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Prevalence, Antimicrobial Susceptibility Pattern and Factors Associated with GBS Colonization in Pregnant Women at the Regional Hospital Bamenda (RHB)

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Abstract

Introduction: Maternal asymptomatic colonization with GBS (Group-B *Streptococcus*) has become a major cause of sepsis, meningitis and encephalopathy in neonates alongside premature births, stillbirths and post-natal infections. Routine screening of pregnant women for GBS carriage and antimicrobial susceptibility are therefore necessary. This study was aimed at evaluating the prevalence, antimicrobial susceptibility pattern and factors associated with GBS colonization in pregnant women at the Regional Hospital Bamenda (RHB). **Materials and Methods:** Vaginal and rectal swab samples were collected from 121 pregnant women in the 3rd trimester at the RHB from December 2017 to May 2018. Sociodemographic, obstetric and neonatal history and some clinical parameters were obtained through a questionnaire approach. Cultures for the isolation and identification of GBS from the samples were done and grouping as well as susceptibility testing of GBS isolates was done. **Results:** The colonisation rates were 5.8% (7), 1.7% (2) and 5.8% (7) for rectal, vaginal and concomitant recto-vaginal carriage. GBS was isolated in the vagina/rectum of 16 participants (13.2% prevalence). Of the 16 GBS strains used for *in vitro* susceptibility test, no resistance to ampicillin, oxacillin, amoxicillin-clavulanate, ceftriaxone, erythromycin, imipenem, aztreonam and clindamycin was recorded. 6.3% (1) of the strains had intermediate susceptibility to ampicillin and amoxicillin-clavulanic acid. Of the isolates examined, 37.5% (6), and 12.5% (2) were respectively sensitive to gentamycin and levofloxacin.

Maternal overweight, HIV positive status, history of PROM and spontaneous abortion, presence of *Gardnerella vaginalis* and *Candida albicans* had a high rate of GBS colonization but only HIV positive status had a statistical significance (p = 0.01). Other microbes isolated were *Gardnerella vaginalis* (55.4%, 67), *Candida albicans* (40.5%, 49), and *Candida spp* (12.4%, 15). **Conclusion:** GBS prevalence was 13.2%. GBS had decreased susceptibility to some antibiotics. Only HIV positive status was significantly associated with GBS colonization.

Keywords

Group B *Streptococcus* (GBS), Prevalence, Anti-Microbial Susceptibility Profiles, Pregnant Women, Carriage, Colonization

1. Introduction

Background

In the 1970s, *Streptococcus agalactiae*, also referred to as Lancefield group B *Streptococcus* (GBS), emerged abruptly as an important pathogen causing invasive bacterial infections. In general, GBS is a harmless commensal bacterium which comprises part of the normal flora of the gastrointestinal and urogenital tracts of up to 30% of healthy human adults in whom it doesn't manifest any symptoms, asymptomatic carriers [1]. Although a part of the normal flora of the gut and genital tract, GBS has become the major cause of bacterial infections in the perinatal period, including bacteraemia, amnionitis, endometritis, and urinary tract infection in pregnant women as well as sepsis and meningitis in neonates and young infants.

Neonatal mortality accounts for over 46% of under-five deaths worldwide [2]. Globally, neonatal mortality is a critical problem resulting in over 3 million deaths per year, that is 7000 new-born deaths everyday with two thirds occurring in the first week of life [2]. The 3rd Sustainable Development Goal of the UN has as one of its targets to end preventable deaths of new-borns, with all countries aiming to reduce neonatal mortality to as low as 12 per 1000 live births by the year 2030 [3]. In Cameroon neonatal mortality rate was 28 per 1000 live births in 2013 with the major causes being neonatal sepsis, prematurity and birth asphyxia [4]. Group B *Streptococcus* is among the leading causes of early-onset sepsis (EOS) worldwide with a 12% global case-fatality rate, which can be 3 times higher in low-income countries [5]. Although EOS from GBS has been successfully reduced by 79% with the use of intrapartum antibiotic prophylaxis (IAP) in most high-income countries [6], low and medium-income countries including Cameroon do not routinely offer such screening and treatment.

Group B streptococcal diseases are however not restricted to new-borns. They are also common in pregnant women and have been recognized as an ever-growing cause of still births and premature delivery. In women during pregnancy or the

postpartum period, genital tract colonization with GBS is usually asymptomatic, but GBS clinical manifestations include urinary tract infections, chorioamnionitis, endometritis, wound infections associated with caesarean delivery or episiotomy, puerperal sepsis and, occasionally, meningitis, septic thrombophlebitis, or other serious complications. All these infections during pregnancy and in the puerperal period make up 11% of the causes of maternal mortality worldwide [7]. The 3rd sustainable development goal has as objective to attain an average global target of Maternal Mortality Ratio (MMR) of less than 70 maternal deaths per 100,000 live births by 2030; a stillbirth rate of 12 per 1000 total births by 2030 [7]. Asymptomatic colonization with GBS is common worldwide, and depending on the population, between 6.5% [8] and 43.6% [9] of pregnant women are colonized with GBS in the vagina or rectum. Maternal GBS colonization varies by population characteristics such as age, parity, socio-economic status, geographic location [10], presence of sexually transmitted diseases [11] and sexual behaviour [12].

Group B *Streptococcus* colonization of the genitourinary tract during pregnancy is common among women from high-income countries and prevalence rates vary from 10% to 30%; however, less is known about its epidemiology in low and medium-income countries [10]. GBS carriage rate among pregnant women in the world is 17.9%, with the highest rates recorded in Africa; 22.4% [13]. In Yaoundé, Cameroon available data places this rate between 6.7% - 14% [14] [15] [16].

Although studies in high-income countries suggest that bacterial culture from rectal and vaginal swabs is superior to risk factor assessment in the implementation of preventive measures [17] [18], it still remains a huge problem due to the low socio-economic status in low and medium-income countries like Cameroon; hence the need to properly assess risk factors and implement preventive measures accordingly [14].

The known risk factors of GBS colonization include; delivery at <37 weeks' gestation, intrapartum temperature ≥ 38.0˚C, or rupture of membranes for ≥18 hours [19]. A study in Brazil reported maternal age, number of sexual intercourse/week, occurrence of previous spontaneous abortion, presence of vaginal candidosis and cytolytic vaginosis to be statistically associated with streptococcal colonization [20]. After conducting a large population-based study, the Center for Disease Control and Prevention (CDC) of the United States currently recommends screening of all pregnant women for GBS between 35 and 37 weeks of gestation with all GBS positive women to receive IAP with beta-lactamines [21]. Additionally, there is the problem of antimicrobial resistance with some betalactamines having decreased susceptibility to isolated GBS strains [22].

Antimicrobial resistance (AMR) has become one of the biggest threats to global health and endangers other major priorities such as human development. All around the world, many common infections are becoming resistant to the antimicrobial medicine used to treat them, resulting in longer illnesses and more deaths. At the same time, not enough new antimicrobial drugs, especially antibio-

tics, are being developed to replace older and increasingly ineffective ones. This pushed global leaders at the United Nations General Assembly in September 2016 to commit to fighting AMR together. This was only the fourth time in the history of the UN that a health topic was discussed at the General Assembly [23].

2. Materials and Methods

2.1. Study Site

The clients were recruited at the ante-natal unit of the Regional Hospital Bamenda while culture and analysis of the specimens were carried out at the Bacteriology Laboratory of the same institution. RHB is a referral hospital in the North West Region of Cameroon.

The RHB has a total bed capacity of about 600 beds. In addition to being the main referral hospital of the region, it is also the teaching hospital of the Faculty of Health Sciences of the University of Bamenda. It has an Obstetrics and Gynaecology unit made up of a; Gynaecology ward, post-natal ward, Maternity, an antenatal unit and a family planning unit. The staff of this service include 2 Gynaecologists/Obstetricians, 5 midwives and 15 nurses.

2.2. Study Type

A cross-sectional hospital based study was carried out.

2.3. Duration of Study

This study was carried out over a period of 6 months: December 2017 to May 2018.

2.4. Study Population

This study involved pregnant women attending the antenatal clinic of the Bamenda Regional Hospital.

2.4.1. Inclusion Criteria

- Pregnant women in the 3rd trimester consulting at the antenatal clinic of the Bamenda Regional Hospital who gave their written or verbal informed consent.

2.4.2. Exclusion Criteria

- All pregnant women who refused to give verbal or written informed consent.
- All pregnant women in the 3rd trimester who had done intimate douching the same day.
- All pregnant women who had taken antibiotics within the previous 2 weeks before recruitment.
- All women who had symptoms of gastroenteritis and haemorrhoids.

2.5. Sampling

Sampling was non exhaustive consecutive and by convenience.

Sample Size

Using the Cochrane formula;

$$n = Z_{1-\alpha}^2 \left[P(1-P) \right] / d^2 ,$$

at 5% type 1 error and a 3rd trimester prevalence of 11.1% as detected by Adawaye *et al.* in 2014 [15], we had a minimum sample size of 152 participants.

2.6. Ethical Considerations

Potential ethical issues related to this study included;

- Non respect of confidentiality.
- Non respect of autonomy.

2.7. Collection and Transportation of Samples

After detailed explanation of the procedure to each participant and using a sterile cotton swab stick, 2 samples per person were collected that is 1 swab from the lower vagina and another from the lower rectum while the patient was lying on the gynaecological bed on which a draw sheet was placed.

Each swab was then immersed into 3 ml of normal saline (in the swab stick tube) and then placed into the specimen transportation flask. The samples were conveyed to the laboratory within 1 hr after collection.

2.8. Laboratory Procedures

2.8.1. Culture Media Preparation

1) Blood agar plates

39 g of the blood base agar was added to 1 litre of distilled water. This was boiled to dissolve the medium completely. It was sterilized by autoclaving at 121˚C for 15 minutes, cooled to 50˚C and 5% sterile Sheep blood was added. One vial of Nalidixic acid (ANC) was then added to every 100 ml of the blood agar. This was then poured into the petri dishes ensuring a depth of 4 mm.

2) Sabouraud agar plates

65 g of the Sabouraud dextrose + Chloramphenicol base was added to 1 litre of distilled water. It was then boiled to dissolve the medium completely, then sterilized by autoclaving at 121˚C for 15 minutes and cooled to 50˚C. This was then poured into the petri dishes ensuring a depth of 4 mm.

After preparation, the dishes were allowed at room temperature for the medium to solidify after which they were kept in the refrigerator at 4˚C. Before using the plates, they were incubated at 37˚C for about 5 minutes, agar side up.

2.8.2. Culturing of the Samples

After sample collection the vaginal specimens were inoculated into the sheep blood agar + ANC plates and Sabouraud + Chloramphenicol plates, while rectal specimens were inoculated only on sheep blood agar + ANC (nalidixic acid). This was usually done before microscopy so as to avoid contamination.

2.9. Microscopy

This involved the combination of a wet amount, whiff shift and a gram stain.

NB: We could equally identify yeast cells on the Gram stain.

2.10. Identification of GBS after Culture

Identifying the different streptococcal species entailed a series of biochemical tests and observing the morphology of the growth on the culture. Identifying GBS was done using the following technics.

2.10.1. Colony Morphology

GBS colonies were small, smooth, greyish or whitish, non-pigmented, convex colonies with their entire margins surrounded by a zone of beta haemolysis. If these colonies were present, the catalase test and a control Gram stain were carried out.

2.10.2. Control Gram Stain

About 2 to 5 colonies were picked up by a loop and placed on a drop of physiologic water on a slide so as to prepare a smear. It was then fixed with the flame and stained as described above. GBS appeared as cocci in short chains in tetrads or triads.

2.10.3. The Catalase Test

Catalase is an enzyme that breaks down hydrogen peroxide (toxic to some bacteria) to oxygen and water. To perform this test, a few suspected colonies were picked from the agar plate using a

Pasteur pipette and immersed into a drop of 3% hydrogen peroxide. If bubbles were emitted, the reaction was said to be positive. Samples of the suspected colonies were carefully picked to make sure that no agar was added. All colonies which tested negative were considered for further analysis (**Figure 1**).

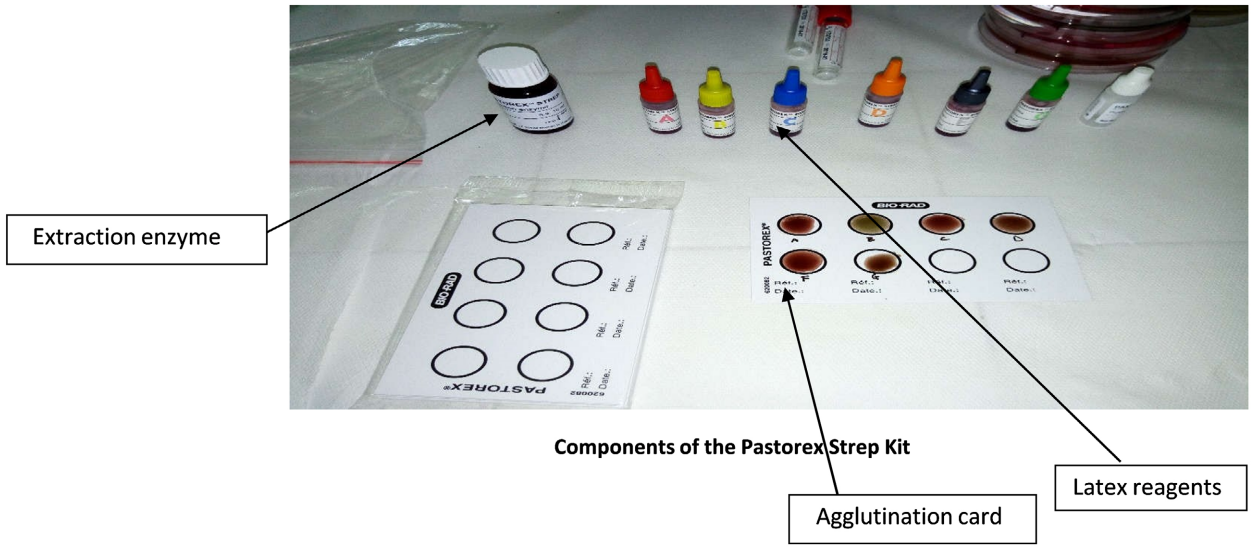

Figure 1. Photo taken by Anouboweh Asaah, the principal investigator.

2.11. Identification of Bacterial Vaginosis

Identification was based on:

Macroscopy: Foul, fishy smelling, thin gray vaginal discharge.

Whiff test: Positive.

Microscopy (*Gram stain*): Presence of Clue cells. These are epithelial cells of the vagina that get their distinctive stippled appearance by being covered with Gram variable bacteria.

2.12. Data Analysis

The questionnaire was checked for completeness and consistency, and the data were entered into the statistic software Epi Info version 7 for different analysis. In addition, the software Microsoft excel was used to design the different tables and figures for the presentation of the result. Descriptive statistics (frequencies, mean and standard deviation) were used to tabulate and describe the data. Chi-Squared test was used for comparisons of categorical variables. A p-value < 0.05 was considered statistically significant.

3. Results

3.1. Socio-Demographic Characteristics of Participants

A total of 121 pregnant women participated in the study giving a total of 242 samples overall. The mean age of participants was 27.60 (±5.5 SD) years old, with the youngest being 17 and the oldest 43 years old. The modal age was 24 years and the most represented age group was 20 to 30 years (83, 68.6%).

A total of 39 (32.2%) had some level of higher education, 62 (51.2%) ended at the secondary level of education and 1 (0.8%) had no formal education.

Socio-economically, the participants' median income was 25,000 (IQR 37,500) FCFA with a minimum amount of 1000 FCFA and a maximum monthly income of 350,000 frs.

With respect to occupation, traders were the most represented, 38 (31.4%), while farmers were the least represented, 6 (5.0%) as shown in Table 1.

The mean BMI was 26.44 (±4.02) with range 18.96 to 48.61. A total of 57 (47.1%) of the participants were overweight with no participant being underweight.

With respect to parity, 46 (38.0%) of the participants had delivered 2 or more times while 36 (29.8%) had delivered just once.

Of the 121 participants, 76 (62.8%) had sexual intercourse 2 - 4 times per week meanwhile 7 (5.8%) had sexual intercourse > 4 times per week.

Based on the participants' immune status, 4 (3.3%) of them were HIV positive as seen in Table 2.

Among the 121 participants, 84 (69.4%) had been pregnant at least once with 37 (30.6%) being pregnant for the first time.

Among the 121 participants, 84 (69.4%) had been pregnant at least once with 37 (30.6%) being pregnant for the first time (Figure 2).

Table 1. Distribution of participants according to socio-demographic factors N = 121.

Variable	Category	Frequency	Percentage (%)
Age group	<20	5	4.1
	20 - 30	83	68.6
	>30	33	27.3
Level of education	Primary	19	15.7
	Secondary	62	51.2
	Tertiary	39	32.2
	None	1	0.8
Occupation	Student	24	19.8
	Housewife	28	23.1
	Farmer	6	5.0
	Trader	38	31.4
	Civil servant	13	10.7
	Private sector	12	9.9
Monthly income	<100,000	107	88.4
	≥100,000	14	11.6

Table 2. Distribution of participants by clinical characteristics N = 121.

Variable	Category	Frequency	Percentage (%)
BMI	Underweight	00	00
	Normal weight	47	38.8
	Overweight	57	47.1
	Obese	17	14.0
Parity	Nulliparous	39	32.2
	Primiparous	36	29.8
	Multiparous	46	38.0
Frequency of sexual intercourse per week	<2 times	38	31.4
	2 - 4 times	76	62.8
	>4 times	7	5.8
HIV status	Positive	4	3.3
	Negative	117	96.7

The mean gestational age of clients was 32 (SD = ±3.7) weeks, with the minimum gestational age being 28 weeks and the maximum 39 weeks. The most frequently recorded gestational age was 28 weeks. As seen in **Figure 3**, the majority of participants, 93 (76.9%) had gestations below term.

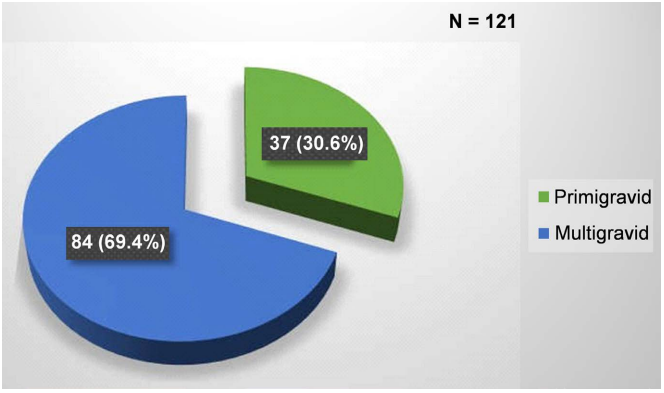

Figure 2. Distribution of participants according to gravidity.

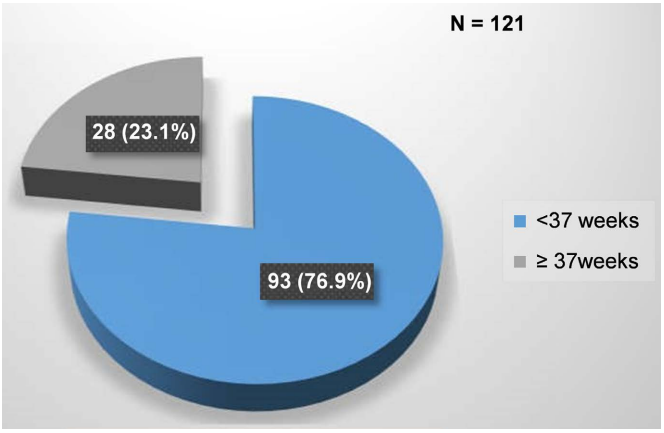

Figure 3. Distribution of participants according to gestational age.

3.2. Obstetric/Neonatal History of Participants

Among the 121 participants, 23 (19.0%) had a history of spontaneous abortions, 15 (12.4%) had a history of preterm deliveries while 2 (1.7%) had a history of still births and 8.3% had a history of neonatal infections (Table 3).

3.3. Prevalence of Rectal and Vaginal GBS and Other Germs

Laboratory analysis revealed the most isolated germs to be *Gardnerella vaginalis*, *Candida albicans*, and *Candida spp* with prevalence rates at 67 (55.4%), 49 (40.5%) and 15 (12.4%) respectively. GBS was identified in 14 (11.6%) and 9 (7.4%) of rectal and vaginal cultures respectively. However, GBS was isolated in the vagina or rectum of 16 participants giving us a prevalence of 13.2% (Table 4).

Among the 16 participants identified to have GBS in either vaginal or rectal samples, isolated rectal colonization rate and concomitant rectal and vaginal carriage rate were 5.8% (44.0% of overall carriage, 7/16) each, while the isolated vaginal carriage was about 1.7% (12.4% of overall carriage, 2/16) as seen in Figure 4. The rectal to vaginal carriage ratio was approximately 4:1.

The overall number of strains identified from rectal and vaginal samples of the 16 participants was as shown below.

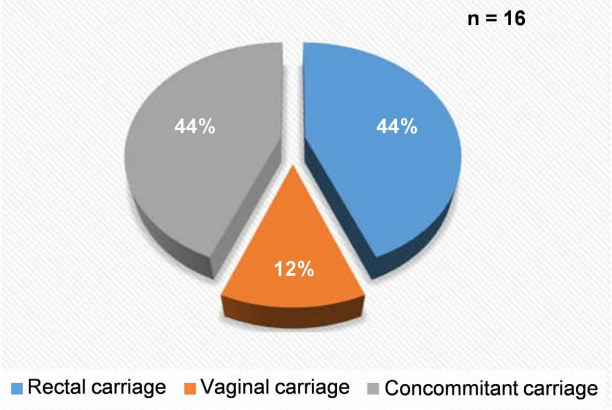

Figure 4. Location of isolated GBS strains.

Table 3. Frequency of Obstetric/Neonatal past history N = 121.

Obstetrical/Neonatal factors	Frequency	Percentage (%)
Preterm births	15	12.4
PROM	18	14.9
Neonatal deaths	9	7.4
Neonatal infection	10	8.3
Still births	2	1.7
Spontaneous abortion	23	19.0

Table 4. Frequency of identified germs N = 121.

Variable	Category	Frequency	Percentage (%)
Germs Isolated	NO	30	24.8
	YES	91	75.2
	Trichomonas vaginalis	3	2.5
	Gardnerella vaginalis	67	55.4
	Candida albicans	49	40.5
	Candida spp.	15	12.4
	GBS in rectal swabs	14	11.6
	GBS in vaginal swabs	9	7.4

3.4. Antimicrobial Susceptibility Trend of GBS Isolated

Of the strains isolated from the 16 participants, all were sensitive to oxacillin and ceftriaxone while 15 (93.8%) were sensitive to ampicillin. However, only 4 (25.0%) of the strains were sensitive to erythromycin. A total of 9 (56.3%) strains had an intermediate sensitivity to gentamycin with just 6 (37.5%) being sensitive and 1 (6.3%) were resistant. A resistance rate of 12.5% was recorded for levofloxacin (Table 5).

Table 5. Antimicrobial susceptibility trend of isolated GBS strains (N = 16).

Antibiotics	Number of Strains (%)		
	Sensitive	Intermediate	Resistant
Oxacilline	16 (100.0)	0 (00)	0 (00)
Amoxicilline + Clavulanic acid	15 (93.8)	1 (6.3)	0 (00)
Ampicilline	15 (93.8)	1 (6.3)	0 (00)
Ceftriaxone	16 (100)	0 (00)	0 (00)
Aztreonam	4 (25.0)	12 (75.0)	0 (00)
Clindamycine	1 (6.3)	15 (93.8)	0 (00)
Erythromycine	4 (25.0)	12 (75.0)	0 (00)
Imipenem	14 (87.5)	2 (12.5)	0 (00)
Gentamycine	6 (37.5)	9 (56.3)	1 (6.3)
Levofloxacin	2 (12.5)	12 (75)	2 (12.5)

Participants who had *Gardnerella vaginalis* present in their vaginal samples, had a higher GBS colonization rate (12, 17.9%) when compared with those who did not have *Gardnerella.* This disparity was approaching statistical significance (OR = 2.72, CI at 95% = 0.82 - 9.00, p = 0.09).

Participants in whom *Candida albicans* was isolated from their vaginas had a higher GBS colonization rate (10, 20.4%) relative to those who did not grow *Candida*. The difference was close to statistical significance (OR = 2.82, CI at 95% = 0.95 - 8.36, p = 0.05) (Table 6).

Multivariate analysis was done using the binary logistic regression model to control the effects of any confounding factors. Variables with statistical significance or close to statistical significance on bivariate analysis were used.

Participants with overweight had a close to statistically significant association with GBS colonization upon logistic regression (OR = 4.33, CI at 95% = 0.96 - 19.53, p = 0.06)

HIV positive status had a statistically significant association with GBS colonization upon logistic regression (OR = 0.06, CI at 95% = 0.01 - 0.92, p = 0.04).

Presence of *Gardnerella vaginalis* had a near statistically significance association with GBS colonization (OR = 0.36, CI at 95% = 0.08 - 1.56, p = 0.17) (Table 7).

3.5. Factors Associated with Gardnerella Vaginalis

Participants belonging to the age group < 20 years had the highest rate of colonization with *Gardnerella vaginalis* (3, 60%) while those belonging to the age group > 30 years had the lowest colonization rate (18, 54.5%).

Participants who had acquired a tertiary level of education had the highest rate.

Table 6. Para clinical factors associated with GBS colonization.

Variables	GBS colonization			
	Yes n (%)	No n (%)	OR (CI at 95%)	p-value
Presence of *Gardnerella vaginalis*	12 (17.9)	55 (82.1)	2.72 (0.82 - 9.00)	0.09
Presence of *Candida albicans*	10 (20.4)	39 (79.6)	2.82 (0.95 - 8.36)	0.05
Presence of *Candida spp*	2 (13.3)	13 (86.7)	1.01 (0.20 - 4.96)	1.00

Table 7. Binary logistic regression of factors associated with GBS colonization N = 121.

Variables	Adjusted OR (CI at 95%)	p-value
Overweight	4.33 (0.96 - 19.53)	0.06
Obesity	1.01 (0.21 - 4.92)	0.99
HIV positive status	16.67 (1.09 - 100)	0.04
PROM	0.44 (0.08 - 2.34)	0.34
Still birth	0.15 (0.03 - 6.80)	0.33
Spontaneous abortion	0.47 (0.09 - 2.32)	0.35
Gardnerella vaginalis	0.36 (0.08 - 1.56)	0.17
Candida albicans	0.50 (0.15 - 1.72)	0.27

Participants with an average monthly income of ≥100,000 frs had a higher *Gardnerella vaginalis* colonization rate (9, 64.3%) although there was no statistical significance (OR = 1.52, CI at 95% = 0.48 - 4.84, p = 0.48).

Gardnerella vaginalis colonization rate was highest among participants with gestational ages before term (53, 57.0%) however, there was no statistical significance (OR = 1.32, CI at 95% = 0.57 - 3.09 and p = 0.51).

Participants with a frequency of sexual intercourse of <2 times per week had the highest rate of colonization (24, 63.2%), however those with a frequency of sexual intercourse of >4 times per week had the lowest colonization rate with *Gardnerella vaginalis* (1, 14.3%). The difference was statistically significant (OR = 0.12, CI at 95% = 0.01 - 1.04, p = 0.04) (Table 8).

All variables with statistical significance or near significance on bivariate analysis were introduced into a binary logistic regression model to control for the effects of any confounding factor (s).

Participants with tertiary level of education had a near statistically significant association with *Gardnerella vaginalis* (AOR = 0.47, CI at 95% = 0.21 - 1.07, p = 0.07).

Participants with a frequency of sexual intercourse > 4 times per week had a statistically significant association with *Gardnerella vaginalis* colonization (AOR = 9.65, CI at 95% = 1.06 - 87.96, p = 0.04) (Table 9).

Table 8. Association between sociodemographic factors and *Gardnerella vaginalis* (N = 121).

Variables	Category	*Gardnerella vaginalis*		OR (CI at 95%)	p-value
		Yes n (%)	No n (%)		
Age	<20	3 (60.0)	2 (40.0)	1.21 (0.19 - 7.56)	1.00
	20 - 30	46 (55.4)	37 (44.6)	1.01 (0.47 - 2.18)	0.99
	>30	18 (54.5)	15 (45.5)	0.96 (0.43 - 2.13)	0.91
Level of education	Primary	9 (47.4)	10 (52.6)	0.68 (0.26 - 1.82)	0.45
	Secondary	32 (51.6)	30 (48.4)	0.73 (0.36 - 1.50)	0.39
	Tertiary	26 (65.0)	14 (35.0)	1.81 (0.83 - 3.96)	0.13
Monthly income	<100,000	58 (54.2)	49 (45.8)	0.66 (0.21 - 2.09)	0.48
	≥100,000	9 (64.3)	5 (35.7)	1.52 (0.48 - 4.84)	0.48
Gestational age	<37 weeks	53 (57.0)	40 (43.0)	1.32 (0.57 - 3.09)	0.51
	≥37 weeks	14 (50.0)	14 (50.0)	0.76 (0.32 - 1.76)	0.51
Frequency of sexual intercourse per week	<2 times	24 (63.2)	14 (36.8)	1.59 (0.73 - 3.50)	0.24
	2 - 4 times	42 (55.3)	34 (44.7)	0.99 (0.47 - 2.08)	0.98
	>4 times	1 (14.3)	6 (85.7)	0.12 (0.01 - 1.04)	0.04
HIV status	Positive	3 (75.0)	1 (25.0)	2.48 (0.25 - 24.59)	0.63

Table 9. Logistic regression for factors associated with *Gardnerella vaginalis* N = 121.

Variables	Category	Adjusted OR (CI at 95%)	p-value
Level of education	Tertiary	0.47 (0.21 - 1.07)	0.07
Frequency of sexual intercourse per week	<2 times	0.74 (0.33 - 1.65)	0.46
	>4 times	9.65 (1.06 - 87.96)	0.04

4. Discussion

4.1. Socio-Demographic Characteristics

The results from the study revealed that out of the 121 participants, the age range was between 17 - 43 years old, with a mean age of 27.6 (SD ± 5.5). The majority were aged 20 - 30 years, while more than half of the participants had completed at least basic and secondary and had at least been pregnant once (Multigravida).

These results are different from those reported by Turner and collaborators in 2012 in a similar study in a refugee population in South East Asia where the majority of the clients were in their 20 s [21].

These results are however similar to those of Adawaye and collaborators in 2014 at the YGOPH, [15], those of Assefa and associates in Ethiopia, 2018 [22] and those of Mengist and associates in Ethiopia in 2016 [24].

These results can be explained by the fact that this study, took place in an urban setting where women tend to acquire a certain level of education before getting pregnant in contrast to the study carried out in the refugee population.

The results also revealed that about 88.4% of the participants had a monthly income of less than 100,000 frs and most of them (47.1%) were overweight with about 3.3% being HIV positive.

These results are similar to those found by Mitima and collaborators in the Democratic Republic of

Congo, 2014 who reported that 71% of the participants in the study had a low level of income with 4 percent being HIV positive [25].

4.2. GBS Colonization Rate among Other Germs Isolated

This study revealed an overall carriage rate of 13.2%, with 7.4% vaginal carriage rate and 11.6% rectal carriage rate. These results are lower when compared with the values reported by Mengist *et al.* in Ethiopia in 2016 [24]; Mohamed and collaborators in Ethiopia in 2012 [26]; Bassir *et al.* in Marrakech in 2016; Ezeonu *et al.* in Nigeria in 2014 [27] and Mitima and collaborators in the Democratic Republic of Congo in 2014 [25]. These rates are equally lower than those documented in a review by Kwatra *et al.* in 2016 where an overall rate of 17.0% and 22.4% was detected globally and for Africa respectively [13]. Other reviews revealed higher carriage rates in Nigeria, Cote d'Ivoire, Togo, Gambia and Zimbabwe [28].

These disparities could be explained by the fact that, rates of GBS colonization vary widely throughout the world. Food habits, climate, maternal hygiene and culture methods, including the number and type of sites cultured and type of medium used, could account for some of these variations [29]. For example, in this study lower vaginal and lower rectal swabs were collected which is however in harmony with other studies, meanwhile other investigators collected upper vaginal swabs only and no rectal swab.

The characteristics of the GBS colonies on blood agar plates with the agglutination test to determine carriage rate was used in this study meanwhile other investigators used more sensitive technics like PCR or hydrolysis of Hippuric acid [24]. This disparity equally lends crdence to the fact that GBS carriage rates vary per country as documented by Stoll *et al.* in 1998 [28].

These results are however similar to those reported in the same setting by Adawaye *et al.* and Foumane *et al.* who reported 7.7% and 6.7% vaginal carriage rates respectively [15]. They are equally similar to those of Kacou and collaborators in Abidjan in 1991 with a vaginal carriage rate of 8.2% [19].

This study equally revealed an isolated rectal carriage rate of 44.0%, 12.4% isolated vaginal carriage and a concomitant recto-vaginal colonisation rate of 44.0%.

These are similar to results documented by Mengist *et al.* in Ethiopia in 2016 with predominant rectal carriage (46.0%) followed by 29.0% concomitant carriage and 25.0% vaginal carriage [24].

Among other germs isolated from vaginal samples of participants, the most predominant were *Gardnerella vaginalis* 67 (55.4%), *Candida albicans* 49 (40.5%) and *Candida spp* 15 (12.4%).

These results are in concordance with Adawaye and collaborators in 2014 at the YGOPH who reported the most predominant germs in the vaginal samples to be *Candida albicans* (45.16%), *Gardnerella vaginalis* (22.58%) and *Candida spp* (11.82%) [15]. These similarities could be explained by the similarities in socio-economic and cultural status of our study population with that of Adawaye.

4.3. Factors Associated with GBS Colonization

From the results, GBS colonisation rates were greatest in participants who were in the >30 years age range when compared with those less than 20 years or 20 - 30 years; same results were recorded amongst participants belonging to the categories; primigravida, multiparas, maternal obesity and overweight, gestational age at term, <2 times of sexual intercourse per week, HIV positive participants and equally amongst those with a history of preterm birth, PROM, still births and spontaneous abortions. However, maternal overweight had a near statistical significant association while HIV positive status had a statistically significant association with GBS colonization.

These results are similar to results reported by Mitima and collaborators in 2014 in DRC and Assefa and collaborators in 2018 in Ethiopia [22] who reported that HIV positive status had a statistically significant association with GBS colonization on one hand and multigravidas, poor obstetric and neonatal histories were not statistically significant on the other hand.

These results are also similar to results reported by Khan *et al.* in Saudi Arabia that majority of carriers of GBS were above 40 years of age.

These results are however different from those reported by Adawaye *et al.* in 2014 and Schuchat A and collaborators in 1998 in clients less than 20 years old and amongst those with a history of neonatal deaths [15].

These disparities could be due to the fact that GBS colonisation rates might be influenced by multiple factors which may vary from one geographical region to another. The lack of association with these factors could equally be explained by the fact that, this study was not a follow up study.

4.4. Antimicrobial Susceptibility Trends

Among the 16 different strains isolated and tested for antimicrobial susceptibility, 100% were sensitive to oxacillin and ceftriaxone, meanwhile 93.8% were sensitive to mmpicillin and amoxicillin-clavulanic acid and the remaining 6.2% having an intermediate susceptibility in each case.

However, the highest level of resistance was recorded with the quinolones antibiotic group (12.5% of strains resistant to levofloxacin); aminoglycoside antibiotic group (6.3% of strains resistant to gentamycin while 56.3% of the strains having an intermediate susceptibility); Macrolide antibiotic group (75% of the

strains had an intermediate susceptibility to erythromycin with 25% being susceptible).

These results showed that Beta-lactamines which constitute the recommended first and second line prophylaxis regimen [26] were active on the isolated strains.

However, erythromycin which is recommended in case of allergy to Beta-Lactamines wasn't active on some strains.

Similar results were reported by Assefa and collaborators in Ethiopia 2018 [22] as with some strains having decreased susceptibility to ampicillin which differed slightly from Mengist and collaborators in Ethiopia in 2016 with 100% susceptibility of strains to penicillin G and amoxicillin and reduced susceptibility (90%) of the later to erythromycin [30].

The expanded use of beta-lactam antimicrobials in the treatment of several infective clinical syndromes and the free accessibility of purchase over the counter might be the cause of the emergence of GBS resistance strains in this environment.

Similar results were reported in Saudi Arabia by Khan and associates in 2015 with reduced susceptibility to macrolides, 16% and 5% resistance to Erythromycine and Clindamycine respectively.

Susceptibility of GBS to Gentamycin as detected by this study closely correlates with those reported by Adawaye and collaborators at the YGOPH with 100% of strains resistant to gentamycine [15]. Increased resistance to aminoglycosides could be explained by the fact that these antibiotics have limited action of streptococci.

4.5. Limitations and Strengths

Due to financial constraints, only 121 patients were recruited with a total of 242 samples collected. However, despite this limitation, this study was the first of its kind in Cameroon (to best of our knowledge) to assess for factors associated with GBS colonization and was among one of the only studies in Cameroon to have evaluated not just vaginal carriage, but rectal carriage of GBS too.

5. Conclusion and Recommendations

5.1. Conclusion

This study revealed the following:

- The prevalence of GBS in vaginal and rectal swabs of pregnant women in the 3rd trimester was 13.2%.
- The most predominant germs in vaginal samples of 3rd trimester women were *Gardnerella vaginalis*, *Candida albicans* and *Candida spp.*
- Some few strains of isolated GBS had decreased susceptibility to some antibiotics predominantly, beta-lactamines which make up the 1st and 2nd line prophylactic regimens and macrolides which make up the recommended prophylactic regimen in case of allergy to Penicillin.
- HIV positive status had a statistically significant association with GBS colo-

nization

5.2. Recommendations

To the Bamenda Regional Hospital

- ✓ To systematically screen all High-risk pregnant women in the third trimester for GBS colonization both in the rectum and vagina.
- ✓ For all the clients presenting in labour without any GBS colonization screening, risk factors assessment be done and intra-partum antibiotic prophylaxis be administered.

To the Health Practitioners

- ✓ To routinely demand a 3rd trimester GBS screening, not just in the vagina, but equally in the anus and rectum among high risk pregnant women considering the cost of the screening.
- ✓ To treat all pregnant women carriers of GBS or high-risk women who did not benefit from screening during pregnancy when they present in labor.

To the scientific community

- ✓ To carry out large-scale epidemiological studies in different parts of the country in order to establish the actual GBS colonization rate and GBS serotypes.
- ✓ To carry out studies to further assess the risk factors associated with maternal GBS carriage.
- ✓ To carry out studies to evaluate the relationship between GBS carriage and adverse obstetrical and neonatal outcomes.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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