DOI Link: https://doi.org/10.61586/ka0KA
Vol.410, Issue.11, Part.1, Nov 2025, PP.2-12

Group B Streptococcus screening program and antibiotic prophylaxis during childbirth in Armenia Hospitals

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Abbreviations
ACOG - American College of Obstetricians and Gynecologists
CDC - Centers for Disease Control and Prevention
EOD - Early-onset Disease
GBS - Group B Streptococcus
LOD - Late onset disease
IAP - Intra-partum antibiotic prophylaxis
NAAT - Nucleic acid amplification test

Received Feb 2025 Accepted Oct 2025 Published Nov 2025

ISSN: 0034-8082

Abstract

Background: *Group B Streptococcus (GBS)* is a gram-positive bacterium that colonizes the gastrointestinal and genitourinary tracts and is a leading cause of neonatal illness and death. This study aimed to assess GBS colonization in pregnant women and their antibiotic sensitivity patterns. **Methods:** The research was conducted at the Research Center of Maternal and Child Health Protection in Yerevan, Armenia. The study comprised two components: prospective microbiological research with antibiotic susceptibility testing involving 857 patients and a retrospective study at the neonatal department of Yerevan State Medical University Muracan Hospital involving 2,216 newborns. **Results:** The study included women aged 19–42 years, with a GBS colonization rate of 16.1% (138 out of 857). The average gestational age at childbirth was 39 1/7 weeks. Among natural births, 67.6% occurred between 38–40 weeks (28.4% at 38 weeks, 21.6% at 39, and 17.6% at 40), while 12.1% occurred after 41 weeks. Since GBS screening is most accurate within 5 weeks of delivery, adjusting the screening in Armenia from 35–37 weeks to 36 0/7–37 6/7 weeks would extend predictive accuracy up to 41 0/7 weeks. Although Penicillin G is the preferred prophylactic, it is unavailable in Armenia; thus, Ampicillin or Ceftriaxone is used. Antibiotic sensitivity tests conducted during the study revealed that (50%) of GBS isolates were sensitive to Ampicillin. Notably, GBS isolates demonstrated (78%) sensitivity to Amoxicillin. Additionally, they exhibited high sensitivity to Ceftriaxone (76%), Cefuroxime (83%), Norfloxacin (68%). (39%) of GBS exhibited intermediate susceptibility to Erythromycin, (27%) to Vancomycin, and (28%) to Ampicillin. (13%) of GBS isolates showed intermediate susceptibility to Cefuroxime, (22%) to Ceftriaxone, (15%) to Norfloxacin, and (7%) to Clindamycin. The majority of GBS isolates demonstrated resistance to Vancomycin (67%), while a considerable number were also highly resistant to Clindamycin (61%) and Erythromycin (41%). Notably, resistance to Erythromycin frequently co-occurred with Clindamycin resistance, though not in all cases. Additionally, (17%) to the analyzed samples showed resistance Norfloxacin and (12%) to Amoxicillin. **Conclusion:** The high prevalence of GBS colonization in pregnant women demands for screening in women attending an antenatal clinic so that intrapartum antimicrobial prophylaxis can be offered to all women who are colonized with GBS, thus prevents its transfer to the newborn.

Keywords: Group B Streptococcus (GBS), perinatal infection, vaginal colonization, rectal colonization, pregnancy, early onset GBS, prevention of GBS, antibiotics prophylaxis.

Introduction:

Group B Streptococcus (GBS), also known as *Streptococcus agalactiae*, is a gram-positive bacterium that colonizes the gastrointestinal and genitourinary tracts. GBS is recognized as the leading infectious cause of morbidity and mortality in neonates. [5,10] It is responsible for both early-onset and late-onset neonatal infections, although current interventions are effective primarily in preventing early-onset (EOD) disease.

The primary means of preventing early-onset GBS (EOGBS) is through the use of intra-partum antibiotic prophylaxis (IAP) for pregnant women who carry GBS. [6] Various prevention strategies are employed globally based on the identification of mothers at risk. Established risk factors include pre-labor rupture of membranes lasting more than 18 hours, an intra-partum fever of 38.0°C or higher, preterm birth (before 37 weeks of gestation), GBS bacteriuria during the current pregnancy, and a previous case of EOGBS infection in a sibling. [17]

Within the risk-based approach, all women displaying one or more established risk factors for EOGBS infection receive IAP. This strategy is straightforward for healthcare providers to implement. However, its impact is limited because more than 40% of babies who develop EOGBS are born to mothers who do not exhibit any risk factors during pregnancy or labor. [21]

In the screening strategy, a culture is collected during the 35-37 weeks of gestation, and all women found to be colonized with GBS receive IAP. Similar to the risk-based approach, healthcare providers can easily apply this strategy. Nevertheless, it faces two challenges: First, the laboratory's accuracy in detecting GBS in pregnant women is not consistent and contributes to a portion of EOGBS cases. [18, 21, 22] Second, a significant number of women receive IAP, which may result in potential adverse effects, including increased maternal and fetal exposure to antibiotics, concerns about antimicrobial resistance, trends in non-GBS neonatal sepsis, and maternal anaphylaxis. [21]

The combination strategy involves a culture taken at 35-37 weeks of gestation, and IAP is administered to women who are both colonized with GBS and have one or more established risk factors for EOGBS. This strategy ensures the most precise use of IAP and consequently leads to the lowest rate of adverse side effects.

There have been concerns about the possibility that intra-partum antibiotic prophylaxis may lead to an increase in infections caused by non-GBS organisms or trigger antibiotic resistance. [1] Studies examining this issue have reported conflicting results. While some studies found no evidence of an increase in early-onset neonatal infections caused by non-GBS organisms, [1, 11] others have reported an uptick in *Escherichia coli* infections among infants with low and very low birth weights. [4,19] Some reports have also indicated an increase in the resistance of *E. coli* to ampicillin, particularly among premature infants. [1]

Screening:

Universal screening conducted at 35 to 37 weeks of gestation to identify maternal Group B Streptococcus (GBS) colonization and the subsequent use of intrapartum antibiotic prophylaxis have led to significant reductions in the

incidence of EOGBS disease in newborns. Notable changes in the latest guidelines from the American College of Obstetricians and Gynecologists (ACOG) now recommend antepartum screening for GBS between 36 0/7 to 37 6/7 weeks of gestation (36 weeks and 0 day to 37 weeks and 6 days), a shift from the previous 2010 CDC consensus guidelines suggesting screening between 35-37 weeks of gestation. This change is prompted by the fact that around 7% of births in the United States occur after 41 weeks of gestation. The updated recommendation of waiting until at least 36 0/7 weeks of gestation expands the window for predicting screening culture results up to 41 0/7 weeks. For the most accurate prediction of GBS colonization status at birth, it is advisable to collect GBS screening specimens within 5 weeks prior to delivery. [12]

Key Aspects of Intrapartum Antibiotic Prophylaxis Agents and Dosage:

Penicillin remains the preferred choice for intrapartum antibiotic prophylaxis, with ampicillin as a suitable alternative (AI). Women with penicillin allergies who have not experienced severe reactions such as anaphylaxis, angioedema, respiratory distress, or urticaria after penicillin or cephalosporin administration should be administered cefazolin (BII). [7]

For penicillin-allergic women at high risk of anaphylaxis due to a history of severe reactions, such as anaphylaxis, angioedema, respiratory distress, or urticaria following penicillin or cephalosporin use, antimicrobial susceptibility testing should be performed on antenatal GBS cultures (AII).

According to the 2019 ACOG guidelines, women with non-severe penicillin allergies can be treated with first-generation cephalosporins, including cefazolin. In cases of severe penicillin allergy, clindamycin is the recommended prophylactic agent. Recent studies have reported increasing rates of resistance to both erythromycin and clindamycin, with reports of 15% to over 40% clindamycin-resistant isolates. Penicillin-allergic women at high risk of anaphylaxis should receive vancomycin if their isolate is intrinsically resistant to clindamycin (as determined by antimicrobial susceptibility testing), if the isolate shows inducible resistance to clindamycin, or if susceptibility to both agents is unknown (CIII). [8,13,20]

The recommended dosing regimen for penicillin G is an initial intravenous dose of 5 million units, followed by 2.5 to 3.0 million units intravenously every 4 hours (AII). The range of 2.5 to 3.0 million units is recommended to ensure sufficient drug levels in fetal circulation and amniotic fluid while minimizing the risk of neurotoxicity. The specific dose within this range should be selected based on the availability of penicillin G formulations to reduce the need for compounding by pharmacies.

Erythromycin is no longer considered a suitable alternative for intrapartum GBS prophylaxis in penicillin-allergic women at high risk of anaphylaxis.

Aim:

In 2017, Armenia adopted guidelines for the prevention of Group B Streptococcus (GBS) infection during pregnancy. However, these guidelines have not been effectively implemented due to insufficient funding. In 2022-2024, a pilot

study was conducted at the Research Center of Maternal and Child Health Protection in Yerevan, Armenia. The study aimed to assess GBS colonization rates among pregnant women, evaluate the effectiveness of GBS screening, and determine the sensitivity and effectiveness of antibiotics in preventing GBS infection.

Methods:

The research was conducted at the Research Center of Maternal and Child Health Protection in Yerevan, Armenia. The study comprised two components: prospective microbiological research with antibiotic susceptibility testing involving 857 patients and a retrospective study at the neonatal department of Yerevan State Medical University Muracan Hospital involving 2,216 newborns. The retrospective study compared outcomes to a group of mothers who had not undergone GBS screening or received prophylactic antibiotics during labor. All patients admitted to the maternity home were in their 36-37th week of gestation. The entire process adhered to the guidelines provided by the Centers for Disease Control and Prevention (CDC) for the Detection and Identification of Group B Streptococcus (2021). Swab specimens were collected using a single swab, first from the lower vagina (near the introitus) by inserting the swab approximately two centimeters, and then from the rectum by inserting the same swab approximately one centimeter through the anal sphincter, without the need for a speculum. [12] GBS colonization often occurs at low bacterial cell concentrations, and vaginal-rectal swabs have been found to yield high bacterial concentrations. To maximize the likelihood of GBS recovery, a single swab was used to obtain specimens from both the vagina and rectum. Collection from the cervix, perianal area, perirectal area, or perineum was not considered acceptable, and a speculum was not used for culture collection. Vaginal-rectal specimens were collected using flocked swabs and placed in a liquid-based transport medium, such as Amies transport media. Flocked swabs are known to release microorganisms more efficiently than traditional fiber swabs. Traditional fiber-wrapped swabs, including Rayon, Dacron, and cotton swabs, have been historically used for culturing GBS from vaginal and rectal specimens. However, these types of swabs hinder the release of microorganisms, reducing GBS recovery. [12] Following vaginal-rectal specimen collection, swabs were immediately placed in Amies transport medium or an equivalent and transported to the microbiological laboratory within 24 hours. In cases where transport and/or culture were delayed, specimens were refrigerated in Amies transport medium at temperatures of 4-8°C, or swab-collected specimens were stored at room temperature. GBS screening specimens were incubated in selective enrichment broth before agar media plating or nucleic acid amplification testing (NAAT). Selective enrichment broth incubation is known to enhance GBS detection compared to non-selective broths, resulting in up to a 2.5-fold increase in GBS-positive screening. Selective broths contain components that inhibit or suppress the growth of enteric organisms and certain Gram-positive bacteria, such as Staphylococcus. The use of non-selective broth can lead to overgrowth of these organisms, making GBS detection challenging. Direct specimen plating without enrichment broth culture was considered unacceptable and was not performed. [2,9,16] For rapid GBS detection,

the ACRO BIOTECH Rapid test was employed, and the results were identified using ChromID CPS Elite clinical culture, based on the chromogenic characteristics of the bacterial colonies. Antimicrobial susceptibility testing was conducted on all GBS isolates from pregnant women using several antibiotics, employing the disk-diffusion method.

Results:

The study encompassed pregnant women aged between 19 and 42 years, with an average age of 29.7. GBS colonization was detected in 16.1% of the participants, accounting for 138 out of 857 women. When compared to CDC data from the USA, it is evident that the prevalence of GBS carriers in Armenia is 1.5 times lower than that in the USA (25%). Researchers have previously demonstrated that the overall percentage of GBS carriers varies between 6.5% and 36% among late-term pregnant women in diverse regions and ethnic groups. [3, 14, 15]

The monitoring results indicated that the average gestational age at childbirth for the studied women was 39 weeks and 1 day. Among women who underwent natural childbirth, 67.6% gave birth between 38 and 40 weeks of gestation (20.2% before 38 weeks, 28.4% at 38 weeks, 21.6% at 39 weeks, and 17.6% at 40 weeks). Approximately 12.1% of births occurred after 41 weeks of pregnancy. To enhance the accuracy of GBS colonization prediction at birth, it is advisable in Armenia to shift the recommended screening interval from 35-37 weeks to 36 0/7-37 6/7 weeks. This adjusted recommendation extends the timeframe for predicting screening results up to 41 0/7 weeks **(Fig. 1.).**

All newborns were delivered at full term, achieved an average Apgar score of 8/9, and were discharged on average after 4.72 days. Importantly, regardless of whether GBS-positive mothers received appropriate antibiotic prophylaxis during delivery, all babies were born healthy and without any signs of EOD.

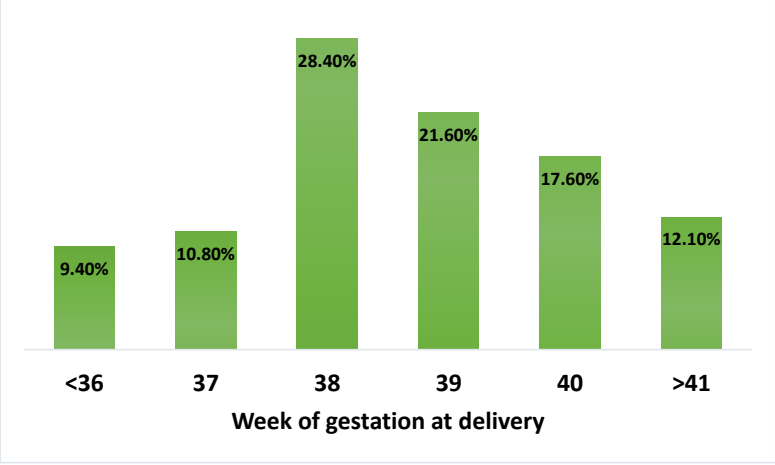

Fig 1. Distribution (%) of women by gestational week at the time of delivery

As mentioned previously, Penicillin G is the preferred drug for prevention. However, it is not imported into Armenia. Consequently, antibacterial prophylaxis for GBS-positive pregnant women was administered using Ampicillin or Ceftriaxone.

Antibiotic sensitivity tests conducted during the study revealed that (50%) of GBS isolates were sensitive to Ampicillin. Notably, GBS isolates demonstrated (78%) sensitivity to Amoxicillin. Additionally, they exhibited high sensitivity to Ceftriaxone (76%), Cefuroxime (83%), Norfloxacin (68%). (39%) of GBS exhibited intermediate susceptibility to Erythromycin, (27%) to Vancomycin, and (28%) to Ampicillin. (13%) of GBS isolates showed intermediate susceptibility to Cefuroxime, (22%) to Ceftriaxone, (15%) to Norfloxacin, and (7%) to Clindamycin. The majority of GBS isolates demonstrated resistance to Vancomycin (67%), while a considerable number were also highly resistant to Clindamycin (61%) and Erythromycin (41%). Notably, resistance to Erythromycin frequently co-occurred with Clindamycin resistance, though not in all cases. Additionally, (17%) to the analyzed samples showed resistance Norfloxacin and (12%) to Amoxicillin **(Fig.2).**

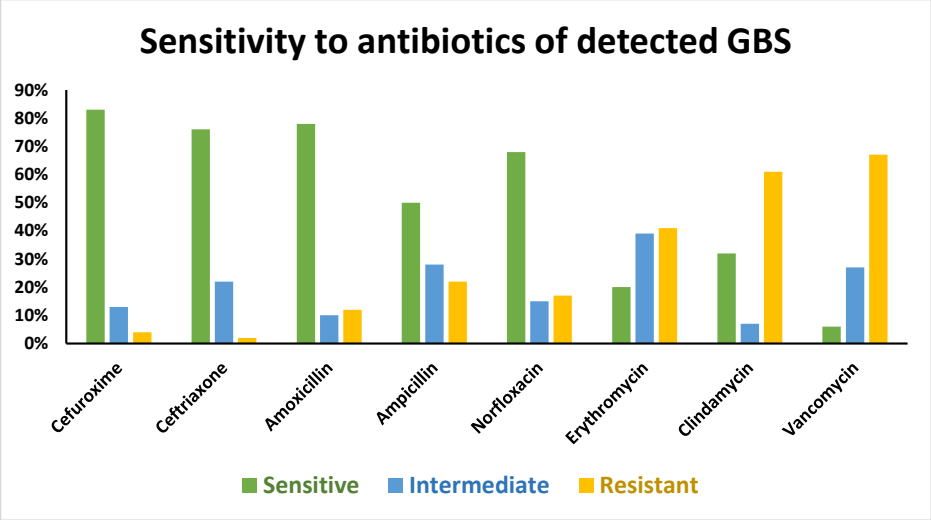

Fig 2: Sensitivity to antibiotics of detected GBS

Impact of GBS Prophylaxis Implementation: In 2021, prior to the implementation of GBS prophylaxis at the Research Center of Maternal and Child Health Protection in Yerevan, Armenia, there were 9.4 cases of EOD per 1000 births recorded. Among these cases, 3.9 resulted in death, accounting for 41% of the affected infants. After the introduction of GBS screening, the rate of early-onset

neonatal infection decreased to 5.3 cases per 1000 live neonates, with 2.1 of these cases ending in death, representing 40% of affected infants. Consequently, the implementation of screening led to a 56.3% reduction in EOD (a 1.8-fold decrease, $p < 0.05$), although the percentage of deaths among affected patients remained unchanged.

Neonatal Department Study: Another study conducted around 2021, prior to the implementation of screening, involved newborns exhibiting early and late onset symptoms from various maternity hospitals in Armenia who were transferred to Muracan Hospital. In the neonatal department of Muracan Hospital, a total of 2,216 newborns were admitted, out of which 637 were aged 0-7 days. Among these newborns, 99 were diagnosed with either early-onset (11%) or late-onset (89%) GBS disease.

Most cases (98%) presented with pneumonia, primarily manifesting as first and second-degree respiratory failure and broncho-obstructive syndrome. A few cases included sepsis (1 early-onset and 1 late-onset), and there was a single case each of meningitis and osteomyelitis. While cultures were not performed in many instances, the results of some cultures indicated the presence of GBS, *alpha hemolytic Streptococcus, S. aureus, and Klebsiella*.

Out of the 99 patients, 20 (20.2%) required admission to the Neonatal Intensive Care Unit and received antibiotic treatment (Amikaton and Cefotaxime). In high-risk sepsis cases, additional antibiotics (Gentamicin and/or Moxicin) were prescribed. Among the 135 patients, 49.6% were discharged from the hospital within 5 to 10 days, 47.4% were discharged within 10 to 20 days, and only 2.2% were discharged after 20 days. The shortest duration of hospital stay was 5 days. Notably, Muracan Hospital did not perform bacteriological examinations to differentiate the strains and evaluate treatment. It is possible that late-onset infections were attributed to medical interventions.

Conclusion:

1.Optimizing Screening Timing: The study highlights that approximately 12.1% of births in Armenia occur after 41 weeks of gestation. In light of this finding, it is recommended to adjust the recommended interval for GBS screening from 35-37 weeks to 36 0/7-37 6/7 weeks. This updated waiting recommendation effectively extends the predictive window for screening results up to 41 0/7 weeks, ensuring a more accurate assessment of GBS colonization.

2.Efficacy of Screening: The implementation of GBS screening, rather than relying solely on risk factor assessment, has significantly reduced the incidence of EOGBS disease in our unit. While there was no difference in the mortality rate of infants with sepsis, it's crucial to note that the absolute number of such cases has decreased. This outcome represents a positive achievement in the realm of neonatal care.

3.Emerging Drug-Resistant Strains: The study identified the presence of drug-resistant strains, particularly against Ampicillin, Erythromycin and Clindamycin. This trend, although rare in the literature, underscores the active circulation of drug-resistant bacteria in Armenia. Vigilance and further research into antimicrobial resistance are essential to combat this emerging challenge effectively.

4.Standardized Microbiological Examination: It is recommended that microbiological examination becomes a standard protocol for all suspicious cases, particularly those in neonatal intensive care units. Implementing such a standardized algorithm for patient management can enhance the early detection and management of infectious cases, ultimately improving neonatal healthcare outcomes.

Limitation of the study.

Limited Clinic Coverage: The research was conducted exclusively in two healthcare facilities, which does introduce some limitations in terms of its generalizability to the entire nation. However, it's worth noting that these two clinics are recognized as leaders in the country within their respective fields and provide medical care to a significant portion of the population.

Small Sample Size: The relatively small number of subjects included in the study may restrict the ability to draw robust evidence-based conclusions. Nevertheless, it's essential to acknowledge that this study serves as a valuable pilot investigation, laying the groundwork for further research. As a result, it can be recommended for replication and expansion to other healthcare facilities throughout the country.

Contributors Manukyan R.R., Saroyan G.E., Ohan G.G, Ugujyan T.P., Simonyan K.H., Avetisyan N.N., Arustamyan K.K. and Ter-Stepanyan M.M. conceptualised the study. Manukyan R.R., Saroyan G.E. and Avetisyan N.N. were responsible for developing the study method and performing data visualisation. Ohan G.G and Ugujyan T.P. performed data cleaning and transformation. Manukyan R.R., Saroyan G.E., Ohan G.G and Ugujyan T.P. did the data analyses, including statistical analysis and machine learning. Manukyan R.R., Saroyan G.E. and Avetisyan N.N. were involved in the investigation. Manukyan R.R., Saroyan G.E., Ohan G.G, Ugujyan T.P., Simonyan K.H., Avetisyan N.N., Arustamyan K.K. and Ter-Stepanyan M.M. were involved in data interpretation. Simonyan K.H., Arustamyan K.K. and Ter-Stepanyan M.M.supervised the study implementation. Administrative and material support was provided by Simonyan K.H., Avetisyan N.N., Arustamyan K.K. and Ter-Stepanyan M.M. Funding was acquired by Simonyan K.H., Avetisyan N.N., Arustamyan K.K. and Ter-Stepanyan M.M.. Manukyan R.R. and Saroyan G.E., prepared the original draft of the manuscript with critical revision of the manuscript for important intellectual content provided by Ohan G.G and Ugujyan T.P. All authors reviewed and approved of the final version of the manuscript. All authors had full access to the raw data in the study. Simonyan K.H., Avetisyan N.N., Arustamyan K.K. and Ter-Stepanyan M.M. accessed and verified the data. All authors had final responsibility for the decision to submit for publication.

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