African Journal of
Medicine, Surgery and Public Health Research

ISSN : 3434-3567

Index: Harvard, Boston, Sydney University, Dimensions, Lens, ResearchGet Scilit, Semantic, Google Scholar, Base etc

https://doi.org/10.58578/AJMSPHR.v1i1.4036

Prevalence of Group B Streptococcus among Individuals in Yenagoa, Bayelsa State, South South, Nigeria

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Article Info:

Submitted:	Revised:	Accepted:	Published:
Sep 25, 2024	Oct 11, 2024	Oct 23, 2024	Oct 31, 2024

Abstract

Streptococcus agalactiae also known as Group B Streptococcus (GBS) is a Gram-positive bacterium that primarily colonizes the gastrointestinal and genitourinary tracts of humans. While it is a commensal organism in many healthy individuals, GBS can become pathogenic, causing a variety of infections. This study investigated the prevalence of Group B Streptococcus (GBS) colonization among adults in Yenagoa, Bayelsa State, Nigeria, to assess the distribution of GBS across various age groups and between sexes. A total of 185 individuals were included in the study which comprises of both male and female across different age groups (16–35 years). The specimens were cultured on Todd-Hewitt broth and Columbia Blood Agar. Morphological and biochemical tests, including Gram staining, CAMP test, and Hippurate Hydrolysis test, were performed to confirm the presence of GBS.Morphological analysis revealed small to medium-sized, grayish-white, beta-hemolytic colonies with characteristic cocci in pairs or chains, confirming *Streptococcus agalactiae*. The overall prevalence of GBS colonization was found to be 5.95%, with 5.17% in males and 6.30% in females. The highest prevalence was observed in the age group 31–35 years among females (16.00%) and in the 26–30 age group among males (9.09%). This study result provides essential

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baseline data on GBS prevalence among individuals in Yenagoa, highlighting the need for continued surveillance and targeted interventions in this area to reduce the risk of GBS transmission.

Keywords: Group B streptococcus, individuals, Prevalence, *Streptococcus agalactia,* Yenagoa

INTRODUCTION

Streptococcus agalactiae also known as Group B Streptococcus (GBS) is a Gram-positive bacterium belonging to the Streptococcaceae family. It is a leading cause of neonatal infections, but its impact on adult populations, particularly the elderly, immunocompromised individuals, and pregnant women, is increasingly recognized. GBS is part of the normal microbial flora of the gastrointestinal and urogenital tracts in approximately 10% to 30% of healthy adults (Akinniyi et al.,2017). While asymptomatic colonization is common, GBS can transition to a pathogenic state, leading to a range of diseases, from mild urinary tract infections (UTIs) to life-threatening conditions like bacteremia, pneumonia, and endocarditis (Batalis et al., 2007). GBS is categorized under the genus Streptococcus, a large group of bacteria known for their role in causing a variety of human diseases. Streptococci are non-motile, non-spore-forming, Gram-positive cocci that appear in chains under a microscope. The Lancefield classification system groups streptococci based on the carbohydrate composition of their cell wall antigens. GBS falls under Lancefield Group B, which distinguishes it from other important streptococci like Group A. Streptococcus (Streptococcus pyogenes) (Whiley & Hardie, 2009). GBS is further classified into ten capsular serotypes (Ia, Ib, II, III, IV, V, VI, VII, VIII, and IX) based on the structure of its polysaccharide capsule (Shabayek and Spellerberg, 2018). This capsule is a critical virulence factor, helping the bacterium evade the host immune system, specifically by inhibiting phagocytosis. Certain serotypes, such as III, are more commonly associated with invasive diseases in neonates, while others like V are increasingly linked to adult infections, particularly in the elderly (Fischer et al., 2021) .

GBS is a pathogen, with its prevalence varying based on geographic location and population group. In high-income countries, routine screening of pregnant women for GBS has significantly reduced neonatal infections through the use of intrapartum antibiotic prophylaxis (IAP). However, in low- and middle-income countries (LMICs) like Nigeria,

GBS screening is not routinely performed, and as a result, neonatal GBS infection rates may significantly remain high. While most studies focus on pregnant women due to the risk of neonatal transmission, GBS prevalence among adults in the general population remains under-researched. In Bayelsa State, there is a dearth of epidemiological data on GBS, particularly regarding its colonization and infection rates among non-pregnant adults. Given the state's high burden of chronic diseases like diabetes and HIV, which are risk factors for GBS infection, understanding the local prevalence of GBS is essential for informing public health interventions.

METHODS

Study Design

A cross-sectional research design was deemed appropriate to achieve the set aims and objectives of this research. A total of 185 individuals comprising of 58 males and 127 females who are 16 years of age but certainly not more than 35 years were included in this experimental research. A stratified sampling technique was used to select the participants within the demography set for this research. Participants were randomly selected to avoid selection bias. Ethical rights of included individuals were considered by anonymizing their identities and other demographic indices.

Ethical Consideration

Before the commencement of this research ethical approval was obtained from the Federal Medical Centre, Yenagoa Research Ethics Committee and other relevant authorities. Informed consent was obtained from individuals participating in the study. Participants had clear understanding of the purpose of the research, potential risks and benefits, and their rights as participants. They were fully informed and voluntarily agree to participate without any coercion. The privacy and confidentiality of participants was protected, and any personal or identifiable information collected during the study was safeguarded and used only for research purposes. The selection of participants was fair and representative of the targeted population.

Isolation of Group B Streptococcus

Upon getting to the laboratory swab samples collected were inoculated into LIM selective enrichment Broth i.e., Todd-Hewitt broth with 15 μg nalidixic acid and 10 μg colistin and

incubated at 35°C with 5% CO2 for 24-48 hrs. The LIM broth was observed for turbidity and subcultured onto Columbia Agar with 5% Sheep's Blood using streak plate method. The agar plates were Incubated in a CO2-enriched atmosphere at 35-37°C for 18-24 hours. After incubation, the agar surface was examined for b-hemolytic and non-hemolytic colonies. Beta-hemolytic colonies with characteristic morphology (small, gray-white, translucent, with narrow zones of beta-hemolysis) was selected as GBS suspect. Upon growth, visible colonies were subjected to biochemical and molecular analysis.

Biochemical Identification Tests

Presumed isolates of Streptococcus agalactiae were subjected to Gram staining (GS), catalase test (CAT), CAMP test, the Hippurate hydrolysis test (HHT) and the Latex Agglutination Test.

Gram Staining

This was performed to identify Gram-positive cocci. A bacterial smear was made on a microscope slide, air-dried, and heat-fixed. Thereafter, the slide was stained with crystal violet and rinsed off with water after one minute. Following this, iodine was placed on the slide and rinsed with water after another minute. Decolorization was performed on the slide with ethanol and washed off again with water. The slide was then counterstained with safranin for a minute. Following another episode of rinsing and drying, the slide was viewed under a microscope.

Catalase Test

This was done by adding a drop of hydrogen peroxide to a microscope slide containing a little amount of the test organism (Streptococcus agalactiae). A vigorous sign of bubbling indicates a positive result, which is inconsistent with Streptococcus agalactiae.

CAMP Test

This was done by streaking a blood agar plate along a line with Staphylococcus aureus down to the middle, and the test organism (Streptococcus agalactiae) was streaked perpendicular to it, but without touching one other. Following incubating at 37°C for 18-24 hours in a CO2 incubator, observation was made for a heightened zone of hemolysis at the intersection indicating a positive result.

Hippurate Hydrolysis Test (HHT): A loopful of Streptococcus agalactiae was added to 0.4 mL of sterile 1% sodium hippurate solution and immediately incubated for 24 hours at

37°C. Subsequently, 0.2 mL of ninhydrin reagent was added and was incubated for another 30 minutes. Observation was made for a deep purple color indicative of a positive result.

Latex Agglutination Test

Latex agglutination tests for GBS antigen detection were utilized for serological identification, employing a Streptococcus latex group kit for serotyping. This kit is capable of identifying the most prevalent streptococci groups: A, B, C, D, F, and G. It comprises latex particles coated with streptococcal group antiserum for these specific streptococci groups, along with extraction reagents, reaction cards, and mixing sticks. The test procedure involved mixing the group antiserum with a specimen obtained from selective broth, which had been inoculated and incubated for 24 hours at 37°C in ambient air, on the reaction card. In cases of no reaction or indistinct reactions, extraction reagent was added. A positive reaction resulted in agglutination within 30 seconds, while a negative reaction showed no agglutination within the same timeframe.

RESULTS

Table 1: provides a detailed description of the morphological and biochemical characteristics of GBS isolates. In Todd-Hewitt Broth, Group B streptococcus Produced uniform turbidity after incubation indicative of bacterial growth. On Columbia Blood Agar, Streptococcus agalactiae appeared as small to medium-sized, grayish-white, and smooth colonies exhibiting beta-hemolysis, characterized by a clear zone of hemolysis around the colonies, indicative of their ability to lyse red blood cells, the isolate presented as cocci occurring in pairs or chains (diplococci or streptococci morphology), displaying non-motile behavior and facultative anaerobic growth conditions.

Table 1: Cultural and Biochemical characteristics of isolates

ISOLATE	Culture Media	Morphological Characteristic	Gram Stain	CAT	CAMP	HHT	LAT	
A	Todd-Hewitt Broth	Produces uniform turbidity after incubation indicative of bacterial growth.	Positive Cocci	-	+	+	+	*Streptococcus agalactiae*
	Columbia Blood Agar	Appear as small to medium-sized, grayish-white, and smooth colonies. The colonies are beta-hemolytic, producing a clear zone of hemolysis around them due to their ability to lyse red blood cells. Appear as cocci occurring inpairs or chains (diplococcior streptococci morphology). These cocci are typically non-motile and facultatively anaerobic.						

Gram staining of the isolate confirmed the presence of gram-positive cocci, definitively identifying it as Streptococcus agalactiae. The negative catalase test indicated the absence of catalase enzyme activity, a distinguishing feature from catalase-positive bacteria such as Staphylococcus spp. The CAMP test yielded a positive result, demonstrating the presence of the Christie-Atkins-Munch-Peterson (CAMP) factor, which aids in differentiation from other β-hemolytic streptococci. Additionally, the latex agglutination test indicated a positive result.

Keys: Cat ; Catalase test

CAMP; Christie-Atkins-Munch-Peterson test

HHT; Hippurate Hydrolysis Test

LAT; Latex Agglutination Test

Table 2: Prevalence of Group B *Streptococcus*

Age Group (%)	Sex	Sample Collected	Positive Cases	Prevalence
16-20	Male	13	0	0.00
	Female	33	1	3.03
21-25	Male	20	1	5.00
	Female	28	1	3.57
26-30	Male	11	1	9.09
	Female	41	2	4.88
31-35	Male	14	1	7.14
	Female	25	4	16.00
Overall	Male	58	3	5.17
	Female	127	8	6.30
Total		**185**	**11**	**5.95%**

Table 2: present data on the prevalence Group B Streptococcus across different age groups and sexes which include a total of 185 participants, including 58 males and 127 females, across four age groups (16-20, 21-25, 26-30, and 31-35 years). No case of GBS was recorded among males aged 16-20, resulting in a prevalence of 0.00%. In contrast, 1 case was positive among the 33 females in this age group yielding a prevalence of 3.03%. 1 out of 20 males tested positive (5.00%) for aged category of 21-25, against 1 out of 28 females who tested positive (3.57%) in the same age demography. None of the 11 males in the 26-30 age group tested positive (0.00%), whereas 2 out of 41 females tested positive (4.88%). Among participants aged 31-35, 2 out of 14 males tested positive (14.29%), and 4 out of 25 females tested positive (16.00%). Overall, the prevalence of GBS among males across all age groups was 5.17%, with 3 positive cases out of 58 participants. Among females, the overall prevalence was 6.30%, with 8 positive cases out of 127 participants. The combined prevalence across all participants was 5.95%, with 11 positive cases out of 185 participants. These findings underscore a notably higher prevalence of GBS infection among females compared to males, particularly evident in the age groups 26-30 and 31-35.

DISCUSSION

Streptococcus agalactiae was isolated from a 11 out of 185 samples examined giving a prevalence of 5.95%. This is a relatively low prevalence when compared to previous studies in several other geographical settings. A prevalence study by Dozie-Nwakile et al., (2022) in

Enugu Nigeria, reported a higher prevalence 15% from neonates and women. Also, another epidemiological study by Takang et al., (2022) reported a seemingly higher prevalence of 13.2% which is higher than the one reported in this research but lesser than the that reported in Enugu. Discrepancies in prevalence as reported by other studies orchestrated by other socioeconomic, demographic and environmental factors (Gonçalves et al., 2022). The considerably low prevalence reported in this experiment is an evidence of enhanced healthcare practices in the region which encourages routine screening, effective surveillance system, prompt management of infections and practicable preventive protocols (Lelei 2023). This is evident in other geographical region such as China with improved health care system. Consistent with our findings, a prevalence study reported an 8.7% colonization rates of GBS in China (Ge et al., 2021). Also, the exclusion of individuals on antibiotic therapy could be another contributory factor to the low prevalence in this study. This is because, those who tested positive from the selected participants may be asymptomatic and did not show any signs of the infection and were yet to be prescribed any form of antibiotics.

Age emerged as an influencing factor on the colonization of the GBS in this research as those within the aged of 16-20 reported lesser prevalence of 3% as opposed to the 13% observed among participants within the ages of 31-35, with the highest prevalence. Similar to the 16-20 age category, participants within the ages of 21-25 and 26-30 recorded a prevalence of 4.1% and 5.7%, respectively. From this prevalence result it is explicit that prevalence has a pattern of increasing with respect to age, and this pattern has been attributed to several behavioral, immunological and socioeconomic determinants (van Kassel et al., 2021). Firstly, individuals belonging to the lower age demography scarcely attend routine health screening compared to adults resulting in lower detection rates of the GBS and other related health conditions (Dozie-Nwakile et al., 2022). This is more evident as demonstrated in this research as the participation rates increased along the age group. Hence infection rates may be due to the frequency of routine health screening rather than age. However, there are existing arguments that, younger individuals are far less associated to risk factors such as multiple pregnancies and other medical conditions that are commonly associated with GBS (Berardi et al., 2021). Hence the slight prevalence of 3% observed among females within the ages of 16-20 which may be more or less attributed to early screening carried out in this research which inadvertently detected GBS colonization in otherwise asymptomatic individuals (McRobbie 2021). Contrary to our findings that

documented higher prevalence rates among higher age demography, Schindler et al, (2020) reported a lower prevalence among older individuals compared to the younger counterparts. The study further argued that, discrepancies may not necessarily be influenced by age but other environmental, geographic and socioeconomic variations. Another angle to look at the higher prevalence with age is the aspect of hormonal changes and increased sexual activities among individuals belonging to the higher age demography (Mollaioli 2020). Increased sexual activity and other lifestyle factors including risky sexual behaviors, having multiple sexual partners significantly contributes to higher rates of GBS colonization observed in this demographic (Quilatan 2020). Increased sexual activities without necessary protections provides enablement for the transmission and colonization. Sexually active individuals have increased probability of exposure to potential carriers. According to McManus et al., (2020), lifestyle factors and bad sexual behaviors increases the chances of GBS transmission. Particularly, lifestyle and environmental factors such as living in shared environments without proper hygiene protocols may increase the possibility of GBS colonization (Quilatan 2020). Contrary to our finding

Gender specific factors particularly health seeking behaviors were intricate in the prevalence as documented in this research. Females exhibited slightly higher prevalence rates of 6.30%, compared to males with 5.17% pervasiveness. This finding is consistent with that of Lemma et al., (2022) who documented that GBS is more prevalent among women particularly among parous women and those withing child bearing demography. The higher prevalence rates seen among females is linked with reproductive health screening prioritized for women across diverse settings (Collin et al., 2020). Routine sexual health screening during antenatal sessions is critical for preventing mother to child transmission of GBS (Gonçalves et al., 2022, Schindler et al., 2020). This further highlights the seemingly poor healthcare seeking behavior of men as evident in this study. The participation rate of males (31.4%) is a far cry compared to female (68.6%). Routine screening for GBS increases the likelihood of early detection of the bacterium for prompt and effective management preventing further complications or transmission (Quilatan, M. J. 2020). While this prevalence is lower compared to females, it indicates that males are still susceptible to GBS colonization and infection (McManus et al., 2020). Nonetheless, low awareness on GBS and other STIs have been blamed for the considerably low utilization of screening opportunities by males (Mollaioli et al., 2020). Therefore, it is important to incorporate males into routine health screening and increase awareness on the risk factors

associated with GBS and other medical condition (Sekeb et al., 2024). Contrary to the finding of this current study, Slotved and Hoffmann (2020) reported a higher colonization rate of GBS among males in Denmark. However, their study had a refined dissimilar focus on individuals older than 60 years. This highlights the need for further study among populations in that age group.

Implications to public health: The epidemiological findings of this study along its demographic distribution offers valuable insights that is relevant in guiding effective public health policies. It will further help in planning regional screening protocols and equitable resource allocation in efforts to contain community the spread of GBS induced infections. Additionally, age-specific findings of this research highlight the importance of health promotion efforts that will particularly effect positive health behaviours among the cohort who lacks health literacy and awareness and knowledge on GBS along its preventive measures.

Limitation of Study: Key strength of this experimental research includes its research methodologies employing standard epidemiological and microbiological protocols. One significant limitation is the small sample size which is relatively small and affects the generalizability of this findings to broader setting. The restricted age demography of 16-35 limits the extrapolation of findings to other age demography without the ones studied.

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