93 | 93.0 |
| Vaginal discharge | 4 | 4.0 |
| Decreased foetal movement | 3 | 3.0 |
| Patients with diabetes mellitus | | |
| Yes | 10 | 10.0 |
| No | 90 | 90.0 |

Table III. Obstetric characteristics of the study sample.

| Obstetric characteristics | Frequency | Percentage |
|---|---|---|
| Gestational age at labour | | |
| 28-31 +6 weeks | 15 | 15.0 |
| 32-33 +6 weeks | 14 | 14.0 |
| 34-36 +6 weeks | 71 | 71.0 |
| Parity | | |
| Nulliparous | 9 | 9.0 |
| Primiparous | 13 | 13.0 |
| Multiparous | 78 | 78.0 |
| History of previous miscarriage | | |
| Yes | 24 | 24.0 |
| No | 76 | 76.0 |
| Type of delivery | | |
| Normal vaginal delivery | 91 | 91.0 |
| Caesarean section | 9 | 9.0 |

Table IV. Neonatal outcomes of the studied sample

| Neonatal outcomes | Frequency | Percentage |
|---|---|---|
| Discharged in <24 h | 60 | 60.0 |
| Discharged within 1 week | 25 | 25.0 |
| Discharged after >1 week | 8 | 8.0 |
| Did not survive | 7 | 7.0 |

labour (from 28 to 36 weeks + 6 days), with gestational age being diagnosed by LMP, confirmed by a report of the first trimester ultrasound. The exclusion criteria were as follows: Women pregnant with a foetus with congenital anomalies, women with premature rupture of membranes, and women with fever, an elevated white blood cell count, or other features suggestive of chorioamnionitis.

*Statistical analysis*. Statistical analysis was performed using the statistical package for social sciences (SPSS version 26; IBM Corp.). The Independent sample t-test was used to compare the continuous variables. Fisher's exact test was used for categorical variables. A two-tailed P-value ≤0.05 was considered to indicate a statistically significant difference.

## Results

A total number of 100 pregnant women experiencing preterm labour were included in the present study sample. The age distribution of the study sample ranged from 19-43 years. The majority of the participants were in the age group of 20-29 years. As regards residence, 83 (83.0%) participants were of urban residence. As regards the estimated pre-pregnancy BMI, 71 (71.0%) women were of normal weight, whereas 29 (29.0%) women were overweight or obese, as demonstrated in Table I.

In relation to the chief complaint of preterm labour, abdominal pain was the most common presentation (93%), followed by vaginal discharge (4%) and decreased foetal movement (3%). As regards a past history of diabetes mellitus (DM), 10 (10%) patients had DM (gestational and non-gestational), as presented in Table II.

Table V. Association between study parameters and group B streptococcal colonisation.

| | GBS, n (%) | | |
|---|---|---|---|
| Parameter | Positive | Negative | P-value |
| Age | | | |
| <20 years | 0 (0.0%) | 1 (1.1%) | 0.831[a] |
| <30 years | 3 (37.5%) | 45 (48.9%) | |
| 30-39 years | 2 (25.0%) | 39 (42.4%) | |
| ≥40 years | 3 (37.5%) | 7 (7.6%) | |
| Mean ± SD | 32.9±7.3 | 31.6±6.2 | |
| Residence | | | |
| Urban | 5 (62.5%) | 78 (84.8%) | 0.133[b] |
| Rural | 3 (37.5%) | 14 (15.2%) | |
| Estimated pre-pregnancy BMI | | | |
| Normal weight (estimated BMI, 18.5-25.0 kg/m$^2$) | 1 (12.5%) | 70 (76.1%) | 0.001[b] |
| Overweight or obese (estimated BMI, >25.0 kg/m$^2$) | 7 (87.5%) | 22 (23.9%) | |
| Diabetes | | | |
| Yes | 5 (62.5%) | 3 (3.3%) | <0.001[b] |
| No | 3 (37.5%) | 89 (96.7%) | |
| Parity | | | |
| Nulliparous | 1 (12.5%) | 8 (8.7%) | 0.815[b] |
| Primiparous | 1 (12.5%) | 12 (13.0%) | |
| Multiparous | 6 (75.0%) | 72 (78.3%) | |

BMI, body mass index. Data were analysed using an [a]independent samples t-test or [b]Fisher's exact test.

The gestational age of the women ranged from 30-36.6 weeks, with a mean of 35.1 weeks (±1.1 SD). A total of 15 (15%) patients experienced preterm labour at 28-32 weeks, while 85 (85%) women experienced preterm labour at ≥32 weeks. As regards parity, the majority of the patients (78%) were multiparous. As regards previous miscarriages, 24 (24.0%) patients had a history of previous miscarriage. As regards the type of delivery, the majority (91.0%) underwent normal vaginal delivery, as presented in Table III.

With respect to neonatal outcomes, 60 (60.0%) neonates were discharged within a few hours, 25 (25.0%) were admitted for <1 week, 8 (8.0%) were admitted for >1 week and 7 (7.0%) did not survive, as demonstrated in Table IV.

In relation to maternal GBS colonisation, 8 (8.0%) patients had a positive infection detected by a vaginal and anal swab, while 92 (92%) tested negative. As demonstrated in Table V, GBS positivity was significantly associated with both a higher

Table VI. Adverse outcomes associated with GBS colonisation.

| Parameter | GBS, n (%) | | P-value |
|---|---|---|---|
| | Positive (n=8) | Negative (n=92) | |
| Gestational age at birth | | | |
| 28-31 +6 weeks | 6 (75.0%) | 9 (9.8%) | <0.001[a] |
| 32-33 +6 weeks | 1 (12.5%) | 13 (14.1%) | |
| 34-36 +6 weeks | 1 (12.5%) | 70 (76.1%) | |
| Mean ± SD | 31.1±2.3 | 35.4±4.6 | |
| Final neonatal outcome | | | |
| Immediate discharge | 0 (0.0%) | 60 (65.2%) | <0.001[b] |
| Admitted for <1 week | 2 (25.0%) | 23 (25.0%) | |
| Admitted for >1 week | 1 (12.5%) | 7 (7.6%) | |
| Did not survive | 5 (62.5%) | 2 (2.2%) | |

Data were analysed using an [a]independent samples t-test or [b]Fisher's exact test.

Table VII. Association of survival with gestational age.

| Gestational age at birth | Neonatal survival, n (%) | | P-value |
|---|---|---|---|
| | Did not survive (n=7) | Survived (n=93) | |
| 28-31+6 weeks | 6 (85.7%) | 9 (9.7%) | <0.001[a] |
| 32-33 +6 weeks | 1 (14.3%) | 13 (14.0%) | |
| 34-36 +6 weeks | 0 (0.0%) | 71 (76.3%) | |
| Mean ± SD | 30.2±1.8 | 35.8±4.5 | |

[a]Data were analysed using an independent samples t-test.

pre-pregnancy BMI and diabetes, as 87.5% of the GBS-positive women were overweight or obese (P=0.001) and 62.5% had diabetes (P<0.001), compared to markedly lower proportions in the GBS-negative group. By contrast, there were no statistically significant associations between the GBS status and age (P=0.831), residence (P=0.133), or parity (P=0.815).

A positive GBS colonisation was significantly associated with a lower gestational age at birth and a poor neonatal survival, as demonstrated in Table VI. Statistical analysis revealed that neonates who had an early neonatal death, had a significantly lower gestational age than neonates who survived, as demonstrated in Table VII. The cause of death in all neonates was respiratory distress.

## Discussion

*Prevalence and association with a low gestational age at birth*. The present study revealed that GBS was identified in 8% of women experiencing premature labour. Notably, GBS colonisation was more likely to be associated with early preterm (28-32 weeks), rather than late preterm (32-37 weeks) labour. A previous study conducted in Denmark by Feikin *et al* (9) found that among 84 women experiencing preterm labour, 14% were colonised at delivery with GBS. The study by Schwab *et al* (10) in Indonesia, included 23 women (term and preterm) and reported that 8/23 (35%) had GBS.

It is important to highlight the low prevalence of GBS that was detected in the present study. The previous meta-analysis by Ashary *et al* (8) identified three parameters that were associated with improving the detection of GBS: i) The site of sample collection: Studies that employed a rectovaginal sample reported higher detection rates than studies relying of vaginal samples alone. ii) Enrichment prior to culture: The meta-analysis reported that enrichment increased the detection rate of GBS by almost 2-fold compared to studies that did not use enrichment. iii) Detection method: The prevalence of GBS determined by the culture approach was calculated at 7.4%, but the immunological method indicated a frequency of 11.6%. Compared to the cultural and immunological approaches, the identification of GBS by molecular techniques yielded a high prevalence of 62% (8).

Although a rectovaginal sample was used in the present study, culture enrichment was not performed with the recommended sheep blood agar that increased detection. Therefore, it is reasonable to assume that the prevalence would significantly increase if culture enrichment and immunological or molecular approaches were employed in Iraq.

*Factors associated with GBS colonisation*. In the present study, obesity was significantly associated with GBS colonisation. This finding is in concordance with the study by Dahan-Saal *et al* (11), who reported that obesity was the most prevalent determinant of GBS colonisation, as it increased the risk of acquiring GBS by 19%. In the USA, the study by Stapelton *et al* (12) included 1,20,000 pregnant women and reported a 20% increase in the risk of miscarriage in moderately obese women (BMI, 30-40) and a 40% increase in cases of morbid obesity (BMI, ≥40). In another study, Kleweis *et al* (13) included 7,711 pregnant women and reported that obese gravidas were still 35% more likely than non-obese women to test positive for GBS. In the USA, Venkatesh *et al* (14) included 1,15,070 women with term deliveries and reported a significant rise in the frequency of GBS colonisation with a higher pre-pregnancy BMI.

The molecular mechanism behind heightened GBS colonisation in obese women remains unclear, while it may be associated with changes in the composition and functional characteristics of the gut microbiota.

In the present study, DM was also significantly linked to a higher rate of GBS infection. This finding is in concordance with the study by Field *et al* (15), who included 305 pregnant women and reported that women with an HbA1c level ≥6.5% were twice as likely to have GBS colonisation than women with an HbA1c level <6.5%. This association between DM and GBS colonisation may be attributed to the following reasons: i) DM alters immune cell function within the reproductive tract; and ii) glucose alters GBS growth and promotes biofilm production, which may promote colonisation (16).

*Vertical transmission*. A notable finding of the present study was that the proportion of vertical transmission of GBS was

found to be 0%. This may be attributed to the study setting, as it was conducted in a tertiary centre, where intrapartum prophylactic antibiotics are administered early. In the USA, the CDC has reported that by using the strategy of maternal screening and the administration of intrapartum antibiotics, GBS infection among newborns has been reduced from 1.7-1.9 per 1,000 live births in the early 1990s, to 0.34-0.37 per 1,000 newborns in 2008 (17). Yadeta (5) reported that all newborns exposed to IAP for ≥4 h were not colonised. However, newborns exposed to IAP for <4 h were colonised. The study by McNanley *et al* (18) demonstrated that the GBS vaginal colony counts decreased by 5-fold during 2 h of intravenous penicillin G administration and decreased by 50-fold within 4 h. Studies conducted in countries, such as Bangladesh and Eastern Ethiopia with limited access to IAP have reported vertical transmission rates as high as 38.0 and 45.02%, respectively (5,19).

Another reason for the low vertical transmission may be attributed to the small sample size of the present study. Other than IAP, Gizachew *et al* (20) reported that commitment to antenatal care follow-up was the most critical factor preventing vertical transmission, as pregnant mothers who attended four to five antenatal care visits throughout their pregnancy were 20.9% less likely to vertically transfer colonised GBS to their babies.

The demonstrated association of maternal GBS colonisation with preterm labour, highlights the importance of vaccination. In 2020, Procter *et al* (21) conducted a global analysis of 140 million pregnant women across 183 countries, and concluded that a GBS vaccination was successful in avoiding up to 4,07,000 preterm births.

In conclusion, although the present study found that vertical transmission can be controlled by the proper administration of IAP, babies of mothers with maternal colonisation had poor outcomes 5/8, (62.5% died), as GBS colonisation was significantly associated with A lower gestational age (which was significantly associated with neonatal mortality). Moreover, the present study effectively consolidated the association between maternal GBS and both DM and obesity. Therefore, women with DM and obesity require special attention for early detection, and therefore, the early management of their condition.

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**Availability of data and materials**

The data generated in the present study may be requested from the corresponding author.

**Authors' contributions**

LRAO collected the data and wrote the manuscript. NSA conceived and designed the analysis. Both authors have read and approved the final manuscript. LRAO and NSA confirm the authenticity of all the raw data.

**Ethics approval and consent to participate**

Informed consent was obtained from all participants prior to data collection. Verbal consent had been obtained from all participants prior to data collection. An official letter of approval (no. 338) was obtained from the Scientific Committee of the Scientific Council of Obstetrics and Gynecology-Iraqi Board for Health Specializations, in accordance with the Declaration of Helsinki.

**Patient consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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