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ORIGINAL ARTICLE

MATERNAL VAGINAL COLONIZATION OF GROUP B STREPTOCOCCUS AND NEONATAL TRANSMISSION DURING DELIVERY IN A REFERAL HOSPITAL IN ADDIS ABABA

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ABSTRACT

Introduction: *Streptococcus agalactiae or Group B Streptococci frequently colonizes the human genital and gastrointestinal tract and a risk factor for subsequent infection in pregnant women and newborns. The study was conducted to determine the prevalence of maternal vaginal colonization of Group B Streptococci during delivery, transmission rate to the newborns, to identify the possible risk factors and determine the antimicrobial susceptibility pattern of Group B Streptococci isolates.*

Methods: *A cross-sectional study design was used to enroll 250 pregnant women coming for delivery at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia. Both maternal vaginal and neonatal oropharyngeal swabs were collected and inoculated onto CHROMagarTM StrepB and 5% sheep blood agar plates. Isolates were identified based on the mauve color on GBS CHROMagar, colony morphology, gram reaction and hemolysis on 5% sheep blood agar. Antimicrobial susceptibility testing was performed by using Kirby–Bauer disk diffusion method according to the Clinical Laboratory Standard Institute guidelines.*

Results: *The overall prevalence of GBS colonization among the participants was 23.6% with a transmission rate to the newborns of 47.4%. Group B Streptococci colonization was significantly associated with occupation and parity ($p< 0.05$) GBS isolates had significantly high resistance level to cefepime (59.4%) and penicillin (57.7%). Highest susceptibility to vancomycin (93.3%), clindamycin (86.4%) and chloramphenicol (79.6%) were observed.*

Conclusions: *There was high rate of maternal colonization and maternal to child transmission of Group B Streptococci with resistance to commonly used antibiotics.*

Keywords*: Antimicrobial susceptibility, Group B streptococcus, Intra-partum antibiotic prophylaxis, maternal colonization*

INTTRODUCTION

Maternal mortality is unacceptably high worldwide. Every day, approximately 830 women die from preventable causes related to pregnancy and child birth. Ninety nine percent of all maternal death occurs in developing countries and it is significantly higher in women living in rural and poorer countries. Majority of deaths occur because of bleeding, infections, high blood pressure, obstructed labor and unsafe abortion (1).

Streptococcus agalactiae (Group B streptococcus; GBS) is one of the leading causes of neonatal sepsis and meningitis. GBS is also associated with preterm labor or membrane rupture, as well as urinary tract infections, postpartum endometritis, postpartum wound infection, septic pelvic thrombophlebitis and endocarditis in females (2).

The vagina and the perianal regions are the major reservoirs for GBS, and the colonization of these regions is a risk factor for subsequent infection in pregnant women and newborns (3). About 50-60% of infants born to colonized mothers have positive GBS cultures from skin and mucous membranes, and 1-2% of these colonized newborns develop invasive GBS infection (4). Colonization of pregnant women by GBS is major risk factors for maternal as well as neonatal infections which contribute significantly to maternal as well neonatal death. It has a significant risk for adverse obstetric outcomes (5-7).

GBS causes two types of infections in neonates, early onset disease which occur during the first week of life, within the first 24 - 48 hours after birth and late onset disease which occurs after the first six days of life. In addition, colonized females may get infections including urinary tract infections, endometritis or chorioamnionitis (8-9).

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Studies from different part of Ethiopia showed high carriage rate of GBS among pregnant women. However, further epidemiological investigations is required in different parts of the country in order to know the actual GBS colonization rate of pregnant women and to consider the possibility of implementing prophylactic treatment to prevent potential adverse maternal and neonatal outcomes 10-12).

There are many potential opportunities for reducing the burden of early onset neonatal bacterial and maternal sepsis. However, the benefit of package of interventions can only be maximized if devised using high quality, reliable data on the burden and causes of morbidity having local data. Moreover, Intrapartum antibiotic prophylaxis (IAP) also raises concerns about emerging antimicrobial resistance and neonatal microbiome development (13). So there is a need to conduct this study. The objectives of the study were to determine the prevalence of maternal vaginal colonization of GBS, transmission rate to the newborns, associated risk factors and determine the antimicrobial susceptibility pattern of GBS isolates.

PATIENTS AND METHODS

A cross-sectional study was used to determine the prevalence of maternal GBS colonization and neonatal transmission rate during delivery and postpartum at Tikur Anbessa Specialized Hospital (TASH), in Addis Ababa, Ethiopia. The sample size was calculated by using the single population proportion estimation formula by taking 20.6% prevalence (p) of maternal colonization with GBS (14). Considering 5% non-response rate the final sample size become 257 pregnant women coming for delivery were included. After written informed consent was obtained from study participants and assents from parents or guardians for newborn infants was obtained, vaginal swabs were collected from the mucosa of the lower third of the vagina without using a speculum as per CDC recommendations and oropharyngeal swabs were collected from the newborn immediately after delivery by the attending midwife (15). The swabs were placed in Stuart transport media (DIFCO) and transported immediately to the post graduate Bacteriology laboratory of the Department of Microbiology, Immunology and Parasitology, Addis Ababa, University.

Inclusion criteria: All consenting pregnant women during labor. Pregnant women with history of antibiotic(s) use within two weeks prior to recruitment, premature rupture of membranes (PROM), active vaginal bleeding, and women with emergency obstetric conditions which need immediate interventions were excluded.

The study protocols were ethically approved by Institutional Review Boards (IRBs) of the College of Health Sciences, Addis Ababa University. Written informed consent was obtained from study participants while assents from parents or guardians for newborn infants were obtained. Identified GBS isolates and their antimicrobial susceptibility pattern results were reported to the attending physician for subsequent treatment and follow up.

Socio-demographic data collection

Demographic data was recorded, including age, marital status, occupation, monthly family income, level of education using a well-designed questionnaire.

Obstetrics factors data collection

Gravidity and parity, history of abortion, gestational age of current pregnancy, number of ANC visit, use of hormonal contraceptives, sex of the baby, weight of the baby, first minute APGAR and fifth minute APGAR also taken from study subjects.

Specimen processing and identifications of GBS

The swab(s) were removed from transport medium and inoculated into CHROMagar TM StrepB base (CHROMagar microbiology, France) and 5% sheep blood agar plates (Oxoid, UK). The inoculated CHROMagar TM StrepB was incubated in aerobic conditions at 37°C for 18-24 hours. On the other hand, the inoculated sheep blood agar medium was incubated aerobically at 35–37 ^{o}C for 18–24 h in 5% CO_2. After incubation period, plates were examined. The suspected GBS colonies on CHROMagar TM StrepB were mauve appearance and on blood agar β-haemolytic and non-haemolytic were picked and sub-cultured onto sheep blood agar and incubated aerobically at 35–37 ^{o} C for 18–24 h in 5% CO_2. A known GBS (ATCC 27956) as a positive control and *Enterococcus faecalis* (ATCC 29212) as a negative control was also streaked along with the samples. GBS isolates were further identified by using conventional methods such as Gram staining (Gram-positive cocci arranged in chains), Catalase test (negative), CAMP test on blood agar (positive), hippurate hydrolysis (purple color), Bacitracin disk (resistant) and latex agglutination test with specific antisera (Strepto B latex kit, Liofilchem, Italy) (2,11,16).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of GBS isolates were performed with seven antibiotics (Oxoid, UK) based on the Kirby-Bauer disk diffusion method on 5% sheep blood containing Mueller-Hinton agar (Oxoid, UK).

The following antibiotic discs and concentrations were selected according to Clinical Laboratory Standards Institute (CLSI) guidelines: penicillin G (PEN, 10IU), ampicillin (AMP, 10 µg), clindamycin (Da, 2 µg), erythromycin (ERT, 15 µg), chloramphenicol (CAP, 30 µg), cefepime (FEP, 5 µg) and vancomycin (VAN, 30 µg). *Streptococcus pneumoniae* ATCC 49619 was used as quality control strain. Plates were incubated at 35 ◦C for 24 h in 5% CO_2, and the diameters of zone of inhibition were measured and results interpreted as sensitive, intermediate and resistant according to CLSI guideline (17).

Statistical Analyses

Data were entered, cleaned and analyzed using SPSS Statistical Software version 25 (IBM company, Comp.soft-sys.stat.spss.). Qualitative data were described using number and percent. Quantitative data were described using mean, standard deviation. Associations between independent and dependent variables were computed using bi-variate analysis. Differences were considered statistically significant for P-value ≤ 0.05, used to evaluate the association of GBS colonization and socio-demographics or clinical obstetric variables

RESULTS

Socio-demographic Characteristics

A total of 250 pregnant women were included in the study. The age were between 18 to 40 years with a mean of 26.42 (4.99) years. Most (53.6%), of the participants were between the age of 25 – 35 years, while 35.6 % and 27 (10.8%) where in age range of less than 25 and above 35 respectively. The majority of the participants was housewives with 194(77.6%) participants and followed by civil servant 23(9.2%) business women 20(8%) and students 13 (5.2%) (Table 1).

Obstetric characteristics and outcome of pregnant women at term

Fifty two percent of the participants were nulliparous, followed by women who are para 2 - 4 which was 108 (43.2%) and grand multiparous was 12 (4.8%). Fifty participants (20%) had history of abortion and 1.9% of parous women had history of stillbirth. Sixty percent of the participants were with gestational age (GA) 40 and above and the rest 37.6% were below 40 weeks GA. Around 96% had ANC follow up four and above. Three fourth of the participants had a history of hormonal contraceptives usage. From all 250 delivery 52% were male and 48% were female with birth weight ranging 2.3 to 4.5kg with a mean birth weight of 3.23kg, there were 12(4.8%) babies weighing <2.5 kg, 221(88.4%) babies weighing 2.5 to 4kg and 17 (6.8%) were babies weighing >4 kg. Majority of babies had APGAR score of ≥ 7 in first and fifth minute with 246(96.8%) and 248(99.2%) respectively and only 4 (3.2%) and 2 (0.8%) had APGAR of < 7 at first and fifth minute (Table 2).

Table 1: Socio-demographic characteristics of pregnant women (n=250) at TASH, Addis Ababa, Ethiopia, 2018.

Characteristics	Frequency	Percent
Age group		
< 25	89	35.6
25-35	134	53.6
> 35	27	10.8
Marital status		
Single	10	4
Married	240	96
Occupation		
House wife	194	77.6
Civil servant	23	9.2
Student	13	5.2
Business women	20	8
Educational status		
No formal education	83	33.2
Elementary school	99	39.6
High school	41	16.4
Collage	27	10.8

Table 2: Obstetric characteristics of pregnant women at term at TASH Addis Ababa, Ethiopia, 2018.

Obstetric factors	Frequency	Percent
Gravidity and parity		
Nulliparous	130	52
Para 2 – 4	108	43.2
Grand multiparous	12	4.8
History of Abortion		
Yes	50	20
No	200	80
History of still birth		
Yes	3	1.9
No	151	98.1
Gestational age of current pregnancy		
37 – 39+6 weeks	94	37.6
40 – 42 weeks	156	62.4
Number of ANC b visit		
< 4	11	4.4
Four and above	239	95.6
Use of hormonal contraceptives		
Yes	193	77.2
No	156	22.8
Sex of the baby		
Female	120	48
Male	130	52
Weight of the baby		
<2.5kg	12	4.8
2.5 -3.99 kg	221	88.4
4 kg and above	17	6.8
First minute and fifth minute APGAR a		
<7	2/4	1.6 / 0.8
Seven and above	246/248	96.8/ 99.2

APGAR [a]: Appearance, Pulse, Grimace, Activity, Respiration, ANC[b]: Antenatal Care

Prevalence of maternal GBS colonization and neonatal transmission rate

The overall prevalence of GBS colonization as determined by chromogenic culture among 250 pregnant women at term during labor was 59 (23.6%). On the other hand, a total of 250 samples from the babies were taken and over all 44 babies found to have positive culture for GBS and of this 28 babies were born from mothers with positive GBS culture with a transmission rate of 47.4 %. In our study, we have found that 15 babies born from GBS negative women were found to have positive culture for GBS.

Associated risk factors for GBS colonization and transmission

The association of the social-demographic factors with GBS colonization and transmission is demonstrated on (Table 3). There was no significant association between age group, marital status, income and educational status with GBS colonization rate ($p>0.05$). However, being a house wife is significantly associated with lesser risk of positivity for GBS ($p=0.024$).

Table 3: Association of GBS and socio-demographic factors of pregnant women at term at TASH, Addis Ababa, Ethiopia, 2018.

Characteristics	GBS n (%) Present	Absent	Total	P-value a	COR b	AOR c
Age Group						
< 25	24 (40.7)	65 (34)	89	0.623	1.292[0.466 –3.587]	1.132 [0.388 – 3.308]
25-35	29 (49.2)	105 (55)	134	0.947	0.967[0.357 –2.617]	0.899 [0.317 – 2.552]
> 35	6 (10.2)	21 (11)	27		1	1
Marital status						
Non married	2 (3.4)	8 (4.2)	10	0.785	0.803[0.166 –3.888]	0.728 [0.129 – 4.117]
Married	57 (96.6)	183 (95.8)	240		1	1
Occupation						
House wife	44 (74.6)	150 (78.5)	194	0.024	0.545[0.205 –1.449]	0.219 [0.064 – 0.754]
Civil servant	6 (10.2)	17 (8.9)	23	0.527	0.655[0.177 –2.424]	0.885 [0.135 – 5.811]
Student	2 (3.4)	11 (5.8)	13	0.228	0.338[0.058 –1.972]	0.202 [0.030 – 1.351]
Business women	7 (11.9)	13 (6.8)	20		1	1
Educational status						
No formal education	22 (37.3)	61 (31.9)	83	0.658	1.262 [0.451 -3.536]	2.602 [0.316 – 21.432]
Elementary school	25 (42.4)	74 (38.7)	99	0.746	1.182[0.429 –3.260]	2.031[0.271– 15 232]
High school	6 (10.2)	35 (18.3)	41	0.425	0.600[0.171 –2.103]	0.985 [0.121 – 8.040]
Collage and above	6 (10.2)	21 (11)	27		1	1

calculated by using binary logistic regression [a]; Crude odds ratio [b], adjusted odds ratio [c,] 1- Reference Group

3.4. Association of obstetrics factors and colonization and transmission of GBS isolates

The results of our study showed that primigravid women had 27.6 % risk of being colonized by GBS as compared to multiparous (para 2 -4) and grand multipara women's which showed 16.6% and 41.6% rate of colonization respectively and it showed that being para 2 -4 has lesser risk for colonization by GBS. It was also seen that women with history of abortion had 22% rate of colonization as compared to those women with no recent history of abortion which was 24.4% but there was not a statistically significant difference between the two groups (p>0.05).

Women who's GA were between 37 – 40 weeks had a colonization rate of 25.5% as compared to women with GA above 40 which was 22.4% of transmission (p>0.05). Moreover, there was no significant association between GBS colonization rate with Obstetrics factors **(**ANC follow up, history of use of hormonal contraceptives, sex of the baby, weight of the baby and babies having APGAR) (p>0.05) (Table 4).

Table 4: Association of GBS and obstetric factors and neonatal transmission of pregnant women at term at TASH, Addis Ababa, Ethiopia, 2018.

	GBS n (%)		Total	P-value b	COR c	AOR d
Obstetric factors	**Present**	**Absent**				
Gravidity and parity						
Nulliparous	36 (61)	94 (49.2)	130	0.313	0.536[0.160– 1.798]	0.507[0.123-2.092]
Para 2 -4	18 (30.5)	90 (47.1)	108	0.047	0.280[0.080– 0.981]	0.284[0.079-1.013]
Grand multiparous	5 (8.5)	7 (3.7)	12		1	1
History of Abortion						
Yes	11 (18.6)	39 (20.6)	50	0.333	0.470[0.102– 2.167]	1.004[0.275-3.662]
No	48 (81.4)	152 (79.4)	200		1	1
Gestational age of current pregnancy						
37 – 39+6 weeks	24 (40.7)	70 (36.6)	94	0.577	1.185 [0.652 -2.153]	1.261[0.6692.379]
40 – 42 weeks	35 (59.3)	121 (63.4)	156		1	1
Number of ANC visit						
Less than four	1 (1.7)	10 (5.4)	11	0.272	0.312[0.039– 2.490]	0.268[0.033-2.209]
Four and above	58 (99.3)	176 (94.6)	234		1	1
Use of hormonal contraceptives						
Yes	44 (74.6)	149 (78)	193	0.583	0.827[0.419– 1.630]	
No	15 (25.4)	42 (22)	59		1	1
Sex of the baby						
Male	27 (45.8)	103 (53.9)	130	0.288	0.728[0.405– 1.308]	0.660[0.357-1.219]
Female	32 (54.2)	88 (46.1)	120		1	1
Weight of the baby						
Less than 2.5kg	2 (3.4)	10 (5.2)	12	0.655	0.650 [0.098 -4.290]	0.502[0.067-3.772]
2.5 -3.99 kg	53 (89.8)	168 (88)	221	0.966	1.025 [0.321 -3.279]	1.207 0.352-4.143]
4 kg and above	4 (6.8)	13 (6.8)	17		1	1
First minute APGAR a						
≤7	2 (3.4)	2 (1)	4	0.236	3.316[0.457– 24.069]	8.042[0.391-165.456]
Seven and above	57 (99.6)	189 (99)	246		1	1
Fifth minute APGAR						
< 7	1 (1.7)	1 (0.5)	2	0.404	3.276 [0.202 -53.194]	0.429[0.006–29.488]
Seven and above	58 (98.3)	190 (99.5)	148		1	1

*APGAR [a]: Appearance, Pulse, Grimace, Activity, Respiration, [b] calculated by using binary logistic regression; Crude odds ratio [c], adjusted odds ratio [d,] 1-Reference Group

3.5. Antimicrobial susceptibility patterns of GBS isolates

Of the 59 identified GBS isolates, the highest resistance level was recorded to cefepime (59.4%) followed by penicillin (57.7%) and ampicillin (54.3%). The most active drugs for GBS isolates were vancomycin, clindamycin and chloramphenicol with susceptibility results of 93.3, 86.4, and 79.6%, respectively. Moreover, 3(5%) isolates of GBS isolates showed intermediate sensitive to erythromycins and chloramphenicol for each (Table 5).

Table 5: Antimicrobial susceptibility patterns of 59 GBS isolates at TASH, Addis Ababa, Ethiopia, 2018.

	No. (%) of isolates (CLSI, 2018)		
Antibiotics	**Susceptible**	**Intermediate**	**Resistant**
Penicillin G	42.3% (25/59)	-	57.7% (34/59)
Ampicillin	45.7% (27/59)	-	54.3% (32/59)
Cefepime	40.6% (24/59)	-	59.4% (35/59)
Erythromycins	64.4% (38/59)	5% (3/59)	30.6% (18/59)
Chloramphenicol	79.6% (47/59)	5% (3/59)	15.4% (9/59)
Vancomycin	93.3% (55/59)	-	6.7% (4/59)
Clindamycin	86.4% (51/59)	-	13.6% (8/59)

DISCUSSIONS

The overall prevalence of GBS colonization in this study was (23.6%). Our results were comparable with other reports in different parts of the world where GBS colonization was found to be (21%) in studies conducted from Netherland (18) and Portugal (24.4%) (19). It is also relatively comparable result were reported in African countries such as Zimbabwe (21%) (21) and South Africa (30.9%) (22). However, the finding of this study is lower when compared to colonization rate from US (41%) (20). Moreover, different regions of Ethiopia showed different figures of antepartum colonization such as in Jimma (19%) (10), Tigray (11.3%) (23), and two studies in Addis Ababa (7.2%) (12) and (14.4%) (24). All the observed difference in colonization rate of GBS could be due to geographical differences, study design, year of study, sample size difference and laboratory methods used.

Assessment of antepartum vaginal GBS colonization is used as an indirect measure of intrapartum colonization rate and intrapartum assessment will give us a clear picture of rate of colonization as well as the neonatal transmission (4,22), thus this study was performed to assess the intrapartum colonization rate and it is showed that, intrapartum colonization rate was found to be (23.6%). Even though, there were no data on intrapartum colonization of GBS in Ethiopia. Comparable results were reported in elsewhere in Zimbabwe (21%) (21), Iran (20.6%) (14) and Portugal (24.4%) (25).

Identifying risk factors and reducing risk is one of the most important steps in prevention of maternal and neonatal GBS colonization (13). In the present study, risk factors associated with maternal and newborn GBS colonization rate was determined; however, most of the variables were not significant association between maternal and neonatal GBS colonization rate and measured risk factors was found ($P>0.05$).

In the current study, we have found that 15 babies born from GBS negative women were found to have positive culture for GBS. This could be explained by that the hospital environments including medical equipment such as monitors and other high touch surfaces have been implicated as the source, indirect transfer of GBS from infant-to-infant transmission via contaminated hands of health care workers especially from newborn units also involved (26,27). However, hand hygiene and environmental cleaning can render healthcare associated late onset GBS disease preventable as well (27,28).

In our study, only occupation and parity were significantly associated with GBS colonization and being house wife has decreased risk of GBS colonization. Moreover, para 2-4 has decreased risk of colonization.

In contrast to our results studies conducted in USA showed that socio-demographic factors such as occupation, race, higher BMI, higher income, and higher education of pregnant women showed significant association with GBS colonization where as women who smokes has less risk of GBS colonization (29). Comparable with our results, studies conducted in different countries such as Brazil [30], Thailand [31], and Netherland (32) there was no association between studied socio-demographic factors and GBS colonization among pregnant women.

In our study, highest resistance level was recorded to cefepime (59.4%) followed by penicillin (57.7%) and ampicillin (54.3%). It could be explained by that the ease of access of this antibiotics in the study setting, the frequent use of antibiotics for therapy and prophylaxis. Such findings coincides with studies conducted in Addis Ababa (penicillin 55% to 81.5%), ampicillin (86% to 91%) (12).

The most active drugs for GBS isolates were vancomycin, clindamycin and chloramphenicol with susceptibility results of 93.3, 86.4, and 79.6%, respectively. Our results were comparable with studies conducted in elsewhere such as from India (33), Zimbabwe (21), Jimma (10) and Addis Ababa (24). These antibiotics are not recommended for routine intrapartum antibiotic prophylaxis in Ethiopia. Therefore, the sensitivity pattern of commonly used antimicrobials like penicillin, ampicillin, showed less sensitive; it account less than 50% which calls for routine culture and sensitivity test and revision of the antibiotics recommendation for GBS colonized mothers in an area with no facility for culture and sensitivity test.

Conclusion

Our results showed high prevalence of GBS colonization among pregnant women in Addis Ababa, Ethiopia. The study also showed 47.4% transmission of GBS from mothers with positive vaginal colonization for GBS. GBS colonization by the mothers and transmission to their offspring showed statistical significant association with occupation and parity. High level of resistance was observed to commonly prescribe antimicrobial agents.

Recommendation

The high rate of maternal GBS colonization and high rate of maternal to child transmission of GBS calls for universal screening of pregnant women during gestational age of 35 -37 weeks. The low sensitivity and high resistance to the commonly used antibiotics calls for routine test for antibiotics susceptibility before administration of antibiotics.

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Competing Interest

The authors declare that this manuscript was approved by all authors in its current form and that no competing interest exists.

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