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# Group B Streptococcus Isolation, Antimicrobial Susceptibility Profile and Associated Factors among Pregnant Women Attending Health Facilities, Hawassa, Ethiopia

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## ABSTRACT

**Background:** Maternal genitourinary tract colonization with group B streptococcus (GBS) is a predominant risk factor for the development of early-onset disease in neonates and puts newborns at increased risk for morbidity and mortality. This study is aimed to determine the prevalence, antibiotic susceptibility profile and associated factors of group B streptococcus among pregnant women at 35-37 weeks of gestation.

**Methods:** A prospective cross-sectional study was conducted from June to July 2022 at selected health facilities in Hawassa, Ethiopia. A total of 329 antenatal clinics attendees, proportionally allocated were recruited consecutively. Three were excluded because of their antibiotic uptake in the last two weeks of the study. Sociodemographic and clinical data were collected using a structured questionnaire. Vagino-rectal swabs were collected and cultured in Todd Hewitt broth and on 5% sheep blood agar. Antimicrobial susceptibility test was done using Kirby-Bauer disk diffusion test. Statistical analysis was performed using logistic regression test.

**Results:** Among the 326, 48 (14.7%) were positive for GBS. The GBS isolates were susceptible to vancomycin (100%) and resistant to penicillin (2.1%), ampicillin (4.2%), erythromycin (8.3%) and clindamycin (12.5%). Maternal GBS colonization was significantly associated with rural residence (AOR = 2.29 (95%CI = 1.17-5.32), $p = 0.032$) and with a single antenatal visit (AOR = 2.49 (95% CI = 1.07-4.93), $p = 0.018$).

**Conclusion:** The high frequency of vagino-rectal GBS colonization, resistance to the commonly used antibiotics and association with rural residence and a single antenatal visit for present study put emphasis on further investigation and accomplishment of routine GBS screening practices.

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**Abbreviations**
**ANC:** Antenatal Care
**AST:** Antibiotic Susceptibility Test
**AGH:** Adare General Hospital
**BMCSC:** Bushulo Mother and Child Specialty Center
**CAMP:** Christie-Atkins-Munch-Peterson
**EOD:** Early-Onset Disease
**GBS:** Group B *Streptococcus*
**HUCSH:** Hawassa University Comprehensive Specialized Hospital
**IRB:** Institutional Review Board
**SNNPR:** Southern, Nations, Nationalities, and Peoples' Region

**Keywords:** GBS Colonization, Antibiotic Susceptibility Profile, Hawassa

## Introduction

Streptococci were classified as α-hemolytic, β-hemolytic, and γ-hemolytic, based on the ability of each group of bacteria to lyse red blood cells on a blood agar plate. Based on the carbohydrate antigens expressed on the cell surface of the bacteria, Rebecca Lancefield in 1930 further classified the β-hemolytic streptococci into different groups. From this classification, *Streptococcus* agalactiae expressed the B antigen and was subsequently named Group B *Streptococcus* (GBS) [1]. Group B *Streptococcus* is non-motile, encapsulated, Gram-positive cocci that form narrow zones of β-hemolytic, gray-white, mucoid colonies on blood agar plates. It is also a fastidious, facultative anaerobic, catalase-negative, and Christie-Atkins-Munch-Peterson (CAMP) test positive bacteria [2].

Group B *Streptococcus* is one of the most common causes of life-threatening bacterial infections in neonates and infants [3]. Group B *Streptococcus* colonization in the genitourinary tract of pregnant women is the major risk factor for early-onset disease in infants and is the leading cause of neonatal sepsis, meningitis and pneumonia, which is now a major cause of maternal and neonatal morbidity and mortality in many parts of the world) [4].

Globally, approximately 10-30% of pregnant women are colonized with group B streptococci in the genitourinary tract, and 60% of newborns acquire the infection during labor and delivery process [5]. GBS is the leading cause of early-onset sepsis (EOS) and accounts for 12% of the global case-fatality rate, which is three times higher in low-income countries [6].

The IAP has successfully reduced GBS EOS by 79% in most high-income countries, but in most developing countries, including Ethiopia, the prevention and treatment strategies for maternal GBS colonization have not yet been adopted [5, 7]. Therefore, this study was conducted to determine the prevalence, assess antibiotic susceptibility profile and identify associated factors of GBS colonization among pregnant women at 35-37 weeks of gestation attending antenatal clinics at Adare General Hospital and Bushulo Mother and Children Specialty Center, Hawassa, Ethiopia.

## Methods

### Study Area and Participants

A prospective cross-sectional study was conducted among pregnant women at 35-37 weeks of gestation attending ANC of Adare General Hospital (AGH) and Bushulo Mother and Children Specialty Center (BMCSC), Hawassa, Ethiopia from June 06 to July 22-2022. The city is located 275 km away from Addis Ababa, capital city of Ethiopia. Pregnant women in the third trimester attending ANC in AGH and BMCSC were included. Three of the participants, two from AGH and one from BMCSC were excluded because of their antibiotic uptake within two weeks prior to the study.

### Sample Size and Sampling Technique

The sample size was calculated based on the prevalence indicated in the previous study using single population proportion formula [9]. Expected margin of error (d) was 0.04 and Confidence interval (z) was 95%.

$$n = \frac{z^2\, p\,(1-p)}{d^2}$$

Where: n = the required sample size

$z^2$= confidence level, 1.962 = 3.8416

p= prevalence from the previous study = 0.146

d= acceptable difference, 0.04

$$n = \frac{(1.96)^2\, 0.146\,(1-0.146)}{(0.04)^2} = 299$$

Considering 10% non-response rate = n + (10/100*299) = 299 + 30 = 329

The calculated sample size was allocated proportionally to each health facilities based on their average of pregnant women attending ANC between 35-37 weeks gestational per day, 14 in AGH and 8 in BMCSC.

*n* = N1/Nt * nt

Where:

*n* = the sample size was allocated.

N1 = average number of ANC attendants per day in one health facility.

Nt = average number of ANC attendants per day in both health facility.

*n*t = determined sample size = 329.

nAGH = 14/22*329 = 209

nBMCSC = 8/22*329 = 120. Therefore, 209 and 120 participants were included from AGH and BMCSC ANC attendees respectively. All eligible study participants were enrolled in the study using a systematic sampling technique.

### Data Source and Data Collection

A well-structured standard questionnaire with review of medical records was used to collect socio-demographic and clinical characteristics of pregnant women attending ANC. Socio-demographic data like age, place of residence, marital status, educational status, and occupational status; clinical data like Antibiotic up-take in last two weeks, ANC visits, gravidity, mode of previous delivery, place of previous delivery, outcomes of a previous delivery and gestational age were collected.

### Specimen Collection and Transportation

Following universal precautions vagino-rectal swab was collected by brushing the lower vagina and rectum with a sterile cotton-tipped swab by trained midwives nurses. The swabs were immediately placed in Amies transport medium with charcoal and transported at room temperature to the laboratory of the SNNPR public institute within 3-4 hours of collection for analysis [4].

### Culture and Identification

The vagino-rectal swabs should be placed into 2 ml Todd Hewitt broth (Oxoid Ltd., Basingstoke, Hampshire, England) supplemented with gentamicin 8µg/ml, nalidixic acid 15µg/ml and 5% sheep blood incubated at 37°C in a candle jar for 18-24 hours. The broth with no visible turbidity after overnight incubation was re-incubated for a further hour and sub-cultured onto 5% sheep blood agar (Oxoid Ltd, Basingstoke, and Hampshire, England) and incubated in a candle jar for 18-24 hours. All Suspected GBS colonies (pink colonies with narrow β-hemolysis) were confirmed by Gram stain, catalase test and CAMP test [4]. Bacteria that were Gram-positive cocci, catalase-negative, and CAMP-positive were confirmed as GBS.

### Antibiotic Susceptibility Testing

Antibacterial susceptibility testing was performed by using the modified Kirby-Bauer disc diffusion method according to the criteria of the CLSI, 2020 guidelines [8]. Direct colony suspension in sterile normal saline equivalent to McFarland 0.5 turbidity was performed. A sterile cotton swab was used to uniformly inoculate a bacterial suspension onto Mueller-Hinton agar (Oxoid Ltd, Basingstoke, Hampshire, England), supplemented with 5% sheep blood. The inoculated plates were left at room temperature for 3 5 minutes and then antibiotic discs were placed on the surface of the inoculated Mueller-Hinton plate. The inoculated plate was incubated at 37°C in a candle jar for 18-24 hours. The antibiotics tested (Oxoid Ltd., Basingstoke Hampshire, England), were, penicillin G (PEN) (10 IU), ampicillin (AMP) (10 µg), erythromycin (ERY) (15 µg), clindamycin (CLN) (2µg), and vancomycin (VAN) (30 µg).

### Quality Control

Standard operating procedures (SOPs) were followed during sample collection, transportation, and processing steps. The sterility of the culture media was checked by incubating un-inoculated media overnight at 35°C to 37°C. *Staphylococcus aureus* (ATCC 25923), Escherichia coli (ATCC 25922), *P. auroginosa* (ATCC 27853), and *S. agalactiae* isolates (ATCC-12386) strains were used as a quality control organism for culture and antibiotic susceptibility testing.

### Data Entry and Analysis

Data were visually inspected, coded and entered into the SPSS version 25.0 software. Descriptive statistics (frequency, median, and percentage) were used to describe the study population with respect to relevant variables. Bivariate logistic regression was performed, then variables with p< 0.25 were further analyzed by multivariate logistic regression, and P< 0.05 was accepted as statistically significant.

## Results

### Socio-Demographic Characteristics of the Study Participants

A total of 326 pregnant women attending ANC were recruited, three of the participants were excluded because they had taken antibiotics in the last two weeks of the study. The age of the study participants ranged from 20-39 years with a median age of 27.5 years (+ 4.1SD), the 25-29 age group was the most frequent one (49.7%). About 72.1% (235/326) were urban dwellers. Majorities were post-secondary educated 33.1% (108/326), housewives 63.2% (206/326) and married 99.4% (324/326) (Table1).

**Table 1: Socio-demographic characteristics of pregnant women investigated for GBS in AGH (*n*=207) and BMCSC (*n*=119), Hawassa, Ethiopia**

| Variables/Category | Frequency (%) | | |
|---|---|---|---|
| | AGH (*n*=207 (63.5)) | BMCSC (*n*=119 (36.5)) | Total (n=326) |
| **Age** | | | |
| 20-24 | 42 (20.3) | 21 (17.6) | 63 (19.3) |
| 25-29 | 100 (48.3) | 62 (52.1) | 162 (49.7) |
| 30-34 | 43 (20.8) | 31 (26.1) | 74 (22.7) |
| 35-39 | 22 (10.6) | 5 (4.2) | 27 (8.3) |
| **Residence** | | | |
| Urban | 158 (76.3) | 77 (64.7) | 235 (72.1) |
| Rural | 49 (23.7) | 42 (35.3) | 91 (27.9) |
| **Level of education** | | | |
| No formal education | 30 (14.5) | 29 (24.4) | 59 (18.1) |
| Primary school (Grade 1-8th) | 72 (34.8) | 32 (26.9) | 104 (31.9) |
| Secondary school (Grade 9th-12th) | 44 (21.2) | 11 (9.2) | 55 (16.9) |
| Post-secondary (Diploma/degree) | 61 (29.5) | 47 (39.5) | 108 (33.1) |
| Occupational status | | | |
| House wife | 131 (63.3) | 75 (63.0) | 206 (63.2) |
| Governmental employee | 35 (16.9) | 40 (33.6) | 75 (23) |
| Merchant | 22 (10.6) | 2 (1.7) | 24 (7.4) |
| Student | 11 (5.3) | 1 (0.85) | 12 (3.7) |
| Daily worker | 8 (3.9)) | 1 (0.85) | 9 (2.8) |
| Marital status* | | | |
| Unmarried | 2 (1) | - | 2 (0.6) |
| Married | 205 (99.0) | 119 (100) | 324 (94.4) |

AGH-Adare General Hospital, BMCSC-Bushulo Mother and Children Specialty Center. (*)- There were no participants with divorced and widowed marital status visited the ANC of AGH and BMCSC during in the study period.

### Clinical Characteristics of the Study Participants

Of the total 326 pregnant women in weeks 35-37, 34.4% (112/326) were in week 35, 25.1% (82/326) in week 36 and 40.5% (132/326) in week 37. About 35.3% (115/326) of the study participants attended ANC once. The majority were multigravida 78.8% (257/326). Of the 257 multigravidas, 81.7% (210/257) had a previous delivery in health facility and 79.8% (205/257) had a vaginal delivery. About 93.8% (241/257) of the participants had a history of healthy babies in their previous delivery. In this study, only one participant had information about the effect of GBS on the newborn (Table 2).

**Table 2: Clinical Characteristics of Pregnant Women Investigated for GBS in AGH (n=207) and BMCSC (n=119), Hawassa, Ethiopia**

| Variables / Category | Frequency (%) | | |
|---|---|---|---|
| | AGH (*n*=207 (63.5)) | BMCSC (*n*=119 (36.5)) | Total (n=326) |
| **Gestational week** | | | |
| 35th | 59 (28.5) | 53 (44.5) | 112 (34.4) |
| 36th | 64 (30.9) | 18 (15.2) | 82 (25.1) |
| 37th | 84 (40.6) | 48 (40.3) | 132 (40.5) |
| **Antenatal visit*** | | | |
| >2 times | 98 (47.3) | 113 (95.0) | 211 (64.7) |
| One time | 109 (52.7) | 6 (5.0) | 115 (35.3) |
| Gravidity | | | |
| Primigravida | 47 (22.7) | 22 (18.5) | 69 (21.2) |
| Multigravida** | 160 (77.3) | 97 (81.5) | 257 (78.8) |
| **Place of previous delivery (*n*=257)**** | | | |
| Home | 31 (19.4) | 16 (16.5) | 47 (18.3) |
| Health institution | 129 (80.6) | 81 (83.5 | 210 (81.7) |
| **Mode of previous delivery (*n*=257)**** | | | |
| Vaginal | 131 (81.9) | 74 (76.3) | 205 (79.8) |
| CS | 29 (18.1) | 23 (23.7) | 52 (20.2) |
| **Outcome of previous delivery (*n*=257)**** | | | |
| Normal health baby | 150 (93.8) | 91 (93.8) | 241 (93.8) |
| Neonatal death | 10 (6.2) | 6 (6.2) | 16 (6.2) |
| **Information about the impact of GBS to neonates** | | | |
| Yes | 1 (0.5) | - | 1 (0.4) |
| No | 206 (99.5) | 119 (100) | 325 (99.6) |

AGH-Adare General Hospital, BMCSC-Bushulo Mother and Children Specialty Center, CS- Caesarean Section, (*)-WHO recommends the minimum of 4 times ANC visit in normal pregnancy, (**)-refers to participants with multigravida (*n*=257).

### Maternal Group B Streptococcus Colonization

The overall prevalence of GBS colonization among pregnant women at 35-37 weeks' gestation was 48/326 (14.7%; 95%CI: 10.8-18.5). The prevalence of GBS in Adare General Hospital 32/207 (15.5%; 95%CI: 10.6-20.4) and in Bushulo Mother and Children Specialty Center (16/119) (13.4%; 95% CI: 7.3-19.5) (Table 3).

**Table 3: Antibiogram of GBS Isolated from Pregnant Women in Selected Health Institutions, Hawassa, Ethiopia**

| Number of isolates (*n*=48) | | | Antibiogram resistant | | | | |
|---|---|---|---|---|---|---|---|
| | | | AMP | CLN | ERY | PEN & CLN | CLN & ERY |
| $R_1$ | Number | 9 | 2 | 4 | 3 | - | - |
| | % | 18.8 | 4.2 | 8.3 | 6.3 | - | - |
| $R_2$ | Number | 2 | - | - | - | 1 | 1 |
| | % | 4.2 | - | | - | 2.1 | 2.1 |

$R_1$-Resistant to one antibiotic, $R_2$-Resistant to two antibiotics, PEN- Penicillin, AMP- Ampicillin, ERY- Erythromycin, CLN-Clindamycin.

### Associated factors for Maternal Group B Streptococcus Colonization

In bivariate logistic regression analysis, five variables residence, education level, ANC visits, gravidity, and mode of previous delivery met the variable screening criteria (p-value of < 0.25) and were entered into multivariate logistic regression analysis (Table 4, Table 5). In multivariate analysis, the maternal GBS colonization showed a statistically significant association with rural residence (AOR = 2.29 (95% CI = 1.17-5.32), $p = 0.032$) and with single ANC visit (AOR = 2.49 (95% CI = 1.07-4.93), $p = 0.018$) (Table 6).

**Table 4: Bivariate Analysis of Socio-Demographic Factors Associated with Maternal GBS Colonization in Two Selected Health Institutions, Hawassa, Ethiopia**

| Variables/Category | GBS culture result | | COR (95%CI) | *P*-value |
|---|---|---|---|---|
| | Negative (*n* = 278) (%) | Positive (*n* = 48) (%) | | |
| **Age** | | | | |
| 20-24 | 56 (88.9%) | 7 (11.1%) | Ref | |
| 25-29 | 135 (83.3%) | 27 (16.7%) | 1.6 (0.66-3.89) | 0.29 |
| 30-34 | 65 (87.8%) | 9 (12.2%) | 1.11 (0.39-3.17) | 0.85 |
| 35-39 | 22 (81.5) | 5 (18.5%) | 1.82 (0.52-6.34) | 0.35 |
| **Residence** | | | | |
| Urban | 208 (88.5%) | 27 (11.5%) | Ref | |
| Rural | 70 (76.9%) | 21 (23.1%) | 2.31 (1.23-4.34) | 0.009 |
| **Level of education** | | | | |
| No formal education | 43 (72.9%) | 16 (27.1%) | 2.31 (1.05-5.09) | 0.039 |
| Primary school (grade 1-8$^{th}$) | 94 (90.4%) | 10 (9.6%) | 0.66 (0.28-1.54) | 0.337 |
| Secondary school (grade 9$^{th}$-12$^{th}$) | 48 (87.3%) | 7 (12.7%) | 0.90 (0.35-2.37) | 0.837 |
| Post-secondary (>12$^{th}$) | 93 (86.1%) | 15 (13.9%) | Ref | |
| **Occupational status** | | | | |
| House wife | 175 (85%) | 31 (15%) | 0.62 (0.12-3.12) | 0.562 |
| Merchant | 21 (87.5%) | 3 (12.5%) | 0.50 (0.07-3.63) | 0.493 |
| Governmental worker | 64 (85.3%) | 11 (14.7%) | 0.60 (0.11-3.28) | 0.557 |
| Student | 11 (91.7%) | 1 (8.3%) | 0.32 (0.02-4.20) | 0.318 |
| Daily worker | 7 (77.8%) | 2 (22.2%) | Ref | |
| **Health institution** | | | | |
| AGH | 175 (84.5%) | 32 (15.5%) | Ref | |
| BMCSC | 103 (86.6%) | 16 (13.4%) | 1.18 (0.62-2.25) | 0.622 |

GBS- Group B *Streptococcus*, COR-crude Odds Ratio, CI-Confidence Interval, Ref- shows reference group

**Table 5: Bivariate Analysis of Clinical Factors Associated with Maternal GBS Colonization in Two Selected Health Institutions, Hawassa, Ethiopia**

| Variables/Category | GBS culture result | | COR (95%CI) | P-value |
|---|---|---|---|---|
| | Negative (*n* = 278) (%) | Positive (*n* = 48) (%) | | |
| **Gestational week** | | | | |
| 35th | 98 (87.5%) | 14 (12.5%) | Ref | |
| 36th | 71 (86.6%) | 11 (13.4%) | 1.09 (0.47-2.53) | 0.851 |
| 37th | 109 (82.6%) | 23 (17.4%) | 1.48 (0.72-3.03) | 0.287 |
| **Antenatal visit*** | | | | |
| >2 times | 188 (89.1%) | 23 (10.9%) | Ref | |
| One time | 90 (78.3%) | 25 (21.7%) | 2.27 (1.22-4.22) | 0.009 |
| **Gravidity** | | | | |
| Primigravida | 55 (79.7%) | 14 (20.3%) | Ref | |
| Multigravida** | 223 (86.8%) | 34 (13.2%) | 0.60 (0.30-1.19) | 0.145 |
| **Place of previous delivery**** | | | | |
| Home | 40 (85.1%) | 7 (14.9%) | 1.19 (0.48-2.91) | 0.71 |
| Health institution | 183 (87.1%) | 27 (12.9%) | Ref | |
| **Mode of previous delivery**** | | | | |
| Vaginal | 181 (88.3%) | 24 (11.7%) | 0.56 (0.25-1.25) | 0.157 |
| CS | 42 (80.8%) | 10 (19.2%) | Ref | |
| **Outcome of pre-delivery**** | | | | |
| Normal health baby | 209 (86.7%) | 32 (13.3%) | Ref | |
| Neonatal death | 14 (87.5%) | 2 (12.5%) | 0.93 (0.20-4.30) | 0.929 |

GBS- Group B *Streptococcus*, CS- Caesarean Section, COR-Crude Odds Ratio, CI-Confidence Interval, Ref- shows reference group (*)- WHO recommends the minimum of 4 times ANC visit in normal pregnancy, (**)-refers to participants with multigravida (*n*=257).

**Table 6: Multivariate analysis of factors associated with maternal GBS colonization in two selected health institutions, Hawassa, Ethiopia**

| Variables/Category | GBS culture result | | COR (95%CI) | P-value | AOR (95%CI) | P-value |
|---|---|---|---|---|---|---|
| | Negative (*n* = 278) (%) | Positive (*n* = 48) (%) | | | | |
| **Residence** | | | | | | |
| Urban | 208 (88.5) | 27 (11.5) | Ref | | Ref | |
| Rural | 70 (76.9) | 21 (23.1) | 2.31 (1.23 4.34) | 0.009 | 2.29 (1.17-5.32) | 0.032 |
| **Level of education** | | | | | | |
| No formal education | 43 (72.9) | 16 (27.1) | 2.31 (1.05-5.09) | 0.039 | 1.72 (0.71-4.19) | 0.231 |
| Primary school (grade 1-8th) | 94 (90.4) | 10 (9.6) | 0.66 (0.28-1.54) | 0.337 | 0.64 (0.26 1.57) | 0.325 |
| Secondary school (grade 9th-12th) | 48 (87.3) | 7 (12.7) | 0.90 (0.35-2.37) | 0.837 | 0.91 (0.34-2.43) | 0.855 |
| Post-secondary (>12th) | 93 (86.1) | 15 (13.9) | Ref | | Ref | |
| **Antenatal visit*** | | | | | | |
| >2 times | 188 (89.1) | 23 (10.9) | Ref | | Ref | |
| One time | 90 (78.3) | 25 (21.7) | 2.27 (1.22-4.22) | 0.009 | 2.49 (1.07-4.93) | 0.018 |
| Gravidity | | | | | | |
| Primigravida | 55 (79.7%) | 14 (20.3%) | Ref | | Ref | |
| Multigravida** | 223 (86.8%) | 34 (13.2%) | 0.60 (0.30-1.19) | 0.145 | 0.53 (0.26-1.09) | 0.085 |
| **Mode of previous delivery**** | | | | | | |
| Vaginal | 181 (88.3%) | 24 (11.7%) | 0.56 (0.25-1.25) | 0.157 | 0.48 (0.21-1.10) | 0.083 |
| CS | 42 (80.8%) | 10 (19.2%) | Ref | | Ref | |

GBS- Group B *Streptococcus*, CS- Caesarean Section, COR-Crude Odds Ratio, AOR-Adjusted Odds Ratio, CI:-Confidence Interval, Ref- shows reference group, (*)- WHO recommends the minimum of 4 times ANC visit in normal pregnancy, (**)- refers to participants

with multigravida (n=257).

**Antibiotic Susceptibility Profile**

The isolates were susceptible to vancomycin 100% (48/48 isolates), penicillin 97.9% (47/48 isolates), ampicillin 95.8% (46/48 isolates), erythromycin 89.6% (43/48 isolates) and clindamycin 83.3% (40/48 isolates). About 12.5% (6/48 isolates), 8.3% (4/48 isolates), 4.2% (2/48 isolates) and 2.1% (1/48 isolates) were resistant to clindamycin, erythromycin, ampicillin, and penicillin respectively. The isolates also showed intermediate results to clindamycin 4.2% (2/48 isolates) and erythromycin 2.1% (1/48 isolates).

**Discussion**

In this study, the overall prevalence of GBS among pregnant women at 35-37 weeks gestation was (14.7%). This finding was relatively similar to reports from Addis Ababa (14.6%), Hawassa (15.7%) and Brazil (14%) [9-11]. However, it was higher than in studies of South Asia (12.5%) and East Asia (11%), Iran (11.8%), Yemen (10.95%), Cameroon (8.69%), Namibia (13.6%), Adama, Ethiopia (13.2%), Arbaminch, Ethiopia (8.5%), Eastern, Ethiopia (13.68%) and in Nekemte, Ethiopia (12.2%) [12-20].

On the other hand, the prevalence of maternal GBS in this study was lower than the study findings in Guatemala, Republic of Central America (17.3%) [5], Kenya (20.5%), Southern African countries (23.8%), Western Cape region of South Africa (16.6%), Gondar, Ethiopia (25.5%), Southwest, Ethiopia (16.3%) and Bahir Dar city health facilities (18.5%) [21-26]. However, variations in maternal GBS colonization between studies may be due to study populations from different parts of the world, sample collection site (vagino-rectal swabs were more colonized than vaginal swabs), sample size, gestational weeks (as gestational weeks increase, colonization rates may also increase), socio-economic status, and geographical areas.

In this study, maternal GBS colonization was significantly associated with rural residence and single antenatal visit. Those pregnant women who lived in rural areas were 2.29 times more likely to be colonized with GBS than those pregnant women who lived in urban areas (AOR = 2.29 (95% CI = 1.17-5.32), p = 0.032). There was no recent literature showing a significant association between maternal GBS colonization and place of residence, other than a higher colonization rate in rural than in urban dwellers. In this study, about 23.1% (21/91) of GBS colonized mothers were rural and 11.5% (27/235) were urban. Similarly, in the study conducted in the University of Gondar Referral Hospital, about 31.8% of GBS colonized mothers were rural and 24.1% were urban dwellers [24], Arbaminch Hospital GBS colonized rural residents were (11%) and urban were (7.7%) [18]. However, the significant association between maternal GBS colonization and rural dwellers in this study may be due to their socio-economic status, information gap, sanitary habit, and personal hygiene.

In the present study, pregnant women who attended ANC once were 2.49 times more likely to be colonized with GBS than those who attended ANC > twice (AOR = 2.49 (95% CI = 1.07-4.93), p = 0.018). Similarly, in the Northwest Ethiopia study, pregnant women who attended ANC less than three times were significantly associated with vertical transmission of GBS, and pregnant women who attended ANC 4 to 5 times were 0.21 times (AOR = 0.209; 95% CI: 0.063, 0.696), p = 0.01) less likely to transmit GBS to their newborn than those who had 0 to 3 ANC visits [7]. However, the significant association between a single ANC visit and maternal GBS colonization in this study may be due to information gap, lack of health education on prevention and management of pregnancy-related illnesses and health promotion by antenatal care providers. This also shows the importance of ANC follow-up in the prevention and management of pregnancy-related maternal GBS colonization.

In the present study, the other factors were not significantly associated with maternal GBS colonization (p> 0.05), rather than increased colonization rate in compression to each category. The pregnant women in the age group 35-39 years old (18.5%) were more colonized than other age groups. This is similar to studies in Adama where the highest rate (13.8%) of GBS positives were in the 37-42 years age group and Gondar (27.3%) was in the> 25 years age group [17, 24]. This shows that as the age of pregnant women increases, so does the rate GBS colonization, which may be due to maternal immunity.

Pregnant woman with no-formal education (27.1%) were 1.72 times more likely to be colonized with GBS compared to those with post-secondary education (AOR = 1.72 (95% CI = 0.71-4.19), but not statistically significant (p = 0.231). This is similar to the study in Gondar (34.5%) were illiterate [24]. About, (15%) of housewives were positive for GBS in this study, which was higher than other occupational categories. Similarly, in the Addis Ababa study (15.1%) of the GBS positives were housewives [9]. The high colonization rate of GBS according to no-formal education level and housewife occupational level may be due to a lack of information on pregnancy-related disease prevention mechanisms.

In this study, GBS colonization was high at 37 weeks (17.4%) compared to 36 weeks (13.4%) and 35 weeks (12.5%). Similarly, in studies in Hawassa, (15.3%) of GBS positives were between 37-42 weeks, which is higher than other gestational weeks [10]. This may be related to the level of maternal immunity. As gestational weeks increase, protective maternal antibodies and neutrophil-mediated defences decrease [27].

In the present study, more of the GBS positives were primigravida (20.3%) than multigravida (13.2%), similar to the study in Adama, (14%) of the GBS positives were primigravida and (12.3%) multigravida and Hawassa (17.9%) were primigravida and (13.7%) were multigravida [10, 17]. In this study, GBS-positive mothers with CS mode of previous delivery (19.2%) were more colonized than vaginal delivery (11.7%), which is comparatively similar to the study, in Adama (20%) were CS, and (12.8%) were vaginal [17] and in Hawassa (12.9%) were CS and (13.9%) were vaginal [10].

In the present study, the antibiotic susceptibility profile was shown low resistance rate in compression to studies done before. About, 12.5% of the isolates were resistant to clindamycin, comparatively this is low from the findings reported from Addis Ababa 26.8%, Arbaminch 29.2%, Kenya 30.4% and Bahir Dar city 33.3% [9, 18, 21, 26]. About, 8.3% of the isolates were resistant to erythromycin, which is similar to the reports from Addis Ababa 7.5% and comparably low to the reports from Bahir Dar 25.9% and Arbaminch 29.2% [9, 18, 26]. About, 4.2% and 2.1% of the isolates were resistant to ampicillin and penicillin respectively. This is comparatively less than reported from Addis Ababa (14.6% and 19.5%) and Kenya (55.2% and 72.4% to ampicillin and penicillin respectively [9, 21]).

In this study, the antibiotic susceptibility profiles of GBS isolates showed 100% susceptibility to vancomycin. This is similar to the reports from Yemen 100%, Arbaminch 100% and Bahir Dar city 96.3% [14, 18, 26]. According to recent studies including the

present study, vancomycin is the most effective antibiotic for GBS. About 97.9% and 95.8% of the GBS isolates showed susceptibility to the first-line antibiotics penicillin and ampicillin respectively. This is consistent with findings from Kenya 100%, Arbaminch 100% and Bahir Dar City 88.9% and 90.7% to penicillin and ampicillin respectively [14, 18, 26].

The differences in the antibiotic susceptibility profile in different studies are due to the misuse and overuse of antibiotics, as well as poor infection prevention and control.

**Limitation of the Study**

This study identified the potential factors for maternal GBS colonization, with rural residence and low antenatal visit being significantly associated with maternal GBS colonization. The limitations of this study, GBS serotypes and molecular characterization of GBS isolates were not performed due to lack of accessibility and the design of the study did not include other bacterial, fungal and viral pathogens.

**Conclusions**

There was a high frequency of maternal GBS colonization at 35-37 weeks' gestation. GBS isolates showed relatively low resistance to commonly used antibiotics penicillin (2.1%), ampicillin (4.2%), erythromycin (8.3%) and clindamycin (12.5%), and all GBS isolates were susceptible to vancomycin. Maternal GBS colonization was significantly associated with rural residence and a single antenatal visit. Therefore, maternal GBS screening at 35-37 weeks' gestation and antibiotic susceptibility testing, promotion of regular antenatal care, and further large-scale studies should be considered more in rural mothers.

**Declaration**

**Ethics Approval and Consent to Participate**

Ethical clearance was obtained from the IRB with a ref. no. IRB/154/14 of Hawassa University, College of Medicine and Health Sciences, A support letter was obtained from Adare General Hospital and Bushulo Mother and Children Specialty Center. The data were collected after obtaining informed consent and confidentiality of the information was kept by using codes instead of any personal identifiers.

**Consent for Publication**

Not applicable

**Availability of Data and Materials**

There are no remaining data and materials, and all information is presented in the main manuscript. The raw data sets used and/ analyzed in the current study are available from the corresponding author upon reasonable request.

**Competing Interests**

The authors declared that there is no competing interest

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No fund was obtained for this research

**Author Contributions**

AA involved in proposal design, study participants selection, provides materials, conducted laboratory investigation, data analysis and manuscript preparation. FD contributed in laboratory investigation and manuscript preparation, DD and GM supervised the laboratory investigation process and contributed in the manuscript preparation and AW contributed in data analysis and revision of the manuscript. All authors read and approved the final manuscript.

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