

# Prevalence of group B *Streptococcus* colonization among pregnant women attending antenatal clinic of Hawassa Health Center, Hawassa, Ethiopia

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## Abstract

**Background:** Group B *streptococcus* (GBS) or *Streptococcus agalactiae* are members of the normal flora of the female genital tract. GBS has become the major cause of bacterial infections in the peri-natal period, including bacteraemia, amnionitis, endometritis, and urinary tract infection in pregnant women as well as sepsis and meningitis in neonates and young infants. Infection of the new born may be acquired by the intra-amniotic route or directly during passage through the birth canal.

**Objectives:** This study was undertaken to determine the prevalence of group B *Streptococcus* (GBS) colonization and to analyze related risk factors among pregnant women attending the antenatal clinic of Hawassa Health centre, Adare Hospital Hawassa, Ethiopia.

**Methods:** A total of 139 pregnant women were screened for GBS colonization between May and June 2010. Standard microbiological methods were used to isolate and identify GBS from vaginal and ano-rectal swabs obtained from study subjects. An antimicrobial susceptibility test was performed for all GBS isolates according to the criteria of the Clinical and laboratory Standards Institute (CLSI) by disk diffusion method.

**Results:** A total of 29 out of 139 (20.9%) pregnant women were colonized by GBS. No statistically significant association was observed for GBS colonization with any of socio-demographic characteristics of the study subjects including age, occupation, type of contraceptive used, parity, number of antenatal clinic visits. All GBS strains were susceptible to penicillin, ampicillin, vancomycin and gentamicin. Resistance was observed against erythromycin (6.9%), tetracycline (48.2%), ceftriaxone (10.3%), chloramphenicol (51.7%), ciprofloxacin (13.8%) and norfloxacin (10.3%).

**Conclusion:** This study showed that prevalence of GBS colonization was 20.9% among the study subjects. The finding of this study was comparable with findings reported from developed and developing countries. However, further epidemiological investigations should be done in different parts of the country in order to know the actual GBS colonization rate in pregnant women and to consider the use of intra-partum antibiotics prophylaxis for prevention of early onset GBS-neonatal diseases. [*Ethiop. J. Health Dev.* 2012;26(1):36-42]

## Introduction

Since the mid-1960s, group B *Streptococcus* (GBS) has become the major cause of bacterial infections in the peri-natal period, including bacteraemia, amnionitis, endometritis, and urinary tract infection in pregnant women as well as focal and systemic infections in newborns (1). It is a relatively rare cause of infection in older children and non-pregnant adult women (2). Initial case series reported case fatality ratios as high as 50% among new born in USA. In the early 1980s, clinical trials demonstrated that administering antibiotics during labor to women at risk of transmitting GBS to their newborns could prevent invasive disease in the first week of life (3).

Since the 1970s, GBS has been recognized as the most important infectious cause of morbidity and mortality in newborn infants. Despite the decline in mortality during the past several decades, early onset of GBS disease remains a serious neonatal condition, which may cause

severe neurological damage (4). Today, it is also the leading cause of early invasive infections in newborns worldwide and can also cause life-threatening infections in pregnant women, in immune-compromised adults, and apparently in the general population as well (5). Group B *Streptococcus* transmission is vertical from mother to child. The gastrointestinal tract is the source of vaginal GBS colonization and many adults are colonization with GBS without showing any symptoms. Approximately 10–30% of women of child bearing age carry GBS in the recto-vaginal compartment (6). Treatment and prevention guidelines developed by the Centers for Disease Control and Prevention (CDC) in 1996 (5) led to a significant decline in the incidence of early onset of neonatal disease in institution that adopted and followed these guidelines strictly (7). The CDC recommendations are to screen the entire pregnant mothers before term (35-37 weeks of gestation) and to administer intra-partum prophylactic antibiotics to all of them. In addition, if the expectant Mother's status is not known at labor, chemoprophylaxis

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should be administered to all cases showing one or more of the main risk factors indicated by the CDC and others as being significantly related to higher rates of maternally transmitted neonatal disease (5).

Treatment of colonized mothers succeeded temporarily eradicating the organism, but most of the women were re-colonized within 6 weeks. At birth, 50 to 65% of infants, who were born from colonized mothers had positive GBS cultures taken from mucus membranes and skin (external ear canal, throat, umbilicus, ano-rectal sites) (8). Approximately 98% of the colonized newborns remained healthy, but 1 to 2% developed invasive GBS infection (9).

Published information about the prevalence of colonization by GBS among pregnant women in Ethiopia is scarce (10). Therefore the present study was conducted to determine its magnitude among pregnant women attending the antenatal clinic the Hawassa Health Center.

## Methods

### Study population

A total of 139 pregnant women attending the routine antenatal clinic of the Hawassa Health Center were screened for GBS colonization from May 2010 through June 2010. The approach was based on universal screening of all pregnant women for GBS colonization between 35 and 37 of weeks of gestation (11). Sample size was calculated based on previous prevalence rates reported from Ethiopia (10), with 95% confidence interval, 0.05 margin of error, and a contingency of 10%.

### Culture and identification of GBS

According to guidelines of the CDC (11) and the American College of Obstetricians and Gynecologists (12), swabs were taken from both the lower one third of the vagina and the anal region using sterile cotton swabs by the attending midwife and placed in Amies transport medium (Oxoid, UK) and immediately transported to the Microbiology Laboratory of Hawassa Referral Hospital for culture.

The vaginal and ano-rectal swabs were placed into 1 ml Todd-Hewitt broth (Oxoid, UK) supplemented with 10 µg/ml colistin and 15 µg/ml nalidixic acid (Biomerieux, France) to prevent growth of contaminants. The broth was incubated for 18–24 hours at 35–37°C and sub-cultured on 5% sheep blood agar (Oxoid, UK) and incubated overnight in 5% CO<sub>2</sub> atmosphere for 18–24 hours. All suspected GBS colonies (pin point, with narrow beta-hemolysis) were sub-cultured on blood agar and subjected for Gram stain and catalase test. All gram positive and catalase negative cocci isolates were tested for CAMP test and latex agglutination assay as a confirmatory testing for GBS (Oxoid, UK).

### Antimicrobial susceptibility testing

All procedures for disk susceptibility were performed according to the methodologies described in the manual of the Clinical and Laboratory Standards Institute (CLSI) (13). Fresh subcultures of GBS were used after overnight growth on blood agar plate (Oxoid, UK). The inoculums were standardized by suspending colonies in sterile phosphate buffered saline (pH 7.2) to achieve a turbidity of 0.5 McFarland standards. A sterile cotton swab was dipped into the bacterial suspension, elevated above the liquid and rotated several times against the inside wall of the tube to remove excess inoculums. Then the swab was inoculated on Mueller–Hinton agar plate (Oxoid, UK) supplemented with 5% de-fibrinated sheep blood to obtain confluent growth; antibiotic disks were placed and incubated at 35°C with 5% CO<sub>2</sub> atmosphere for 20 hours.

Ten antibiotic disks (Oxoid) used were: penicillin G (P) (10 IU), erythromycin (E) (15 µg), ceftriaxone (CRO) (30 µg), gentamicin (CN) (10 µg), norfloxacin (NOR) (10 µg), chloramphenicol (C) (30 µg), vancomycin (VA) (30 µg), tetracycline (TE) (30 µg), ampicillin (AMP) (10 µg) and ciprofloxacin (CIP) (5 µg).

The zones of growth inhibition were measured to the nearest whole millimeter using a sliding caliper. The sizes of the inhibition zones were graded according to the CLSI approved standard (13). Each isolate was classified as susceptible, intermediate or resistant to each antibiotic tested.

### Quality control

To maintain the quality of data, pre-test was done before the actual work to check the protocol for isolation of GBS and the questionnaire. In actual work, every sample was processed in triplicates and every result was cross checked by the principal investigator and the co-investigator.

*Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 24923), *Streptococcus pyogenes* (ATCC 19615) were used as quality control throughout the study for culture and antimicrobial susceptibility testing. All the strains were obtained from the Ethiopian Health and Nutrition Research Institute (EHNRI).

### Statistical analysis

Data entry and analysis was done using SPSS version 11.5 software. Prevalence figures were calculated for the total study population and separately by age groups. Chi-square test was used to compare results between the pregnant women with different age groups and with the previous findings from the literature. P-value less than 0.05 were considered statistically significant.

### Ethical Considerations

The research project was ethically cleared by the Institutional Review Board (IRB), Faculty of Medicine of Addis Ababa University study site as well as written informed consent from all participants were also obtained.

**Results****Socio-demographic characteristics**

The socio-demographic characteristics of 139 pregnant women screened for GBS colonization are presented in Table 1. The mean age of the participants was 25.6 years ranging 17-40 years. The majority of the participants were between the ages of 25-29 years (41.0%). Most of them were from the Hawassa area (95.0%) and most of them were house wives (91.4%) while the remaining were students, teachers, and other.

**Overall prevalence**

Out of 139 pregnant women screened, 29 (20.9%) were found to be colonized with GBS.

**Risk factors analysis**

Overall, no statistically significant association of GBS colonization was observed in connection with any of the socio- demographic characteristics of the study subjects as shown in Table 2.

Table 1: **Socio-demographic characteristics of 139 women investigated for GBS, at Hawassa Health Centre, Hawassa, Ethiopia June, 2010**

| Socio-demographic characteristics | Frequency | Percent (%) |
|-----------------------------------|-----------|-------------|
| <b>Age groups in years</b>        |           |             |
| 15-19                             | 9         | 6.5         |
| 20-24                             | 51        | 30.7        |
| 25-29                             | 57        | 41.0        |
| 30-34                             | 17        | 12.3        |
| 35-39                             | 3         | 2.2         |
| 40-44                             | 2         | 1.4         |
| <b>Address</b>                    |           |             |
| Algie                             | 1         | 0.7         |
| Hawassa                           | 132       | 95.0        |
| Tikur Wuha                        | 4         | 2.9         |
| Wondo                             | 2         | 1.4         |
| <b>Occupation</b>                 |           |             |
| House wife                        | 127       | 91.4        |
| Teacher                           | 3         | 2.2         |
| Student                           | 6         | 4.3         |
| Lawyer                            | 1         | 0.7         |
| Accountant                        | 1         | 0.7         |
| Merchant                          | 1         | 0.7         |

Table 2: **Variables associated/not associated with Group B Streptococcus colonization in pregnant women, Hawassa Health center, Hawassa, Ethiopia June, 2010**

| Variables                        | Total | GBS colonization | Percentages (%) | P value |
|----------------------------------|-------|------------------|-----------------|---------|
| <b>Age (in years)</b>            |       |                  |                 |         |
| 15-19                            | 9     | 4                | 44.4            | 0.48    |
| 20-24                            | 51    | 8                | 15.7            |         |
| 25-29                            | 57    | 12               | 21.1            |         |
| 30-34                            | 17    | 4                | 23.5            |         |
| 35-39                            | 3     | 1                | 33.3            |         |
| 40-44                            | 2     | 0                | 0               |         |
| <b>Occupation</b>                |       |                  |                 |         |
| House wife                       | 127   | 25               | 19.7            | 0.12    |
| Teacher                          | 3     | 0                | 0               |         |
| Student                          | 6     | 2                | 33.3            |         |
| Lawyer                           | 1     | 1                | 100             |         |
| Accountant                       | 1     | 1                | 100             |         |
| Merchant                         | 1     | 0                | 0               |         |
| <b>Address</b>                   |       |                  |                 |         |
| Algie                            | 1     | 0                | 0               | 0.49    |
| Tikur wuha                       | 4     | 1                | 25              |         |
| Wondo                            | 2     | 0                | 0               |         |
| Hawassa                          | 132   | 28               | 21.2            |         |
| <b>Type of Contraceptive use</b> |       |                  |                 |         |
| Injectable                       | 85    | 17               | 20              | 0.84    |
| Pills                            | 11    | 3                | 27.3            |         |
| Injectable & pills               | 6     | 1                | 16.7            |         |
| Loop                             | 2     | 0                | 0               |         |
| None                             | 35    | 8                | 22.9            |         |
| <b>Number of ANC Visit</b>       |       |                  |                 |         |
| One time                         | 1     | 1                | 100             | 0.05    |
| Two times                        | 19    | 4                | 21.1            |         |
| Three times                      | 21    | 8                | 38.1            |         |
| Four times                       | 98    | 16               | 16.3            |         |
| <b>Type of Gravida</b>           |       |                  |                 |         |
| Primigravida                     | 39    | 7                | 17.95           | 0.59    |
| Multigravida                     | 100   | 22               | 22              |         |

**Antimicrobial susceptibility data**

The susceptibility pattern of 29 GBS isolated from pregnant women against 10 antimicrobial agents is presented in Table 3. All strains were susceptible to penicillin, ampicillin, vancomycin and gentamicin.

Resistance was observed against erythromycin (6.9%), tetracycline (48.2%), ceftriaxone (10.3%), chloramphenicol (51.7%), ciprofloxacin (13.8%) and norfloxacin (10.3%).

Table 3: Antimicrobial susceptibility pattern of 29 GBS isolated from pregnant women, of Hawassa Health Center, Hawassa, Ethiopia (May 2010-June-2010)

| Antibiotics     | Susceptible   | Intermediate | Resistant     |
|-----------------|---------------|--------------|---------------|
| Penicillin G    | 100% (29/29)  | -            | -             |
| Erythromycin    | 89.7% (26/29) | 3.4% (1/29)  | 6.9% (2/29)   |
| Tetracycline    | 39.3% (11/29) | 14.3 (4/29)  | 48.2% (14/29) |
| Ampicillin      | 100% (29/29)  | -            | -             |
| Vancomycin      | 100% (29/29)  | -            | -             |
| Ceftriaxone     | 89.7% (26/29) | -            | 10.3 (3/29)   |
| Chloramphenicol | 20.7% (6/29)  | 27.6% (8/29) | 51.7% (15/29) |
| Ciprofloxacin   | 86.2% (25/29) | -            | 13.8% (4/29)  |
| Gentamicin      | 100% (29/29)  | -            | -             |
| Norfloxacin     | 89.5% (26/29) | -            | 10.3%(3/29)   |

### Discussion

In the present study the overall prevalence of Group B *streptococcus* (GBS) colonization among pregnant women was found to be 20.9%. Similar findings have been reported in other African countries such as in Malawi, Egypt, Zimbabwe, Gambia, and Tanzania; the prevalence of GBS in these countries ranges from 16.5-23% (14-18).. However low colonization rate was reported in a previous study conducted in Ethiopia (9%) (10) and Mozambique (1.8%) (19).

Different studies conducted in Latin America and the Caribbean also showed results similar to the present study such as in Brazil (17-33%) (20-23). However lower prevalence was reported in some Latin American countries such as Peru (6%) (24) and Argentina (3.2%) (25).

The rate of GBS colonization in this study is almost similar to the findings reported in some European countries. Two studies from Italy found a GBS colonization rate of 17.9% (26), and 18% (27). Studies from Poland and Switzerland also found a colonization rate of 17.2% (28) and 21% (29), respectively. Another study in a multicultural population of pregnant women in the Netherlands also showed a colonization rate of 21% (4). However, lower GBS colonization rate have been reported in some Mediterranean countries such as those e.g. Istanbul and Elazin in Turkey giving 8% (30) and 8.7% (31), respectively. A study conducted in a city of Northern Greece also found a low colonization rate of 6.6% (32).

Among 21 studies conducted in pregnant women in 13 European countries, GBS vaginal colonization rates ranged from 6.5 to 36%, with one third of the studies reporting rates of 20% or greater. The regional rates were Eastern Europe 19.7–29.3%, Western Europe 11–21%,

Scandinavia 24.3–36%, and Southern Europe 6.5–32% (33), which is in agreement with the present study.

The rates obtained in the present study one, more or less, similar with findings reported from most Asian countries like Thailand, 16-18% (34, 35), Iran 20-26% (36, 37) and Saudi Arabia 27.6% (38). However, lower prevalence rates were reported in by some Asian countries; in Hong Kong 10.4% (39) and Korea 3.9% (40), with similar findings from Australia (12.9%) (41).

Knowledge about the risk factors contributing to GBS colonization in pregnant women is relevant to minimize the morbidity and mortality associated with maternal and neonatal GBS infections. In the present study, no statistically significant association was observed for GBS colonization in the study subjects with any of the socio-demographic characteristics as outlined in Table 2. Similar findings have been reported in studies conducted elsewhere (22, 24, 42). However studies conducted in Athens and Hong Kong showed that GBS colonization rate was high among pregnant women who work outside their homes and those who had frequent visits of antenatal clinics (32, 39).

In our study, the susceptibility pattern of 29 GBS isolated from pregnant women against 10 antimicrobial agents is presented in Table 3. All strains were susceptible to penicillin, ampicillin, vancomycin and gentamicin. However resistance was observed against chloramphenicol (51.7%), tetracycline (44.8%), erythromycin (6.9%), ceftriaxone (10.3%), ciprofloxacin (13.8%) and norfloxacin (10.5%). Similar findings have been reported in studies conducted in Tanzania (18), USA (43, 44), Canada (45) and Lebanon (46).

The use of intra-partum vertical transmission of sepsis has increased si

Disease Control (CDC) published guidelines in 1996 and to increase the efficiency of the existing guideline, the revised one was released in 2002. The antibiotic of choice is either penicillin G or ampicillin (44).

The current CDC guidelines recommend that patients not allergic to penicillin should receive penicillin or ampicillin. Individuals with a minor allergy to penicillin should receive cefazolin while individuals with a major allergy should receive clindamycin or vancomycin if GBS is known to be sensitive for those antibiotics (44).

One of the limitations of the present study is that serotyping of GBS was not done because of the lack of anti-sera. GBS can be sub-typed into 9 serotypes; type Ia, Ib, I a/c, I b/c, II, III, IV, V and VI based on their capsular polysaccharide using specific anti-sera. Knowledge about the prevalent GBS-serotypes in a given country is very important to develop and implement effective vaccine for prevention of neonatal GBS disease. However, there is a big difficulty in developing group B streptococcal vaccines because of the existence of multiple serotypes in different locations and geographic variations in serotype distribution (47).

In conclusion, the present study revealed a colonization rate of 20.9% with GBS among pregnant women attending the antenatal clinic of the Hawassa Health Center, Hawassa, Ethiopia. No statistically significant association was observed with any of the socio-demographic characteristics of the study subjects with GBS colonization. All Resistance was observed against erythromycin (6.9%), tetracycline (48.2%), ceftriaxone (10.3%), chloramphenicol (51.7%), ciprofloxacin (13.8%) and norfloxacin (10.3%). Large-scale epidemiological studies should be carried out in different parts of the country in order to know the actual GBS colonization rate and GBS serotypes. Further, assessment of risk factors associated with maternal GBS colonization is required. As more data regarding GBS and its serotypes in Ethiopia became available, it is possible to consider implementation of prevention guideline used by other countries. In the long run it is also possible to develop vaccine to prevent early onset neonatal disease caused by GBS.

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