PREVALENCE OF STREPTOCOCCUS AGALACTIAE SEROTYPES ASSOCIATED WITH ANOGENITAL COLONIZATION AMONG PREGNANT WOMEN IN JOS

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Authors' contributions

This work was carried out in collaboration among all authors. Author DAS designed the study, performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript and managed the analyses of the study. Authors DZE and KOTY managed the literature searches, supervised and monitored the entire work. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Aim

The aim of this study was to determine the prevalence of GBS serotype distribution in Jos University Teaching Hospital.

Materials and Methodology

This was a hospital based descriptive cross-sectional study of 300 women receiving health care at the Jos University Teaching Hospital between July 2017 and November 2017. Systematic sampling technique was employed in recruiting consenting subjects for this study. High vaginal and anorectal swabs were collected from each subject after obtaining their consent by signing a structured consent form. The identified *Streptococcus agalactiae* (GBS) isolates were serotyped using immuLex strep-B antisera from SSI Diagnostica, 2 Herredysvejen, DK-3400 Hillerod Demnark to identify the different serotypes. The results obtained were computed using SPSS version 21.

Results

A total of 300 women obtaining health care in Jos University Teaching Hospital (JUTH) were enrolled in this study between the months of July, 2017 and November, 2017. In all, vaginal and anorectal swabs were taken from 200 pregnant women and 100 non-pregnant women. The age range of the study population was between 16 years to 48 years with a mean age of 31.9 year (SD ± 6.6). The prevalence rate among the study population was 6.3%. The colonization rate among pregnant and non-pregnant women was 6.5% and 6.0% respectively with no significant statistical difference. Serotype Ia was the commonest isolate responsible for 42.1% of the GBS isolates. Serotype III accounted for 31.6% of the isolates, followed by serotype V (15.8%). Serotype II was less common, responsible for only 10.5%.

Conclusion

This study showed that GBS colonization rate among the study population was 6.3%. Approximately, 6.5% and 6.0% prevalence rate were found among pregnant and non-pregnant women respectively. Of

all the GBS isolates, serotypes Ia, II, III, and serotype V were isolated with serotype Ia being the most prevalent serotype. This knowledge of serotype distribution will help in instituting serotype specific GBS vaccines for the prevention of GBS diseases in Jos.

Key words: Streptococcus agalactiae, Pregnant women, Serotypes

INTRODUCTION

Streptococcus agalactiae (Group B *Streptococcus*, GBS) is a normal flora of the genitourinary tract and the rectum of humans [1]. *Streptococcus agalactiae* colonizes the human gastrointestinal and genital tracts of 20-30% of healthy humans [2]. The colonization of the vagina by GBS can be transient, chronic or intermittent and serves as potential sources of infection to the new born [3,4]. From this site, the bacterium can colonize 50% - 75% of infants of colonized mothers via the amniotic fluid or the mucous membranes during birth and produce an early onset neonatal disease such as neonatal sepsis, neonatal meningitis, pneumonia 24-48 hours after birth [5,6]. About 1-2% of the new born of GBS colonized mothers may develop early onset neonatal disease with the maternal genital area serving as source of infection [7,8].

Several maternal risk factors can significantly increase the possibility of development of GBS disease in neonates. This is an important fact, since the presence of an isolated risk factor increases the probability that a pregnant woman will have a child with early neonatal GBS disease by 6.5 times [9]. The identification of a high-risk population to be screened for GBS colonization is a considerable challenge, since colonization is also observed in mothers who do not have the classically known risk factors, and it represents 25% to 30% of neonates that develop early neonatal GBS diseases [10].

GBS colonization rate has been found to be associated with prolonged labour, premature rupture of membrane and preterm delivery [11]. Other factors equally associated with GBS colonization are low levels of circulating maternal antibodies against GBS, maternal age of less than 20 years, and maternal diabetes mellitus [10,12].

The frequency of GBS colonization is highest among black women, followed by Hispanic women and white women [10]. However, in some countries, in view of the high degree of racial miscegenation, it is difficult to establish a precise classification of a patient sample in terms of race or ethnic group making it difficult to differentiate among races. Similar study in Saudi Arabia has reported no difference in GBS

prevalence among blacks and white Saudi women [13]. One important virulence factor of this GBS is the capsular polysaccharide (CPS). This CPS antigen has been studied extensively and is important antigenic marker. There are ten antigenically distinct serotypes identified, namely Ia, Ib, II, III, IV, V, VI, VII, VIII, and IX [14]. Capsular type distribution varies with geographical region and ethnic origin of the population [15]. In the United States and Europe, the most prevalent serotypes in human infections are Ia, II, III, and V, whereas serotypes VI and VIII are the most predominant in Japan [16,17].

A study conducted in Gabon revealed that serotypes III and V are the most prevalent serotypes in Gabon similar to what was found in South Africa [18,19]. There is dearth of knowledge about the *Streptococcus agalactiae* serotypes in Jos and this study will help reveal the different serotypes distribution. This knowledge of serotype distribution is necessary for the selection and development of serotype-based vaccine for the prevention of invasive disease in a given country [20,21,22].

2.0 MATERIALS AND METHODS

2.1 Study Area

The study was carried out in Jos University Teaching Hospital (JUTH). Jos University Teaching Hospital is located in Jos the Plateau State capital. The hospital is a tertiary health institution with a 600 beds capacity serving Plateau State and majority of the states in the North-central and part of North-east geopolitical zones of Nigeria. Jos University Teaching Hospital is also a centre for AIDS Prevention Initiative in Nigeria (APIN) that cater for most people leaving with HIV (PLHIV) from within and the bordering states. The main occupation of the people is farming with majority of them in the city being civil servants and businessmen and women.

2.2 Study Population

The study population included 200 pregnant and 100 non-pregnant women attending antenatal clinic at the Jos University Teaching Hospital between July 2017 and November 2017.

2.3 Study Design

The study was a hospital based descriptive, cross-sectional study were 300 pregnant and non-pregnant women attending antenatal and gynaecology clinics at the Jos University teaching Hospital were recruited consecutively as the came into the clinics by signing a structure consent form.

2.4 Sample Collection

Anorectal and vaginal swabs were carefully collected from 200 pregnant women and 100 non-pregnant women using sterile swab sticks by the attending physicians after given them appropriate instructions on how the sample should be collected [23].

2.5 Specimen Transport

The collected specimens were immediately inoculated into a selective enrichment broth, Todd-Hewitt broth (Oxoid LTD) supplemented with gentamycin (8ug/ml), nalidixic acid (15ug/ml) and 5% sheep blood to increase the recovery rate of GBS [16,23]. These were transported to the laboratory within three hours of inoculation.

2.6 Culture and Incubation

The tubes of inoculated Todd-Hewitt broth were incubated aerobically at 37^0C for 18 to 24 hours. After an overnight incubation, the broths were subcultured onto 10% sheep blood agar and chromatic Strepto B agar (Liofilchem, Italy), a selective medium for GBS.

The inoculated 10% sheep blood agar plates were incubated aerobically in 5-10% CO2 (candle extinction jar) at 370C for 18 to 24 hours, while the inoculated chromatic Strepto B agar plates were incubated aerobically at 37^0C for 18 to 24 hours [24]. The Streptococcus agalactiae control strain was also inoculated onto 10% sheep blood agar and chromatic Strepto B agar and incubated as stated above respectively.

2.7 Identification of GBS Isolates

GBS isolates were identified by their beta haemolytic pattern on 5% sheep blood agar and blue-green colour on chromatic Strepto B agar. The isolates were further subjected to Gram staining, catalase test, and Serogrouping using streptococcal grouping kit (DR0585A OXOID) from Oxoid.

2.8 Serotyping of Isolates

The group B B-haemolytic (GBS) isolates were serotyped using immuLexTM strep-B antisera from SS1 Diagnostica, 2 Herredysvejen, DK-3400 Hillerod Denmark to identify the different serotypes according to manufacturers guidelines.

2.9 Data Analysis

The data obtained from the study were analysed using Statistical Package for Social Sciences (SPSS) version 21 (IBM SPSS Inc, USA). Proportions were compared using Chi-square with confidence limit (p-value) of < 0.05 considered significant.

3.0 RESULTS

A total of 300 women obtaining health care in Jos University Teaching Hospital (JUTH) were enrolled in this study between the months of July, 2017 and November, 2017. In all, vaginal and anorectal swabs were taken from 200 pregnant women and 100 non-pregnant women. The age range of the study population was between 16 years to 48 years with a mean age of 31.9 years (SD 16.6).

The prevalence rate among the study population was 19(6.3%). The pregnant women were colonized in 6.5% while the colonization rate was 6.0% among the non-pregnant women. The difference in colonization between pregnant and non-pregnant was not statistically significant (Table 1) ($X^2 = 0.028$, $P = .87$).

Out of the 19 isolates of GBS isolated, 42.1% belonged to serotype Ia, which was the most prevalent serotype among the study population. Serotype III accounted for 31.6% of the isolates, followed by

serotype V (15.8%). Serotype II was less common responsible for only 10.5% of the 19 isolates. Serotypes Ib, IV, VI, VII, VIII and IX were not isolated.

Among the pregnant women, serotypes Ia and III were isolated in 53.8% and 30.8% respectively while serotype II and V accounted for 7.7% each. In non-pregnant women, serotype III and V were the most common serotypes accounting for 33.3% each while serotype Ia and II had equal distribution, responsible for 16.7% each. The serotypes distribution among the pregnant and non-pregnant was not statistically significant (Table 2) ($X^2 = 3.380$, $P = .34$).

Table I

Group B Streptococcal carriage rates among pregnant and non-pregnant women in Jos University Teaching Hospital.

Category of women	No. tested	No. Positive	% Positive
Pregnant	200	13	6.5
Non-Pregnant	100	6	6.0
Total	300	19	6.3

$X^2 = 0.028$ $P = .87$ df = 1

Table 2
Serotypes distribution of Streptococcus agalactiae isolates from pregnant and non-pregnant Women in Jos University Teaching Hospital

Serotype	Pregnant (%)	Non-pregnant (%)	Total
Ia	7(53.8)	1(16.7)	8(42.1)
II	1(7.7)	1(16.7)	2(10.5)
III	4(30.8)	2(33.3)	6(31.6)
V	1(7.7)	2(33.3)	3(15.8)
Total	13(6.5)	6(6.0)	19(6.3)

$\chi 2 = 3.380$ $P = .34$ df = 3

4.0 DISCUSSION

The study revealed GBS colonization rate of 19(6.3%) among the population tested. The carriage rate was 6.5% in pregnant women and 6.0% in non-pregnant women. This result is lower than the 7.0% previously reported by Nsagha et al (1997) in Jos [25]. The slight decrease in the colonization rate may be attributed to improve health awareness among the general public and, improvement in culturing technique as Todd-Hewitt broth and chromatic Strepto B agar which are selective media for Streptococcus agalactiae were used in this study making identification and differentiation easy rather than just blood agar.

In other parts of Nigeria, 19.0% was reported in Ibadan by Onile (1990) [26] and Uhiara (1993)

reported a carriage rate of 9.0% in Calaber [27]. Onipede and his colleagues in 2012 reported a higher prevalence rate of 11.3% in Ile-Ife [28] while 9.8% was reported by Okon et al 2013 in North-eastern Nigeria [29].

The group B streptococcal colonization rate observed in this study was also lower compare to colonization rates reported from several African countries. About 31.6% was reported in Zimbabwe by Moyo et al (2000) [30], 25.3% in Egypt by Shabayek et al (2014) [31] and 23% in Tanzania [6].

When compared with studies conducted in developed countries, it was observed that the result of this study was lower to what was obtained in United States of America [32]. Tor-Udom et al (2006) reported a carriage rate of 16.0% in Thailand [33] while 24.0% was reported in Belgium in 2009 [34]. A similar study conducted in Poland by Brzychczy-Wloch et al (2013) reported 29.5% carriage rate [35]. These results are higher to 5.7% obtained in Israel [36]. The variations between countries could possibly be due, at least in part, to differences in sampling and culturing techniques, types of media used as Well as the population studied [37]. For instance, in this study, samples were collected from HIV positive and HIV negative pregnant women regardless of gestational age.

Serotyping of the GBS isolates revealed that the isolates belong to serotypes Ia, II, III, and V whereas serotypes Ib, IV, VI, VII, VIII and IX were not isolated. As indicated in table 8 above, serotype Ia (42.1%) was the predominant serotype followed by serotype III (31.6%). Serotypes II and V were isolated in 10.5% and 15.8% respectively. The isolated serotypes have been reported as the predominant causes of human infection worldwide [38] and serotype III as the most prevalent and invasive of all the serotypes [39]. Capsular type distribution varies between countries, geographical region and ethnic origin of a population [15]. A study conducted in Gabon revealed that serotype V and III are the most common, which is in consistent with findings in South Africa [18,19]. Serotypes Ia, Ib and II have been reported in Brazil [40] as the predominant serotypes causing most of the infections there. In United States of America and Europe, serotypes Ia, II, III, and V have been isolated in 80-90% of clinical infections while serotypes VI and VIII are the most predominant in Japan.

This knowledge of serotype distribution is necessary for the selection and development of serotype-based vaccine for the prevention of invasive disease in a given country [20].

5.0 CONCLUSION

This study showed that GBS colonization rate among the study population was 6.3%. Approximately, 6.5% and 6.0% prevalence rate were found among pregnant and non-pregnant women respectively. Of all the GBS isolates, serotypes Ia, II, III, and serotype V were isolated with serotype Ia being the most prevalent serotype. Serotype Ib, IV, VI, VIII and IX were not isolated in this study. This knowledge of serotype distribution will help in instituting serotype specific GBS vaccines for the prevention of GBS diseases in Jos Plateau state, Nigeria.

ETHICAL APPROVAL

This study was approved by the research ethical committee of Jos University Teaching Hospital with reference number JUTH/DCS/ADM/127/XIX/6583 and complies with the norms. Written informed consents were also signed by all subjects before enrolment in the study.

CONSENT

All the authors reviewed and gave their consent for this article to be submitted for publication.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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