

Prevalence of *Streptococcus agalactiae* among Women Resident in Calabar, Cross River State, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AIK, RUBE and ARV designed the study and wrote the protocol. Authors UOE and RCE performed the statistical analysis. All authors wrote the first draft of the manuscript. Authors AIK, UOE and ARV managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Prevalence of *Streptococcus agalactiae* infection in women resident in Calabar, Cross River State of Nigeria was investigated. Following informed consent, one hundred and twenty two (122) high vaginal swab samples comprising 72 from pregnant women and 50 from non-pregnant women were collected using standard aseptic microbiological technique. In addition, the pregnancy status, age, health status, and other sociodemographic factors of the participants were also collected using an open ended questionnaire. Samples were aseptically collected and microbiological analysis carried out using standard microbiological analysis. Out of the 122 samples screened, 26 were positive for *S. agalactiae* giving an overall prevalence rate of 21.3%. Furthermore, prevalence rates of 23.6% and 18.0% were obtained for the pregnant and non-pregnant women, respectively.

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Based on the age groups of the participants, prevalence rates of 13.7%, 17%, 26.5%, and 40% were obtained from age groups of ≥ 20 , 21-29, 30-39 and ≥ 40 years, respectively. According to the health status of the participants, 15.4% and 55.6% prevalence were obtained from clinically healthy and ill participants, respectively. These findings suggest that the infection could be wide spread in Nigeria, amongst the pregnant and non-pregnant women. There is need for further studies that would evaluate the circulating strains.

Keywords: Prevalence; *Streptococcus agalactiae*; women; Calabar.

1. INTRODUCTION

Streptococcus agalactiae, also known as group B *streptococcus* (GBS) is a facultative anaerobic gram positive cocci, arranged in chains. They are commensal flora of the intestine and migrate down to rectum, vagina and urinary tract. Approximately 10-35% of women are symptomatic carriers of GBS in the genital and gastrointestinal tracts [1,2,3,4,5] GBS remains a leading cause of serious illness and death in newborn population where it causes meningitis, pneumonia and sepsis in newborns. Thus, the detection of GBS disease in the vaginal anorectal area is critical to the prevention of neonatal GBS disease [6,7]. However, it can cause urinary tracts infections (UTI) in women [5], adult males [8] and those who have preterm births are 1.7 times more likely to be colonized with the organism during labour than woman who do not have preterm birth [9,10]. Furthermore, it has been revealed that this pathogen is a possible agent of skin and soft tissue infections with particular preference for extreme age groups such as neonates and the elderly above 65years of age [11,12]. Others include pregnant woman during delivery and those chronically debilitated such as diabetics and cancers of malignant neoplasms. With greater prevalence in sexually active individuals, this suggests that it can be acquired by sexual contact. Other risk factors include infrequent hand washing; tampon use and prolonged use of immunosuppressive agents [1,2,3,4,5]. The organism also causes bovine mastitis in cattle. Bovine strains can be differentiated from human strains in that they ferment lactose but not salicin while the human stains ferment salicin and rarely lactose [13].

There are two main types of *S. agalactiae* infections in new born; Early-Onset Disease (EOD) and Late Onset Disease (LOD). The Early infection occurs in the first 7days after birth and symptoms usually appear within the first 12- 48 hours [14,15]. These babies are presented with fever, breathing problems with grunting sound,

bluish-coloured skin (cyanosis), seizures, lameness or stiffness, loss of appetite, heart rate and blood pressure abnormalities, vomiting and diarrhea. In the study of 148,000 infants born in Europe between 2005-2011, it was observed that almost all of the 95% infants who developed early the EOD probably began before birth [16]. In Nigeria approximately 19,800 cases of this infection occurs annually across all groups [16]. CDC [9] estimated that the death rate from EOD infection is 2-3% for full term infants. However, the rate is much higher about 20-30% for infants who are born at less than 33weeks gestation. Report has it that 44% of infants who survive infection with meningitis end up with long term health problems including developmental abnormalities, paralysis, seizure disorder, hearing loss, impaired vision, and small brains [9]. Penicillin remains the drug of choice for the treatment of infection [17]. Different susceptibility and resistance pattern have been reported by many studies [18,19]. Humoral immunity has also been implicated in the clearance of GBS in a classical study [20]. Babies born to infected mothers without treatment are more at risks than those without [6,7,9]. A number of studies around the world and within Nigeria have reported varying prevalence rates for pregnant and non-pregnant women [19,21-25]. However, there is no report of its prevalence in Calabar, Cross River State.

Given the shortage of information, the aim of the study was to investigate the prevalence to *S. agalactiae* in the genitourinary tract of pregnant of and non pregnant women in Calabar, Cross River State in addition to their sociodemographic factors and underlying medical conditions.

2. METHODOLOGY

2.1 Experimental Design and Sample Size Determination

This design of this study was based on descriptive study. Sample size was determined following a previous study (Arif et al., 2015).

Sociodemographic data were sought and obtained using open ended questionnaires. In addition, information on clinical status and underlying medical conditions such as diabetes, Human Immunodeficiency Virus (HIV), edema, urinary tract infection and so on were also obtained. Only women aged 16 and above were included in the study after informed consent. Furthermore, those on antibiotics therapy were also excluded from the study. All participants gave their informed consents before inclusion in the study.

2.2 Study Location

This study was conducted at Asi Ukpo Diagnostic and Medical Centre located along Mary Slessor Avenue (Opposite College of Health Technology), Calabar, Cross River State, Nigeria. Cross River State is located in the Niger Delta region of Nigeria and share common boundaries with Benue State to the North, Ebonyi and Abia State to the West and to the East by Cameroon Republic.

2.3 Collection of Sample

Samples were collected using simple random sampling technique. The procedure for the collection was explained to the participants before the samples were collected from patient. Duly filled questionnaires were also obtained from each participant. A total of 122 samples were collected from 72 pregnant women and 50 non-pregnant women who visited the center. High vaginal swab (HVS) samples were collected from each of the participants. Afterwards, the swabs were allowed then transported immediately at 2-4^oC to the microbiology laboratory for analysis using Giostyle box. This was done as described previously [26].

2.4 Inoculation of the Samples

The high vaginal swabs were inoculated on freshly prepared Todd Hewitt broth with nalidixic acid (15 µg/ml) and colistin (10 µg/ml) and sheep blood agar media. They were then incubated at 37°C in 10% CO₂ for 24-48 hours. The broths were then examined for turbidity and also for plates showing beta haemolytic activity. Negative plates were re examined for haemolytic activity by re incubation. Positive colonies were sub-cultured for serological grouping as described by Artz et al. [27].

2.5 Identification of the Isolates

The isolates were identified using standard microbiological techniques. Cultural morphology, Gram reaction and a battery of biochemical tests were used to identify the isolates. These include haemolytic, catalase and coagulase tests, and Christian Atkins Munch Peterson (CAMP) reaction. These were done according to the methods previously described [28].

3. RESULTS

A total of 122 samples were screened for the presence of *S. agalactiae* and 26 samples tested positive, giving an overall prevalence of 21.3% (Table 1). Out of the 72 pregnant women screened, 17 were positive giving a prevalence of 23.6% while for the 50 non-pregnant women tested, 9 were positive giving a prevalence of 17%.

Table 1. Prevalence of *S. agalactiae* according to pregnancy status

Pregnancy status	Positive (%)	Negative (%)	Total
Pregnant	17 (23.61)	55(76.39)	72
Non-pregnant	9(18.80)	41(81.20)	50
Total	26(21.31%)	96 (78.69)	122

The prevalence of *S. agalactiae* according to the various age groups is presented in Table 2. From the result, prevalence rates of 13.7%, 17.9%, 26.50% and 40.0% respectively were observed for age brackets ≥ 20, 21-29, 30-39 and ≥ 40 years, respectively.

Table 2. Prevalence of *S. agalactiae* according to age

Age group (Years)	Positive	Negative	Total
≥ 20	3(13.7%)	19	22
21-29	10(17.7%)	46	56
30-39	9(26.5%)	25	34
40 and above	4(40.0%)	6	10
Total	26(21.3%)	96	122

Table 3 shows the prevalence rate of GBS according to health status. As expected, more than half (55.6%) of the clinically ill tested positive. A total of 16 (15.40%) out of 104 that were clinically healthy were positive. The clinically associated medical conditions are listed in Table 4.

Table 3. Prevalence of *S. agalactiae* according to the health status

Health status	Positive	Negative	Total
Clinically healthy	16(15.4%)	88	104
Clinically ill	10(55.6%)	8	18
Total	26(21.3%)	96	122

Table 4. Prevalence of *S. agalactiae* according to the associated medical condition

Associated med. condition	Positive	Negative	Total
Diabetes patient	3 (60%)	2 (40%)	5
Patients with high B. P	1(50%)	1 (50%)	2
Patients with UTI	2(33.3%)	4 (66.67%)	6
Patients with edema	0 (0%)	1 (100%)	1
Patients with hepatitis	1(100%)	0 (0%)	1
HIV Patients	2(100%)	0 (0%)	2
Patients with Kidney disease	1(100%)	0 (0%)	1
Total	10	8	18

HIV = Human Immunodeficiency Virus, B.P = Blood pressure, UTI = Urinary tract infection

The prevalence rate was also evaluated based on their sociodemographic factors and result is as shown on Table 5.

Table 5. Prevalence of *S. agalactiae* according to socio-demographic factors

Sociodemographic factors	Positive	Negative
Marital status		
Never married	3/18(16.7)	4/34(11.8)
Married	9/37(24.3)	4/12 (33.3)
Widow	3/11 (27.3)	1/4 (25.0)
Divorce	2/6 (33.3)	0
Occupation		
Civil servant	2/20 (10.0)	1/11 (9.1)
Business	14/39 (35.9)	5/21 (23.8)
Student	1/13 (7.7)	3/18 (16.7)
Education status		
Primary	4/7 (57.1)	3/8 (37.5)
Secondary	9/38 (23.7)	4/22 (18.2)
Tertiary	4/27 (14.8)	2/20 (10.0)

4. DISCUSSION

The prevalence of *S. agalactiae* amongst pregnant and non-pregnant women is

widespread [21-25]. The prevalence rates observed in this study were slightly different amongst the different parameters such as pregnant and non-pregnant women, age group, clinical status and associated medical conditions. In pregnant and non-pregnant women the prevalence rates stood at 23.61 and 18.80%, respectively. Our prevalence is lower than the prevalence rates of 80% [21] and 64% in pregnant women in south western Nigeria [23] reported earlier.

However, this is also consistent with the findings of Regan et al. [1] which reported that 20-35% of women are asymptomatic carriers of GBS in the genital tract. It was higher than the 11.30% reported amongst 150 pregnant women attending ante natal [22] and the 13.70% reported in Ethiopia by Alemseged et al. [19]. Furthermore, it was also higher than the 18.00% and 5.50% reported earlier for pregnant and non-pregnant women in Nigeria [25]. It was also higher than the 4% reported by Arif et al. [29] and 14.60% for Chang et al. [30].

The higher prevalence of 23.6% from pregnant women may account for the increased incidence of neonatal GBS disease as described by Baker [31] who reported that maternal carriage of *S. agalactiae* in the genital tract is a prerequisite for early onset of disease, with vertical transmission occurring just prior to birth. Since the infection can be transmitted from asymptomatic pregnant women to neonate, there is a possibility that a lot of neonates presents with clinical manifestations of the disease in Nigeria without receiving the appropriate treatment due to poor knowledge about the aetiology.

The higher prevalence rate found among the participants who are married when compared to the "never married" also suggests that the organism may be found more in the sexually active people. The result also shows a stepwise increase in the rate of prevalence among the age groups with lowest prevalence of 13.3% for women belonging to the age group of 16-20 years, and the highest rate of prevalence of 40% for the women of 40 years and above. This was consistent with the findings of Salou et al. [32] who reported a prevalence rate of A prevalence rate of 15.4% was obtained from the participants who were clinically healthy while 55.6% prevalence was obtained from the clinically ill participants. The higher prevalence rate found among the clinically ill participants is suggestive of the possibility of these associated medical

conditions being predisposing factors to *S. agalactiae* infections.

The prevalence rate of the participants found according to their educational status shows that those who acquired only primary education had the highest rate of prevalence while the lowest prevalence was found among those with tertiary education. This was also consistent with the reports of Salou et al. [32] who reported prevalence rates of 12.5%, 30.5%, 49% and 8% which corresponds to uneducated, primary, secondary and university level, respectively. This reveals the positive impact of education on the infection rate and possibly, the higher standard of hygienic measures among the educated. According to Oddie and Embleton [32] local hygiene or poor sexual practice can increase the risk of vaginal colonization by *S. agalactiae*. Other factors include use of tampons and other intrauterine devices.

5. CONCLUSION

The high prevalence rate (21.3%) of *S. agalactiae* infection among the women resident in Calabar as demonstrated in this study is a call for concern. In order to reduce the incidence of disease outbreak among infants in Nigeria, there is need for continuous national screening of pregnant women for the presence of infection. Furthermore, serotyping should be carried out to identify the circulating serotypes and strains.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

Ethical approval was sought for and obtained from the Management of Asi Ukpo Diagnostic and Health Center.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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