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# Prevalence of Group b Streptococcus, Its Associated Factors and Antimicrobial Susceptibility Pattern Among Pregnant Women Attending Antenatal Care at Arbaminch Hospital, South Ethiopia

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**Abstract:** Background: Group B Streptococcus colonization of the gastrointestinal and genital tracts of pregnant women usually remains asymptomatic; even if it is the critical determinant of infection in neonates and young infants. It causes early and late onset of invasive Group B Streptococcus (GBS) disease manifesting as septicemia, meningitis and pneumonia. Now it is recognized as an important cause of maternal and neonatal morbidity and mortality in many parts of the world including Ethiopia where the magnitude of the problem has been little studied. Objectives: The aim of this study was to determine the prevalence of GBS colonization, to identify associated risk factors and antimicrobial susceptibility pattern of GBS isolates among pregnant women attending antenatal care at Arbaminch General Hospital, Arbaminch, Ethiopia. Methods: A cross sectional study was conducted from March - July, 2016 among 281 pregnant women on their antenatal care (ANC) visit at Arbaminch General Hospital (AGH). Consented participants' information was collected using structured questionnaire. Recto-vaginal swab samples were collected by consecutive sampling technique and inoculated directly onto 5% sheep blood agar (SBA) for isolation of GBS. Antimicrobial susceptibility testing was performed according to the clinical and laboratory standard institute (CLSI) guideline, 2014 by disk diffusion method. Data was coded and entered into EPidata version 3.1 and analyzed by SPSS version 21.0. Bivariate and Multivariate logistic regression analysis were used to ascertain the association between explanatory and outcome variable considering p-value <0.05. Result: The colonization rate of GBS among pregnant mothers was 8.5%. The overall recto-vaginal GBS colonization in this study was not significantly associated with any of socio-demographic and obstetric factors. All of the GBS isolates were susceptible to penicillin, ampicillin and vancomycin. Resistance to ciprofloxacin, ceftriaxone, clindamycin, erythromycin, chloramphenicol and gentamycin was found to be 37.5%, 29.2%, 29.2%, 20.8%, 8.3%, and 4.2%, respectively. From a total of twenty four GBS isolates, two showed multidrug resistance. Conclusion and recommendation: This study found that GBS colonization rate was rationally high and most isolates were resistant to the commonly used antibiotics.

**Keywords:** Group B Streptococcus, Recto-vagina, Antibiotic Susceptibility, Arbaminch

## 1. Background

Group B Streptococci (GBS) are part of the normal flora of the mucous membranes of humans, mainly colonizing gastrointestinal and genitourinary tracts [1]. The colonization of these regions is a risk factor for subsequent infection in

pregnant women and newborns [2]. The prevalence of GBS isolation is highest in the rectum, intermediate in the vagina, and lowest in the cervix. A combination vaginal-rectal culture is now recommended to detect GBS in pregnant women [1, 3].

Group B Streptococcus causes invasive disease primarily in infants, pregnant or postpartum women, older adults, and immunocompromised peoples with the highest incidence among young infants [4, 5]. Many adults are asymptomatically colonized with GBS in the genital and gastrointestinal tracts but colonized pregnant women are at increased risk of adverse obstetric outcomes, premature delivery and perinatal transmission to their neonates. Furthermore, GBS is one of the main causes of infection in pregnant women with cystitis, chorioamnionitis, endometritis, genitourinary tract and surgical wound infection. Genital infection is responsible for almost one-third of preterm deliveries, and it produce protease activity resulting in cervical ripening [6, 7].

Group B *Streptococcus* is also known to infect the newborn and hence increase the neonatal morbidity and mortality. In pregnancy, GBS can infect the amniotic fluid, and the neonates get colonized with it by aspiration of infected amniotic fluid or by vertical transmission during the passage through colonized vaginal canal and later on also from the hospital environment or through breast feeding, leading to neonatal sepsis and meningitis. Maternal Intrapartum GBS colonization is the most important risk factor for developing disease in the newborn. Of neonates that are colonized, 1–3% develops disease caused by group B *Streptococci*. This infection is associated with two distinct clinical syndromes. The first one referred to as early-onset disease, i.e. disease appearing in their first week of life (age 0–6 day, mainly (90%) in the first 12 hours), which is a leading cause of invasive bacterial infection among newborns. The other is late-onset disease that occurs at the age of 7–89 days [3, 8, 9].

In the recent decade, Group B Streptococcus (GBS) has been one of the common causes of the early onset of sepsis among the newborns, which leads to high rate of morbidity and mortality. Infection by this organism may result in neonatal death due to severe neonatal infections such as septicemia, meningitis and pneumonia with a mortality rate of 10 - 20% [10, 11].

Despite decrease in mortality during the last decades, Early-Onset Group B Streptococcal Disease (EOGBSD) remains a serious neonatal condition, which may result in severe neurological damage [12, 13]. Population-based surveys of bacteremia have raised concerns about the growing incidence of GBS disease in neonates [14]. Because the colonization of this microorganism is common among pregnant women; there is a need of having sufficient data on the prevalence of GBS colonization, its associated risk factors and antimicrobial susceptibility pattern of the isolates. Therefore the present study was conducted in attempt to expand on data regarding the problem in the study area and contribute to the solution.

# 2. Methods

### *2.1. Study Setting and Design*

The study was conducted at Arba Minch General hospital in Arba Minch town from March to July, 2016. Arba Minch town is found in Gamo Gofa zone, South Nations, Nationalities' and Peoples' Region (SNNPR); Southern Ethiopia. The town is 505 km to South of the Ethiopian capital city Addis Ababa and 275 km south west of Hawassa, the regional capital. The total area of the town is estimated about 1095 hectares and it lies at an altitude of 1300 meters above sea level, its average temperature is 29°C and the average annual rainfall is 900 mm. Arba Minch hospital is a general hospital originally built to house 50 beds but has now expanded to 300 beds and serving a population of two million. The Hospital provides general outpatient service, emergency, surgical, ophthalmological, dental, psychiatric, obstetric, fistula, Internal medical, and pediatric-neonatal as well as leishmania in-patient services. It was recorded that a total of 4,070 pregnant women attended the antenatal clinic and 3,428 deliveries were attended in the year 2008 E. C [15]. A hospital based cross sectional study design was conducted

### *2.2. Sample Size and Sampling Procedure*

Sample size was calculated by taking the prevalence of colonization rate (p=20.86%) which was indicated in the previous study in 2010 in Hawassa, South Ethiopia [16]. Expected margin of error (d) is 0.05 and confidence interval (z) is 95%. It was calculated by using a single population proportion formula $n= (Z\alpha/2)^2\ p\ (1-p)/d^2$ and considering 10% non-response rate and the total sample calculated was 281.

Sampling Procedure

All consecutively identified pregnant women in 35-37 weeks of gestation period attending routine antenatal clinics at Arbaminch general hospital during the study period who fulfilled the inclusion criteria were enrolled.

### *2.3. Data Collection Procedure*

The data on socio-demographic variables and other relevant informations were collected by using predesigned and pretested structured questionnaire and by reviewing medical records. The questionnaire was adapted from other similar studies and initially prepared in English and was translated to Amharic and then translated back to English by other translator to check for consistency. Informed consent was obtained from each study participants after explaining the purpose and procedure of the study.

#### *2.3.1. Recto – vaginal Swab Collection*

Two swab samples were collected from each woman by using two different sterile Dacron swabs (Medical Wire and Equipment, USA); one swab from the lower vagina (vaginal introitus) and the other from the rectum (i.e., by inserting swab through the anal sphincter). The swabs were collected

by the attending midwife and nurses and transported immediately by Amies transport media to Arba-Minch general hospital microbiology laboratory for inoculation to 5% SBA and for further analysis, with in maximum of 4-6 hours [10, 17].

### *2.3.2. Culturing, Isolation and Laboratory Identification of GBS*

The swab samples were inoculated directly into 5% SBA (Oxoid England) supplemented with 8 µg/ ml gentamicin (CSPC Ouyi pharmaceutical co., Ltd) and was incubated aerobically by using candle jar at 37°C for 24 hours. When there were no colonies over 24hours, re-incubation for an additional 24 hours was done, before discarding the plate as negative.

Colonies were presumptively identified as GBS by colony morphology and hemolytic activity on sheep blood agar plates (grey mucoid colonies, surrounded by a small zone of beta-haemolysis) and typical streptococcal morphology on Gram stain. For confirmation, colonies from the screening BA plates were sub cultured onto nutrient agar (Oxoid, England) and defined as GBS on the basis of catalase negative reaction, bacitracin resistance and CAMP test [18]. (Figure 1) Subcultures that are negative after the 1st incubation should be incubated again overnight and re-examined.

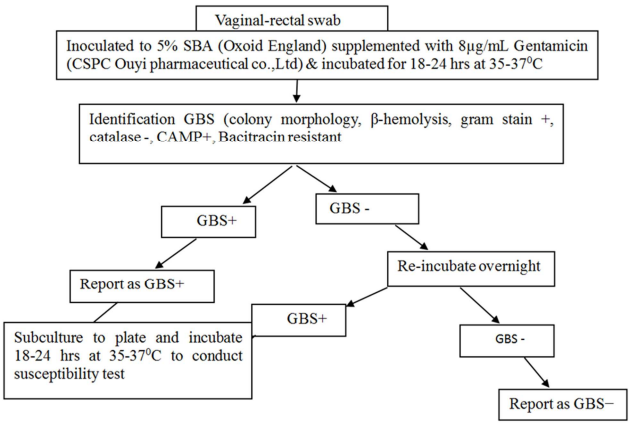


***Figure 1.*** *A Flow chart diagram showing Culturing, isolation and Laboratory identification of GBS.*

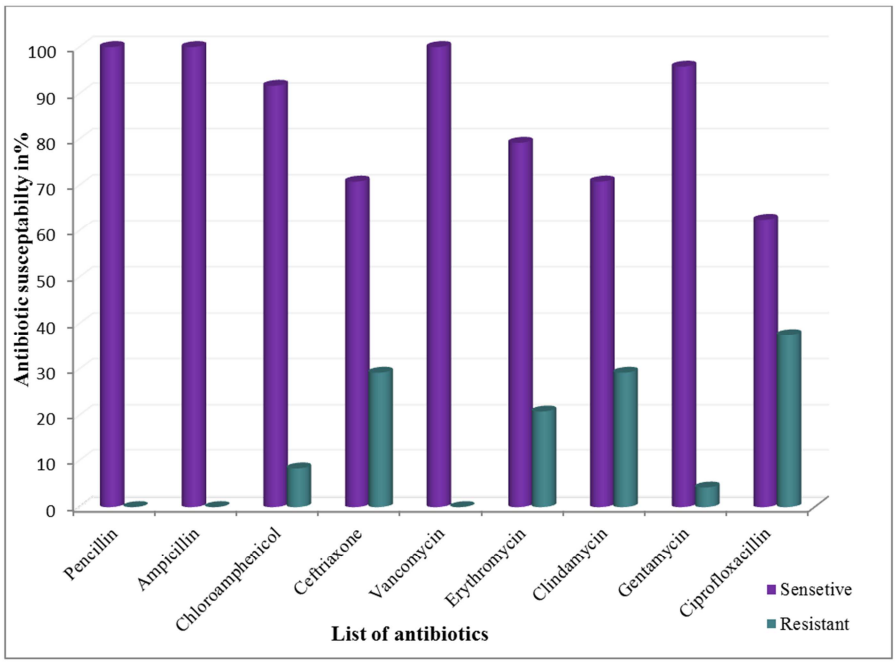


***Figure 2.*** *Bar graph showing antimicrobial susceptibility pattern of GBS isolates from pregnant women attending ANC in AGH from March to July, 2016 (n=24).*

### *2.3.3. Antimicrobial Susceptibility Testing*

Antimicrobial susceptibility testing was performed according to Clinical and Laboratory Standard Institute Guidelines (CLSI) 2014 for disk diffusion [19]. A suspension of the test organism was prepared by removing 3-5 colonies from a pure culture plate by emulsifying in 3 ml of sterile physiological saline and was diluted with saline until the turbidity of the suspension become matched with turbidity standard equivalent to 0.5 McFarland and inoculated on Muller-Hinton agar (MHA) with 5% sheep's blood using a sterile cotton swab. After the excess suspension was removed by gentle rotation of the swab against the surface of the tube, the swab was then used to distribute the bacteria evenly over the entire surface of Mueller Hinton agar (Oxoid, England) supplemented with 5% sheep blood.

The inoculated plates were left at room temperature to dry for 3-5 minutes and a set of 6 antibiotic discs in each plate were placed with the concentration of penicillin (P) (10µg), ampicillin (AMP) (10µg), erythromycin (E) (15µg), clindamycin (DA) (2µg), vancomycin (VA) (30 µg), ceftriaxone (CRO) (30 µg), gentamicin(CN) (10 µg), Chloramphenicol (C) (30µg), ciprofloxacin (CIP) (5µg) [20, 19] (All of the antibiotics are product of Oxoid, England and

HIMEDIA) used in the investigation. Clindamycin and erythromycin antibiotic disks placed 12 mm from each other in order to detect inducible resistance to clindamycin (D-zone test) and incubated at 35-37 °C with 5% $CO_2$ atmosphere by candle jar for 18-24 hours. The zone of growth inhibition was measured using rulers. The sizes of the inhibition zones was graded according to the CLSI 2014 and interpreted as susceptible, intermediate or resistant [19].

Data were collected using self-administered questionnaires. The data collection tool was adapted from the literature on maternal health surveys [22, 2, 21, 1, 4]. The tool contained four sections which assessed socio-demographics of HCWs, knowledge and perceptions of HCW on pediatric emergency triage, factors associated with quality of pediatric emergency triage as to HCWs perspective, and observation checklists for facility visits. Data collectors and supervisors with a nursing background were hired and given four days training on data collection techniques and study objectives. The triage material and physical assessment were done via the use of a checklist on basic triage equipment, medicines and consumables (glucometer, IO needle, IV /rectal diazepam) as well as triage assessment forms, triage guidelines, sick child flow charts, the presence of a separate triage area for children and whether or not pediatric-specific treatment algorithms were present.

### *2.4. Data Quality Management*

Two days training was given to the data collectors on the purpose of study, study participants selection, on the questionnaire, how to get informed consent, and on swab collection and processing. Properly designed data collection tools and protocol manuals were used. Every day the collected data was cross checked for completeness, consistency and on site correction action was taken. Standard operating procedures (SOPs) were followed during sample collection, transportation, and processing steps and protocols were followed strictly. Stored isolates were sub-cultured before use. Well-characterized Standard American Type Culture Collection (ATCC) reference strain of *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922), *S. agalactiae* isolates (ATCC 12386) were used to check the quality of the culture media and antimicrobial disks, which were obtained from SNNPR regional laboratory, Hawassa. Quality controls including selection of satisfactory reagents, preparations, sterility and performance of media checked according to specific manufacturer's instructions

The data were coded, edited and entered into Epi-data version 3.01, cleaned and analyzed by SPSS for windows version 20.

### *2.5. Data Analysis*

After checking the data for completeness and missing values, it was coded, entered into EPidata version 3.1 and analyzed using SPSS statistical software version 21.0. Proportions were calculated for categorical variables and summaries were presented in terms of counts and percentages. Explanatory variables were individually cross tabulated with the outcome variable and statistical significance was assessed using logistic regression model. Odds ratio (OR) and 95% confidences interval (CI) were calculated to determine the strength of association. P-value less than 0.05 were considered statistically significant.

### *2.6. Ethical Issues*

Ethical clearance was obtained from Jimma Institutional Review Board (IRB). Written permission was obtained from Gamo-Gofa zone health department and Arba Minch general hospital administration. During data collection all respondents were asked their permission and informed consent was obtained from each study participants. In addition, the clinical specimens collected during the study period were used for the stated objectives only and pregnant women who are colonized by GBS were linked to the health professionals in the hospital in charge for possible intervention

### *2.7. Operational Definitions*

Colonization: the presence and multiplication of microorganisms without tissue invasion or damage

Contraceptive use is women who had ever used a contraceptive method to delay or prevent pregnancy

Lancefield grouping: is a method of grouping catalase and coagulase-negative bacteria based on the carbohydrate composition of bacterial antigens found on their cell walls

Multi drug resistance: resistant to three or more antimicrobial classes [23].

Resistant: Isolates that are resistant or intermediate resistant to antimicrobial categories [24]

## 3. Result

### *3.1. Socio Demographic Characteristics of the Subjects*

A total of 281 pregnant women in gestational age of 35-37 weeks were participated in this study. The response rate was 100%. Of the 281 pooled samples cultured, 24 (8.5%) were positive for GBS.

The age of the study participants ranged from 15 to 40 years with a mean age of 25.64 and standard deviation of + 4.42 years. Majority of the study participants 115 (40.9%) were in the age group of between 25-29 years. Most of the study participants 265 (94.3%) were married and large proportion of the study participants 208 (74%) were urban residents. Most of the study participants' ethnic group was Gamo 189 (67.3%) and almost half of the study participants were house wives 133 (47.3%) concerning their occupational status. Majority of the study participants have the educational status of Elementary school 81 (28.8%), and high Grade (College or University) 80 (28.5%). (Table 1)

***Table 1.*** *Socio demographic Characteristics of pregnant women.*

| Variables | Category | Frequency | Percent (%) |
|---|---|---|---|
| Age (in years) | 15-19 | 20 | 7.1 |
| | 20-24 | 88 | 31.3 |
| | 25-29 | 115 | 40.9 |
| | 30-34 | 48 | 17.1 |
| | ≥ 35 | 10 | 3.6 |
| Ethnicity | Gamo | 189 | 67.3 |
| | Gofa | 16 | 5.7 |
| | Amhara | 35 | 12.5 |
| | Wolaita | 13 | 4.6 |
| | Others | 28 | 10 |
| Marital status | Single | 14 | 5 |
| | Married | 265 | 94.3 |
| | divorced / separated | 2 | 0.7 |
| Residence | Urban | 208 | 74 |
| | Rural | 73 | 26 |
| Occupation | Civil servant | 64 | 22.8 |
| | Student | 25 | 8.9 |
| | Farmer | 8 | 2.8 |
| | House wife | 133 | 47.3 |
| | Merchant(Business women) | 46 | 16.4 |
| | Daily Laborer | 5 | 1.8 |
| | Unable to read and write | 46 | 16.4 |
| Educational Status | Elementary (1–8) | 81 | 28.8 |
| | Secondary (9–12) | 74 | 26.3 |
| | High grade (College and University) | 80 | 28.5 |

### *3.2. Clinical and Obstetric Data of ANC Attendants*

Great majority of the study participants 200 (71.2%) were in their multigravida. Among these 87 (31%), 50 (17.8%), 29 (10.3%), 22 (7.8%), 8 (2.8%), 3 (1.1%) and 1 (0.4%) were in their second, third, fourth, fifth, sixth, seventh and ninth gravida respectively. The parity of the women ranged from zero to six. Of the 281 study participants 100 (35.6%) were multipara. Fifteen (5.3%) and Ten (3.6%) of the study participants have history of premature child birth and history of PROM respectively.

History of abortion was reported from 52 (18.5%) of the study participants. Among this 41 (78.8%), 9 (17.3%) and 2 (3.8%) mothers experienced abortion once, twice and three times respectively in their life. From the total of 281 participants only 19 (6.8%) had a history of still birth or neonatal loss. Regarding their gestational age of pregnancy, most of the study participants were in their 36$^{th}$ 100 (35.6%) and in their 35$^{th}$ 96 (34.2%) weeks of gestation during the study period. More than half of the study participants 172 (64.9%) attended ANC four times.

From the total of 281 study participants 31 (11%) used antibiotics in the two week time of their enrollment on our study. Among these 9 (29%) used Amoxicillin, 6 (19.4%) used HAART, 3 (9.7%) used Ciprofloxacin, while the rest used combination of different antibiotics. More than half of the study participants 163 (58%) had a history of Contraceptive use. Of which 102 (62.6%) used Injectable, 25 (15.34%) used implant, 10 (6.1%) used oral contraceptives (OCs), 7 (4.3%) used both injectable and implant, 3 (1.84%) used both injectable and OCs, while Loop/IUCD, both implant and OCs, and all contraceptives except loop were each used by 2 (1.2%) of the contraceptive users among the study participants. Fifty one (18.1%) of the study participants has been diagnosed of having UTI during pregnancy. Fourteen (5%) of the study participants were known to be diabetic and Ten (3.6%) of participants were positive for HIV/AIDS. (Table 2)

***Table 2.*** *Clinical and/obstetric features of pregnant women at 35-37 weeks of gestation, who were investigated for GBS in AGH, from March to July, 2016.*

| Variables | Category | Frequency | Percent (%) |
|---|---|---|---|
| History of Gravida | Primigravida | 81 | 28.8 |
| | Multigravida | 200 | 71.2 |
| Parity | Nullipara | 91 | 32.4 |
| | Primipara | 89 | 31.7 |
| | Multipara | 100 | 35.6 |
| | Other | 1 | |
| History of premat. child birth | Yes | 15 | 5.3 |
| | No | 266 | 94.7 |
| History of PROM | Yes | 10 | 3.6 |
| | No | 271 | 96.4 |
| History of previous Abortion | Yes | 52 | 18.5 |
| | No | 229 | 81.5 |
| still birth or Neonatal loss hist. | Yes | 19 | 6.8 |
| | No | 262 | 93.2 |
| Gestational age in weeks | 35 week | 96 | 34.2 |
| | 36 week | 100 | 35.6 |
| | 37 week | 85 | 30.2 |
| Number of prenatal visit | First | 5 | 1.9 |
| | Second | 23 | 8.7 |
| | Third | 65 | 24.5 |
| | Fourth | 172 | 64.9 |
| | Other | 16 | 5.7 |
| Recent use of any antibiotic R | Yes | 31 | 11 |
| | No | 248 | 88.3 |
| | Other | 2 | |
| Contraceptive use history | Yes | 163 | 58 |
| | No | 118 | 42 |
| Dx. Of UTI during pregnancy | Yes | 51 | 18.1 |
| | No | 230 | 81.9 |
| Being Diabetic | Yes | 14 | 5 |
| | No | 267 | 95 |
| Recent HIV status | Positive | 10 | 3.6 |
| | Negative | 270 | 96.1 |
| | Other | 1 | |

### *3.3. Prevalence of Group B Streptococci*

The overall prevalence of GBS colonization among pregnant women's participated in our study at 35-37 weeks of gestation was found to be 8.5% (24/281). Two hundred eight of the study participants were urban residents, from which 16 (7.7%) were positive for GBS and the remaining Seventy three were rural residents, among these 8 (11%) were positive for GBS.

### *3.4. Factors Associated with GBS Colonization*

#### *3.4.1. Socio-demographic Factors*

The table below (Table 3) summarized the rate of GBS

colonization by socio-demographic characteristics. The Group B streptococcal colonization rate was higher among pregnant mothers in the age group of 20-24 years (10.2%) and lower in the age group of 15-19 years (5%). However, the difference was not statistically significant (p>0.05).

***Table 3.*** *Bivariate analysis of the association between socio-demographic factors and GBS colonization among pregnant women attending ANC in AGH, from March to July, 2016 (n=281).*

| variables | GBS result | | OR (95% C. I) | P-value |
|---|---|---|---|---|
| | Positive (%) | Negative (%) | | |
| Age group | | | | |
| 15-19 | 1 (5) | 19 (95) | 1.00 | |
| 20-24 | 9 (10.2) | 79 (89.8) | 0.46 (.055-3.87) | 0.476 |
| 25-29 | 10 (8.7) | 105 (91.3) | 0.55 (.067-4.57) | 0.582 |
| 30-34 | 3 (6.3) | 45 (93.8) | 0.79 (.077-8.08) | 0.842 |
| ≥35 | 1 (10) | 9 (90) | 0.47 (.027-8.46) | 0.611 |
| Ethnicity | | | | |
| Gamo | 18 (9.5) | 171 (90.5) | 1.00 | |
| Gofa | 1 (6.3) | 15 (93.8) | 1.6 (.197-12.66) | 0.667 |
| Amhara | 4 (11.4) | 31 (88.6) | 0.816 (.26-2.57) | 0.728 |
| Wolaita | 0 (0.0) | 13 (100) | 0.22 (0.032-12.7) | 0.96 |
| Others | 1 (3.6) | 27 (96.4) | 2.84 (.36-22.17) | 0.319 |
| Marital status | | | | |
| Single | 1 (7.1) | 13 (92.9) | 1.00 | |
| Married | 23 (8.7) | 242 (91.3) | 0.81 (.10-6.47) | 0.842 |
| divorced / separated | 0 (0.0) | 2 (100) | 0.7 (0.069-14.3) | 0.91 |
| Residence | | | | |
| Rural | 8 (11) | 65 (89) | 1.00 | |
| Urban | 16 (7.7) | 192 (92.3) | 1.48 (.604-3.61) | 0.393 |
| Occupation | | | | |
| Merchant(Business women) | 4 (8.7) | 42 (91.7) | 1.00 | |
| Civil servant | 8 (12.5) | 56 (87.5) | .667 (.188-2.36) | 0.530 |
| Student | 1 (4) | 24 (96) | 2.28 (.24-21.64) | 0.471 |
| Farmer | 0 (0.0) | 8 (100) | 0.47 (0.09-17.1) | 0.93 |
| House wife | 11 (8.3) | 122 (91.7) | 1.06 (.32-3.496) | 0.929 |
| Daily Laborer | 0 (0.0) | 5 (100) | 0.49 (0.51-9.74) | 0.89 |
| Educational Status | | | | |
| Unable to read and write | 3 (6.5) | 43 (93.5) | 1.82 (.47-7.08) | 0.39 |
| Elementary (1–8) | 7 (8.6) | 74 (91.4) | 1.34 (.474-3.8) | 0.581 |
| Secondary (9–12) | 5 (6.8) | 69 (93.2) | 1.75 (.56-5.48) | 0.337 |
| High grade completed | 9 (11.3) | 71 (88.8) | 1.00 | |

In this study, the highest GBS colonization rate was detected from married pregnant mothers (8.7%). The difference in GBS colonization rate based on marital status was not statistically significant (p>0.05). Regarding residence, rural residents have higher GBS colonization rate (11%) than pregnant mothers who live in urban area (7.7%). However, the difference was not statistically significant (P > 0.05). On the basis of occupation, in our study civil servants have higher GBS colonization rate (12.5%), followed by merchant/business women (8.7%). No GBS detected among pregnant mothers who were farmers and daily laborers. However, the difference in GBS colonization rate based on the occupational status was not statistically significant (p>0.05).

In the present study, even if the difference was not statistically significant, GBS colonization rate was found to be higher among pregnant mothers who have had educational status of high grade (college and university) (11.3%) and followed by elementary school (8.6%). Generally, in this study there was no statistically significant association observed between socio-demographic factors and GBS colonization rate, in bivariate analysis.

### *3.4.2. Obstetric/or Clinical Factors Association with GBS Colonization*

The association between GBS colonization rate and maternal obstetric and /clinical factors is summarized in Table 4 below. Variables candidate for multivariate logistics regression were selected by considering p<0.25 from bivariate model. In this view some of the maternal obstetric and /clinical factors, such as parity, number of prenatal visit, history of abortion and history of contraceptive use were selected from bivariate analysis. Whereas, in a multivariate analysis none of them showed significant association (p>0.05). Maternal obstetric and/ clinical factors other than them were also not significantly associated statistically with GBS colonization rate.

Regarding gravidity, the GBS colonization rate was almost the same in both primigravida (8.6%) and multigravida (8.5%) mothers. Based on parity, nullipara had higher GBS colonization rate (11%) than multipara (9%) and primipara (5.6%). However, the difference was not statistically significant (p>0.05). This study indicated that pregnant mothers with previous history of PROM has slightly higher rate of GBS colonization (10%) than pregnant mothers who

had no history of PROM (5.9%); even if the difference was not statistically significant (P > 0.05).

In our study, although it was not significantly associated (p>0.05), maternal GBS colonization was about four fold higher in mothers with history of abortion than those without history of abortion (15.4% vs 3.9%). Pregnant women who had previous history of still birth or neonatal loss has somewhat higher rate of GBS colonization (10.5%) than those with no history of still birth or neonatal loss (5.7%). However, this difference was not statistically significant (P > 0.05). Group B streptococcal colonization rate was higher in those pregnant mothers who visited ANC once (40%) than those visited four times (11.6%), twice (4.3%) and three times (0.0%) during pregnancy in this study. But, the difference was not statistically significant (P > 0.05).

On the basis of history of recent use of any antibiotic treatment, the GBS colonization rate was higher among pregnant mothers who had no history of antibiotic treatment (8.9%) than those who had history of antibiotic treatment (6.5%); even if the difference was not statistically significant(p>0.05). In this study pregnant mothers who had no history of any contraceptive use were colonized by GBS largely (11%) than those mothers who had a history of contraceptive use (6.7%). However, the difference was not statistically significant (p>0.05).

It was showed that in the current study, pregnant women who had not been diagnosed of having UTI during pregnancy had slightly higher rate of GBS colonization (8.7%) than those who had been diagnosed of having UTI during pregnancy (7.8%). However, this difference was not statistically significant (P > 0.05). Although, there was no statistically significant association (p>0.05), the GBS colonization rate was higher among diabetic pregnant mothers (14.3%) in relation to non-diabetic pregnant mothers (8.2%). Pregnant women's who were positive for HIV/AIDS were a little highly colonized with GBS (10%) than those mothers who were negative for HIV/AIDS infection (8.5%). But the difference was not statistically significant (p>0.05) (see Table 4).

***Table 4.*** *Association between clinical/obstetric factors and GBS colonization among pregnant women attending ANC in AGH, from March to July, 2016 (n=281).*

| Variable | n | GBS result | | COR (95% C. I) | p-value[x] | AOR (95% C. I) | p-value[y] |
|---|---|---|---|---|---|---|---|
| | | Pos. (%) | Neg. (%) | | | | |
| History of Gravida | | | | | | | |
| Primigravida | 81 | 7 (8.6) | 74 (91.4) | 0.98 (.39-2.466) | 0.97 | | |
| Multigravida | 200 | 17 (8.5) | 183 (91.5) | 1.00 | | | |
| Parity | | | | | | | |
| Nullipara | 91 | 10 (11) | 81 (89) | 1.00 | | 1.00 | |
| Primipara | 89 | 5 (5.6) | 84 (94.4) | 2.07 (.68-6.33) | 0.200 | 2.99 (.408-21.95) | 0.281 |
| Multipara | 100 | 9 (9) | 91 (91) | 1.25 (.483-3.22) | 0.647 | 1.85 (.317-10.79) | 0.494 |
| Others | 1 | - | - | - | - | | |
| History of prematu. child birth | | | | | | | |
| Yes | 15 | 1 (6.7) | 14 (93.3) | 1.00 | | | |
| No | 266 | 16 (6) | 250 (94) | .773 (.095-6.28) | 0.81 | | |
| History of PROM | | | | | | | |
| Yes | 10 | 1 (10.0) | 9 (90) | 1.00 | | | |
| No | 271 | 16 (5.9) | 174 (94.1) | 1.11 (13- 9.46) | 0.924 | | |
| History of Abortion | | | | | | | |
| Yes | 52 | 8 (15.4) | 44 (84.6) | 1.00 | | 1.00 | |
| No | 229 | 9 (3.9) | 220 (96.1) | 2.82 (1.02-7.8) | 0.045 | 1.9 (571-6.33) | 0.295 |
| History of still birth/Neonatal loss | | | | | | | |
| Yes | 19 | 2 (10.5) | 17 (89.5) | 1.00 | | | |
| No | 262 | 15 (5.7) | 247 (94.3) | 1.41 (.29-6.8) | 0.671 | | |
| Gestational age | | | | | | | |
| 35 week | 96 | 10 (10.4) | 86 (89.6) | 1.00 | | | |
| 36 week | 100 | 9 (9) | 91 (91) | 1.18 (.46 -3.03) | 0.74 | | |
| 37 week | 85 | 5 (5.9) | 80 (94.1) | 1.86 (.61-5.68) | 0.276 | | |
| No. of ANC visit | | | | | | | |
| First | 5 | 2 (40) | 3 (60) | 1.79 (.03-1.254) | 0.085 | 0.267 (.036-1.98) | 0.197 |
| Second | 23 | 1 (4.3) | 22 (95.7) | 2.89 (37-22.66) | 0.311 | 2.65 (3-23.4) | 0.381 |
| Third | 65 | 0 (0.0) | 65 (100) | 0.2 (.15-16.84) | 0.89 | 0.11 (.23- 9.65) | 0.93 |
| Fourth | 172 | 20 (11.6 | 152 (88.4) | 1.00 | | 1.00 | |
| Other | 16 | - | - | - | | | |
| Recent antibiotic Rx | | | | | | | |
| Yes | 31 | 2 (6.5) | 29 (93.5) | 1.41 (.315-6.31) | 0.652 | | |
| No | 248 | 22 (8.9) | 226 (91.1) | 1.00 | | | |
| Others | 2 | - | - | - | - | | |
| Contraceptive use history | | | | | | | |
| Yes | 163 | 11 (6.7) | 152 (93.3) | 1.00 | | 1.00 | |
| No | 118 | 13 (11) | 105 (89) | 0.585 (25-1.36) | 0.211 | 0.438 (13-1.48) | 0.184 |
| UTI during pregnancy | | | | | | | |
| Yes | 51 | 4 (7.8) | 47 (92.2) | 1.00 | | | |

| Variable | n | GBS result | | COR (95% C. I) | p-value[x] | AOR (95% C. I) | p-value[y] |
|---|---|---|---|---|---|---|---|
| | | Pos. (%) | Neg. (%) | | | | |
| No | 230 | 20 (8.7) | 210 (91.3) | 905 (295-2.78) | 0.861 | | |
| Being Diabetic | | | | | | | |
| Yes | 14 | 2 (14.3) | 12 (85.7) | 1.00 | | | |
| No | 267 | 22 (8.2) | 245 (91.8) | 1.856 (39-8.83) | 0.437 | | |
| Recent HIV status | | | | | | | |
| Positive | 10 | (10.0) | 9 (90) | 1.00 | | | |
| Negative | 270 | 23 (8.5) | 247 (91.5) | 1.2 (145-9.84) | 0.87 | | |
| Other | 1 | - | - | - | - | | |

### *3.5. Antimicrobial Susceptibility Testing*

All GBS isolates were susceptible to penicillin, Ampicillin and Vancomycin. Utmost isolates were susceptible for Gentamycin 23 (95.8%) and chloramphenicol 22 (91.7%). Resistance to Ciprofloxacin, Ceftriaxone, Clindamycin, Erythromycin and Gentamycin was found to be 37.5%, 29.2%, 29.2%, 20.8% and 4.2% respectively. Of the seven isolates found to be resistant to clindamycin 2 (28.6%) were found by inducible clindamycin resistance test (D-zone test) and the remaining 5 (71.4%) were found directly from disk diffusion test. 29.2% and 8.3% intermediate sensitivity to Ceftriaxone and Erythromycin respectively was also found in our study. Two of the GBS isolates (2/24) showed multidrug resistance against Ceftriaxone, Erythromycin, Clindamycin and ciprofloxacin. (Table 5)

***Table 5.*** *Antimicrobial susceptibility pattern of GBS isolates from pregnant women attending ANC in AGH from March to July, 2016 (n=24).*

| Antimicrobial | Disc potency (µg) | Sensitive N (%) | Resistant N (%) |
|---|---|---|---|
| Penicillin G | 10 | 24 (100) | 0 |
| Ampicillin | 10 | 24 (100) | 0 |
| Chloramphenicol | 30 | 22 (91.7) | 2 |
| Ceftriaxone | 30 | 17 (70.8) | 7 (29.2) |
| Vancomycin | 30 | 24 (100) | 0 |
| Erythromycin | 15 | 19 (79.2) | 5 |
| Clindamycin | 2 | 17 (70.8) | 7 (29.2) |
| Gentamycin | 10 | 23 (95.8) | 1 |
| Ciprofloxacillin | 5 | 15 (62.5) | 9 (37.5) |

## 4. Discussion

The current investigation indicates an overall prevalence of 8.5% *S. agalactiae*. The finding is similar to the initial study done in Ethiopia which was carried out in Gonder with the colonization rate of 9%, studies at two hospitals in Addis Ababa with the colonization rate of 7.2% and Adigrat (11.3%) in 2012, [25-27]. But, it is lower than the prevalence reports from studies in different regions of Ethiopia; like Mekelle (13.7%), Hawassa (20.86%) and Jimma with overall carriage rate of 19% [16, 20, 28]. This variation between the regions could possibly be due to differences in method used, sample size, population variation and geographical difference. The result of this study is also consistent with reports from other developing African countries such as Yaoundé, Cameroon in (7.7%), and North eastern Nigeria (9.8%) [29, 30]. The finding is higher than the prevalence report from some African countries, such as Maputo, Mozambique (1.8%) [31] and lower than the prevalence rates described for some African countries, such as Egypt (17.89%), Democratic Republic of Congo (20%), Zimbabwe (21%) and Tanzania (23%) [32-35]. The difference in frequency could be due to variation in sample size, method used, culture media used and geographic differences. For example in our study we haven't used the primary isolation media, Todd-Hewitt broth (THB) media, which is selective for GBS.

In the current study no risk factors is associated with GBS colonization. This finding similar with studies conducted in Brazil; Hedayat Hospital of Tehran; Dares Salam-Tanzania; a pilot study in Ghana; in Hawassa, in Mekelle and in Jimma [16, 33, 36-39, 20, 28]. This could be due to small sample size in the current study.

Contrarily, in a study conducted in Daejeon- Korea, GBS colonization was significantly associated with hospital type, age group, education, frequency of pregnancy, gravidity, history of spontaneous abortion and PROM [40]. This difference could probably be due to difference in sample size, methodology and. geographic variation.

Even if it is not statistically significant, the GBS colonization rate in our study was higher among rural resident than urban residents (11% vs 7.7%). This could be due to personal hygiene and environmental sanitation difference between rural and urban population. However, two studies conducted in Zimbabwe showed significant association of GBS colonization among rural residents compared to urban residents [31, 41]. The difference may be related with awareness and behavioral variation. In the current study, GBS colonization didn't vary significantly with occupational status in which, civil servants have higher GBS colonization rate (12.5%), followed by merchant/business women (8.7%); which is parallel with study conducted in Hong Kong that showed high GBS colonization rate among pregnant women who work outside home [42]. This may partially be explained by their exposure difference. No GBS detected among pregnant mothers who were farmers and daily laborers in our study; which could be due to very small number of participants with these occupations.

Group B *Streptococcus* colonization in our study is almost similar in primigravida (8.6%) and multigravida (8.5%). This finding is somewhat different from studies reported from Nigeria, Ethiopia and Ghana, in which there is substantial variation in GBS colonization based on gravidity; even if it is not statistically significant [16, 43, 39]. However, in other studies conducted at Jawaharlal Institute of Postgraduate

Medical Education and Research (JIPMER), South India (p=0.05) and Mount Hope Maternity hospital and the San Fernando General Hospital of Trinidad (P<0.001), colonization rates were found to be significantly greater among multigravida women than primigravida women. In a study conducted in Daejeon-Korea higher gravidity was associated with lower prevalence of GBS colonization (p=0.009) [44, 40, 45]. This could fairly be due to geographical variation.

In our study, although it was not significantly associated, maternal GBS colonization was about four fold higher in mothers with history of abortion than those without history of abortion (15.4% vs 3.9%). The finding is in line with studies conducted in Daejeon, Korea and Bukavu, Democratic Republic of Congo [40, 35]. This could be facilitated due to unnatural disruption of the hormonal changes which accompany pregnancy and scaring or damage to the mucous membranes of genital area or to increased stress and the negative impact of stress on the immune system.

In the current study, GBS colonization rate was slightly higher among pregnant mothers with previous history of still birth or neonatal loss (10.5%) than those mothers without the history of still birth /neonatal loss (5.7%). Which may indicate the relation of colonization with maternal complications. But it seems to be contrary to the finding of study from Mumbilli Tanzania in which still birth or neonatal loss did not influence GBS colonization [33]. So there need to have further studies to explain the existence of correlation between still birth/neonatal loss and GBS colonization among pregnant mothers.

In our study approximately two fold higher rates of GBS colonization was showed in pregnant mothers with diabetes mellitus (14.3%) when compared to those without diabetes mellitus (8.2%); but it is not statistically significant. This is due to the fact that diabetes is associated with an increased tendency for infections which is caused by the hyperglycemic environment that favors immune dysfunction (e.g., damage to the neutrophil function, depression of the antioxidant system, and humoral immunity).

In our study, antimicrobial susceptibility patterns of twenty four GBS isolates from pregnant women against nine antimicrobial agents have been detected. All strains were susceptible to penicillin, ampicillin and vancomycin. The finding is also in line with the results of study conducted in Jimma (2016), in Hawassa (2010) and in Mekelle [16, 20, 28].

In our study, resistance to clindamycin and erythromycin was found to be 29.2% and 20.8% respectively. This is comparable with: study conducted in US, in Juiz de Fora, Brazil, in Geneva Switzerland, at the University Hospital of Bern in Switzerland, at Thammasart Hospital of Thailand, Korea, in Beijing-China, Dares Salaam-Tanzania, at Adigrat and Jimma-Ethiopia in [4, 33, 46-48, 27, 28, 49, 50, 51]. In this study resistance to ceftriaxone (29.2%) was also found; which is in line with studies from Hawassa and Jimma [16, 28].

In the present study, resistance was also observed against ciprofloxacin (37.5%), Chloramphenicol (8.3%), and gentamycin (4.2%). The finding is contrary to study conducted in Bali-Indonesia, in which all GBS isolates were sensitive to chloramphenicol and ceftriaxone; whereas in the study carried out in Brazil greatest resistance was to gentamicin (76.1%), followed by clindamycin (17.4%) [37, 52]. Resistance to ciprofloxacin, ceftriaxone, clindamycin, erythromycin and chloramphenicol in this study could be explained by the difference in strain, clinical history of participants, socio-demographic characteristics and geographical variation. The unusual resistance of ceftriaxone could possibly be due to wide and indiscriminate use of these antibiotics.

# 5. The Study limitation

Absence of THB, primary selective broth media for isolation of GBS, and Nalidixic acid, one of the antibiotics which make the primary media to be selective for isolation of the bacteria, makes our isolation inadequate to indicate maximum carriage rate.

Failure to assess the outcome on neonates, whose mother detected to be colonized by GBS on the study. The response of study participants about risk factors due to the threat of recall bias might not be always right.

# 6. Conclusion

The current study presented the overall GBS colonization rate of 8.5% among 281 pregnant mothers with 35-37 weeks of gestation, attending ANC of AGH, Arbaminch, Ethiopia. Even if the prevalence of GBS detected in our study is low when compared to most of similar studies conducted in different parts of the country and also in Africa, it is rationally high enough to warrant the need for screening of pregnant mothers near term delivery and to determine their antibiotic susceptibility so as to set appropriate intervention mechanisms. All GBS isolates in our study were susceptible for penicillin, ampicillin and vancomycin, which are the first line drugs for IAP's; that is in line with CDC guideline and many other similar study reports. And except one isolate, the remaining all isolates of GBS were susceptible to gentamycin (95.8%). Low level of resistances against ciprofloxacin, ceftriaxone, clindamycin, erythromycin and chloramphenicol with 37.5%, 29.2%, 29.2%, 20.8%, 8.3% respectively was found. Two (8.33%) of the isolates showed multidrug resistance.

# 7. Recommendation

Resistance to the commonly used antibiotics such as clindamycin, erythromycin, ceftriaxone and ciprofloxacin was observed in this study, which calls for performing susceptibility testing and careful use of any of these antibiotics. Awareness about the prevalent GBS serotypes in a given country is very important to develop and implement effective vaccine for prevention of neonatal GBS disease. Therefore;

Serotyping of GBS ought to be performed in future researches, because it is an effective epidemiological tool for studying GBS. The current study was conducted in small sample size; therefore further comprehensive epidemiological survey to establish the GBS colonization rate among pregnant mothers at different gestational ages and the effects of it on both maternal and neonatal outcome of pregnancy need to be conducted to introduce national guideline.

## Competing Interests

The authors have declared that no competing interests exist.

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## Authors' Contribution

SS, MM, DB, ZS and TL
All authors equally contributed to this research work
*These authors read and approved the final manuscript

## Abbreviation and Acronyms

AGH. Arbaminch General Hospital, ANC Antenatal care, ATCC. American Type culture collection, BA Blood Agar, CAMP Christie, Atkins, and Munich-Prevention, CDC . Center for Disease Control and prevention, I Confidence Interval, CLSI Clinical and Laboratory Standard Institute, EOGBSD Early-Onset group B streptococcal Disease, GBS Group B streptococcus/Streptococci, HIV Human Immunodeficiency Virus, IAP Intrapartum Antibiotics Prophylaxis, IRB Institutional review board, LOGBSD. Late-Onset Group B Streptococcal Disease, MHA Late- Onset Group B Streptococcal Disease, OR Odds ratio, PMROM Premature Rupture of Membranes, SBA Sheep Blood Agar, SNNPR Southern Nations, Nationalities and Peoples Region, SOS Standard Operating Procedures, SPSS Statistical Package for Social Sciences, THB Todd Hewitt Broth, UTI Urinary Tract Infection.

## Availability of Data & Materials

The data for this research is available, so we can contact you when you need our data for the future process.

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