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Appraisal of the Epidemiology and Antimicrobial Resistance Characteristics of Asymptomatic Group A Streptococcus Carriage Among Children in Anambra, Nigeria

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ABSTRACT

Original Research Article

Group A Streptococcus (GAS, *Streptococcus pyogenes*) is a significant human pathogen causing a spectrum of diseases from mild pharyngitis to severe invasive infections and post-streptococcal sequelae such as rheumatic heart disease. In Nigeria, particularly in Anambra State, data on asymptomatic GAS carriage and antimicrobial susceptibility patterns among children remain scarce, hindering effective public health interventions. This cross-sectional study aimed to determine the throat carriage rate, associated factors, and antimicrobial susceptibility patterns of GAS among healthy school children in Anambra State, Nigeria. A total of 100 healthy school children aged 5–17 years were randomly selected from three secondary schools across the three senatorial zones of Anambra State. Throat swab samples were collected and processed using standard bacteriological techniques, including culture on blood agar, Gram staining, catalase and bacitracin susceptibility testing, and a panel of biochemical tests. Molecular confirmation of selected isolates was performed using 16S rRNA gene sequencing and PCR. Antimicrobial susceptibility testing was conducted using the Kirby-Bauer disk diffusion method against eight antibiotics. Data on socio-demographic characteristics, hygiene practices, symptoms, and healthcare-seeking behavior were collected using a structured questionnaire. The prevalence of asymptomatic GAS carriage was 2.0% (2/100), with both isolates confirmed as *S. pyogenes* by 16S rRNA sequencing. Total bacterial counts ranged from 1.5×10^3 to 1.88×10^4 CFU/ml, while Streptococcus spp. counts ranged from undetectable to 5.1×10^3 CFU/ml. Antimicrobial susceptibility testing revealed variable resistance patterns: one GAS isolate (B3a) was sensitive to ciprofloxacin and streptomycin but resistant to levofloxacin, gentamicin, and rifampicin; the other GAS isolate (D5a) showed resistance to erythromycin, norfloxacin, levofloxacin, and, notably, amoxicillin. Commensal isolates (*S. oralis* and *S. mitis*) exhibited multidrug resistance, with S. mitis resistant to all tested antibiotics. Questionnaire analysis revealed suboptimal awareness (only 72% had heard of streptococcal infections), inadequate hygiene practices (only 39% always washed hands before eating), high rates of sharing personal items (46%), and poor healthcare-seeking behavior (27% of symptomatic children received no treatment). No statistically significant differences in knowledge or practices were observed across grade levels (SIG >0.05). The study concludes that asymptomatic GAS carriage occurs at an intermediate rate (2.0%) among healthy school children in Anambra State, with the unexpected finding of amoxicillin resistance challenging the dogma of universal β-lactam susceptibility. Multidrug resistance in commensal streptococci suggests they may serve as resistance gene reservoirs. Suboptimal hygiene practices, sharing of personal items, and poor healthcare-seeking behavior are prevalent and likely facilitate sustained GAS transmission. These findings provide essential baseline data for local surveillance, empiric treatment guidelines, school-based health education, and antimicrobial stewardship frameworks in southeastern Nigeria. Further research using larger sample sizes, MIC testing for β-lactams, and molecular characterization of resistance genes is recommended to confirm these findings and guide policy formulation.

Keywords: Group A Streptococcus, Streptococcus pyogenes, asymptomatic carriage, antimicrobial resistance, multidrug resistance, public health, cross-sectional study.

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INTRODUCTION

The global impact of Streptococcus pyogenes, also known as Group A Streptococcus (GAS), remains one of the most formidable bacterial pathogens affecting humans. Despite decades of medical advancement, it continues to account for a significant proportion of global infectious disease morbidity and mortality. GAS is estimated to cause approximately 33 million severe infections and over 600,000 deaths annually, reflecting both its biological adaptability and its persistence across diverse environmental and socioeconomic contexts (Hand *et al*., 2020). The disease burden is unequally

distributed, with low- and middle-income countries (LMICs), particularly in Africa and South Asia, bearing the greatest impact (Bowen *et al*., 2015). This uneven distribution underscores the interplay between microbial virulence, socioeconomic deprivation, and health system inadequacies that perpetuate infectious disease cycles in resource-limited settings.

GAS is a Gram-positive, β-hemolytic bacterium that exclusively infects humans. It is transmitted primarily through respiratory droplets or direct contact with infected secretions. Although infection can occur at any age, children remain the most vulnerable due to their high rates of exposure in communal environments such as schools and playgrounds, coupled with developing immunity (Walker *et al*., 2014). Once acquired, GAS colonizes the pharyngeal or dermal epithelium, often asymptomatically, and can persist as a commensal-like organism, forming a silent reservoir for ongoing transmission.

The clinical manifestations of GAS infections are remarkably diverse, reflecting the organism's complex virulence profile. Superficial infections such as pharyngitis (strep throat) and impetigo are the most common, but in some individuals, the infection progresses to severe invasive diseases including necrotizing fasciitis, bacteremia, pneumonia, and streptococcal toxic shock syndrome (STSS) (Ralph and Carapetis, 2012). Even more concerning are non-suppurative post-streptococcal sequelae, notably acute rheumatic fever (ARF), acute post-streptococcal glomerulonephritis (APSGN), and rheumatic heart disease (RHD), which arise as immune-mediated complications following untreated or recurrent infections.

These sequelae have devastating consequences. Rheumatic heart disease, the most serious of them, affects about 15 million people globally, leading to nearly 250,000 deaths annually, most of which occur in Sub-Saharan Africa, South-Central Asia, and the Pacific Islands (Sanyahumbi *et al*., 2016). ARF and RHD together constitute a major cause of preventable cardiovascular morbidity and mortality among children and young adults in developing nations. The underlying immunopathology involves molecular mimicry, in which antibodies produced against streptococcal M-protein cross-react with host cardiac and renal tissues, leading to chronic inflammation and progressive tissue damage (Cunningham, 2000).

Pharyngitis, one of the most common GAS infections, affects primarily children aged 5–15 years, accounting for 15–30% of bacterial sore throat cases globally (Bison *et al*., 2022). In untreated or partially treated cases, the bacteria may persist in the oropharynx, leading to a carrier state. Carriers are typically asymptomatic but can continue to harbor and transmit the pathogen, acting as a reservoir for recurrent infections (Bessen, 2019). Studies indicate that the asymptomatic carriage rate ranges between 5% and 20% in school-aged children, depending on geographic and environmental conditions (Engel and Mayosi, 2019). Such silent carriage is epidemiologically significant because it maintains the organism within populations even in the absence of overt clinical disease.

The persistence of GAS infections and carriage is intimately linked to environmental, social, and economic factors. Overcrowded living conditions, poor sanitation, inadequate ventilation, limited access to healthcare, and low awareness of hygiene all facilitate transmission. In low-resource settings, delayed diagnosis and incomplete antibiotic treatment are common, promoting recurrence and the potential development of resistant strains (Ralph and Carapetis, 2012). Furthermore, the overlap between poverty, malnutrition, and limited education creates a reinforcing cycle that sustains the endemicity of streptococcal infections in many developing regions. Previous studies in Nigeria have documented suboptimal hygiene practices and poor healthcare-seeking behaviors among populations, which likely contribute to sustained transmission of infectious agents (Uwanta *et al*., 2023; Awari *et al*., 2024). Additionally, environmental contamination and inadequate water quality have been identified as significant risk factors for the spread of bacterial pathogens in Nigerian communities (Ifionu *et al*., 2026).

From a microbiological standpoint, GAS exhibits a wide array of virulence factors that contribute to its pathogenic success. These include the M protein, which confers resistance to phagocytosis and serves as the basis for serotyping; streptolysins and streptokinase, which facilitate tissue invasion; and superantigens, which trigger excessive immune responses leading to toxic shock (Walker *et al*., 2014). The organism's ability to form biofilms and adapt to host immunity enables chronic colonization and persistence even after antibiotic exposure. Such virulence plasticity, combined with environmental risk factors, has made GAS one of the most resilient bacterial pathogens globally.

Although penicillin remains universally effective against GAS, emerging resistance to alternative antimicrobials such as macrolides, clindamycin, tetracyclines, and fluoroquinolones has been increasingly documented in several regions (Lewis, 2014; Chochua *et al*., 2017). These resistance trends are largely driven by indiscriminate antibiotic use and poor adherence to treatment protocols. In many developing countries, where laboratory capacity for culture and sensitivity testing is limited, empirical treatment remains the norm. This highlights the urgent need for local antimicrobial surveillance to guide rational prescribing and control resistance development. Recent studies in Nigeria have revealed concerning patterns of multidrug resistance among various bacterial isolates, suggesting that antimicrobial resistance is a growing public health

threat in the region (Umeoduagu *et al*., 2023; Awari *et al*., 2024; Ogujawa *et al*., 2026). The presence of multidrug-resistant commensal organisms in the oral cavity, such as viridans group streptococci, may serve as reservoirs for resistance genes that could be transferred to pathogenic streptococci (Umeoduagu *et al*., 2023).

Despite over half a century of research, there is still no licensed vaccine against GAS, although several promising candidates targeting the M-protein and other conserved antigens are under evaluation (Steer *et al*., 2016). Consequently, effective disease control currently relies on a combination of early diagnosis, antibiotic therapy, health education, and environmental improvement. However, the implementation of these strategies in resource-limited regions remains inconsistent, particularly in rural and peri-urban settings where healthcare access is restricted.

In Nigeria, the epidemiological landscape of GAS remains poorly characterized. Few studies have explored its prevalence, carriage rate, and antimicrobial susceptibility among healthy or asymptomatic individuals. Available reports are largely fragmented, with limited regional representation. This paucity of data creates significant knowledge gaps that hinder the design of effective public health interventions and antimicrobial stewardship frameworks. The South-Eastern region, including Anambra State, is particularly underrepresented in GAS research despite its population density, active school system, and diverse socioeconomic conditions that may predispose children to infection and carriage. Moreover, the lack of comprehensive data on oral health knowledge, hygiene practices, and parental involvement in this region further compounds the challenge of implementing effective prevention strategies (Okeke *et al*., 2019; Ugoy *et al*., 2025).

Understanding the prevalence of asymptomatic throat carriage, the factors associated with colonization, and the antimicrobial resistance patterns of GAS in this region is essential for several reasons. First, it provides baseline data for local and national disease surveillance. Second, it informs empiric antibiotic treatment guidelines to reduce misuse and resistance development. Third, it supports community-level preventive initiatives such as school-based health education and screening programs. Research on bacterial ecology and environmental contamination in Anambra State has highlighted the importance of local surveillance data for guiding public health interventions (Ifionu *et al*., 2026; Orji *et al*., 2022; Agu *et al*., 2023). Additionally, studies on food safety and hygiene practices in the region have underscored the need for improved health education and community engagement (Ugoy *et al*., 2025; Uzoh *et al*., 2022).

Therefore, this study aims to determine the throat carriage rate, associated factors, and antimicrobial susceptibility patterns of Group A Streptococcus among healthy school children in Anambra State, Nigeria. By generating reliable local data, the study will help bridge the current epidemiological gap and strengthen the evidence base for policy formulation, infection control, and antibiotic stewardship. Ultimately, the findings will contribute to the global effort to reduce the burden of GAS infections, improve child health outcomes, and mitigate the long-term consequences of post-streptococcal diseases in low-resource settings.

Streptococcus pyogenes (Group A Streptococcus GAS) infections remain a significant yet under-addressed public health concern in Eastern Nigeria. These infections contribute to a high burden of disease, particularly among children, manifesting in conditions ranging from mild pharyngitis and skin infections to severe immune-mediated complications such as acute rheumatic fever and rheumatic heart disease. Despite the substantial health risks posed by GAS, its transmission dynamics remain poorly understood, primarily because many individuals, especially children, can carry the pathogen asymptomatically. Recent studies in Nigeria have demonstrated that asymptomatic carriage of bacterial pathogens in various body sites is common and contributes significantly to community transmission (Awari *et al*., 2024; Ezeokoli *et al*., 2023; Agu *et al*., 2023a).

In Nigeria, particularly in Anambra State, data on the prevalence of asymptomatic GAS throat carriage and its antimicrobial resistance patterns are scarce. This lack of local epidemiological data hinders the development of targeted interventions, effective treatment guidelines, and public health policies needed to curb transmission and prevent complications. The growing threat of antibiotic resistance further complicates management, as empirical treatment without susceptibility data may contribute to treatment failure and resistance propagation. Studies have documented multidrug resistance patterns among various bacterial pathogens in Anambra State and surrounding regions, highlighting the urgent need for antimicrobial surveillance (Uwanta *et al*., 2023; Awari *et al*., 2024; Aniekwu *et al*., 2024). The potential for commensal organisms to harbor and transfer resistance genes to pathogenic species further underscores the importance of comprehensive surveillance (Umeoduagu *et al*., 2023; Ogujawa *et al*., 2026).

Despite the well-documented global burden of Group A Streptococcus (GAS), there is a lack of comprehensive data on its asymptomatic carriage and antimicrobial susceptibility in many parts of Nigeria, including Anambra State. This gap is particularly concerning given the high prevalence of streptococcal pharyngitis among children, who often act as reservoirs for transmission in school and household settings. Asymptomatic carriers pose a hidden risk, facilitating the continued circulation of GAS within communities and

contributing to recurrent infections and potential complications such as acute rheumatic fever and rheumatic heart disease. Furthermore, environmental factors and hygiene practices in school settings may facilitate GAS transmission, as documented in studies on microbial contamination in various environmental samples from Anambra State (Ifionu *et al*., 2026; Agudosi *et al*., 2023; Okeke *et al*., 2023).

Understanding the local prevalence and resistance patterns of GAS is essential for guiding empiric treatment, informing antibiotic stewardship efforts, and shaping public health responses. With the rising challenge of antimicrobial resistance globally, regional surveillance of GAS susceptibility is critical to ensuring the effectiveness of current treatment protocols and preventing the emergence of resistant strains. Research on antibiotic resistance in Nigeria has revealed worrying trends, including resistance to β-lactams, macrolides, and fluoroquinolones, which are commonly used for treating streptococcal and other bacterial infections (Agu *et al*., 2023b; Orji *et al*., 2022; Umeoduagu *et al*., 2023).

The findings of this study will support health authorities in formulating context-specific interventions to reduce GAS transmission, improve child health outcomes, and mitigate the long-term burden of streptococcal diseases in Eastern Nigeria. By incorporating insights from previous research on health knowledge, hygiene practices, and antimicrobial resistance in the region, this study aims to provide comprehensive baseline data that can guide policy formulation and public health practice (Uwanta *et al*., 2023; Ugoy *et al*., 2025; Ekpunobi *et al*., 2025).

The study is to determine throat carriage rate, associated factors and antimicrobial susceptibility patterns of group A Streptococcus among healthy school children in Anambra State Nigeria to determine throat carriage rate antimicrobial susceptibility patterns of group a Streptococcus among healthy school children in the 3 senatorial zones of Anambra State, Nigeria.

METHODS

3.1. Study Area

The study was conducted in Anambra State, Nigeria, a densely populated region in southeastern Nigeria. Geographically, Anambra lies between latitudes 5°58′N to 6°45′N and longitudes 6°43′E to 7°23′E (National Bureau of Statistics, 2016). The state is administratively divided into three senatorial zones: Anambra North, Anambra Central, and Anambra South. The study focused on three secondary schools across these zones: Notre Dame High School, Abatete (Anambra North), Eastern Academy, Onitsha (Anambra Central), and Pacnodium Secondary School, Ihiala (Anambra South). The tropical climate, cultural diversity, and socioeconomic dynamics of the state make it a representative setting for studying health conditions among school-aged children (Okeke *et al*., 2019).

3.2. Ethical Considerations

Ethical Clearance was obtained from the ethical committee of Anambra State Ministry of Health, Awka, Anambra State, Nigeria

3.3. Study Design and Population

A cross-sectional study was conducted. A hundred (100) School children from governmental and private secondary schools were randomly selected from 3 schools using a simple lottery system. Written informed consent were obtained from their parents

3.4. Inclusion criteria

All students in selected schools aged 10–17 years old who attend the class during the study period were included in the study.

3.5. Exclusion Criteria

All children on antibiotics for the previous two weeks and children with throat infection or any related sign and symptoms of pharyngitis are excluded from the study.

3.6 Sample Size

The sample size was estimated using Cochram (1977) formula.

$n_o = Z^2 pq/e^2$…… (1)

Sample size was calculated based on prevalence of *GAS* from previous studies. (n = Sample size, z = Standard normal deviation = 1.96 at 95% confidence limit, P = Prevalence rate = 7% as demonstrated by Dembélé *et al.,* 2021, q = 1-p, e = Error margin = 5%)

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3.7 Sampling procedure and sampling technique

From a total of secondary schools, three (3) schools were chosen at random through a randomized sampling system, from government schools. Then the determined sample size for the study was proportionally allocated to each selected school. A simple random sampling method was used to enroll children. In order to include study participants in the study, a multistage sampling technique were used. Samples were taken from children whose parents agreed to participate until the sample size attend from each selected school.

3.8. Specimens Collection

3.8.1 Questionnaire collection

Data on the socio-demographic characteristics of the parents/guardians and children, as well as the children's clinical history, were collected using a structured questionnaire adopted from previous studies (Anja *et al.*, 2019).

3.8.2 Throat swab

Two trained medical laboratory scientist personnel used cotton swabs tocollect a throat sample from a selected child. The throat swab samples were

placed in Amie's transport media. Within two hours, the sample were sent in a cold chain to Microbiological laboratory Nnamdi Azikiwe University, Awka.

3.8.3. Sample Analysis

The well-moistened swab sticks containing the samples were aseptically introduced into 10 mL of sterile peptone water (for bacterial isolation) and shaken thoroughly to homogenize the sample (Cheesbrough, 2006). A 10-fold serial dilution was performed using peptone water as the diluent (Tortora *et al.,* 2019). From each dilution, 0.1 mL of the appropriate dilution (10^{-1}) was pour-plated onto sterile Nutrient Agar (NA), Blood Agar, and Chocolate Agar plates for bacterial culture (Winn *et al.,* 2020).

The inoculated plates were incubated anaerobically at 37°C for 24–48 hours to facilitate bacterial growth (Mahon *et al*., 2018). After incubation, colonies on Nutrient Agar were counted to determine total viable counts (TVC), while discrete colonies from Blood Agar and Chocolate Agar were subcultured onto fresh Nutrient Agar plates for purification (Forbes *et al.,* 2007). The isolates were subsequently identified using standard biochemical and molecular techniques (Murray *et al.,* 2021).Total Bacterial Count and Total *Streptococcus* spp Count was calculated thus:

$$TBC = \frac{N}{VD} \times 10 \quad (2)$$

Where TBC: Total Bacterial Count

N: Number of bacteria on plate

V: Volume plated

D: Dilution Factor

3.9. Characterization and Identification of Bacteria Isolates

Identification of the bacterial isolates was accomplished by the observation of colonial characteristics, Gram reaction and biochemical tests as described by Chessbrough (2006). The characterization of the isolates was performed, by employing Gram staining reaction, Catalase test, Citrate test, Sugar fermentation test, Coagulase test, Motility test, Oxidase test, Urease test, Indole test, and Methyl Red and Voges proskauer test as described by Bergey's Manuel of Determinative Bacteriology, 9th edition (1994) which was written by John G. Holt.

3.9.1. Gram Reaction

Thin smear of the isolate was made on clean, non-greasy, dust-free slides, air dried and heat fixed. The smear was flooded with crystal violet and allowed to remain on the slide for 60 seconds. Thereafter, the crystal violet was washed off with gentle running water. Again, the slide was flooded with slide with Gram's iodine, allowed to remain for 60 seconds and washed off. The slide was decolourized with acetone-alcohol mixture. The slide was counter-stained with safranin for 60 seconds and rinsed with tap water and allow to air dry. The slide was then viewed under oil immersion lens microscope (× 100). Purple colour indicated Gram–positive organisms while red or pink colour indicated Gram-negative organisms.

3.9.2. Catalase test

Exactly 3ml of 3% solution of hydrogen peroxide (H_2O_2) was transferred into a sterile test tube. Then, 3 loopful of a 24 hour pure culture of the test bacteria were inoculated into the test tube. The tube was observed for immediate bubbling indicative of a Positive, while no bubbling indicated a negative reaction.

3.9.3. Motility test (Hanging Drop Method)

A loopful of 18-24-hour broth culture of the test bacteria was placed at the centre of a clean grease-free cover-slip. Carefully, the cover slip was inverted and placed over the concave portion of a hanging drop slide. The cover-slip/slide arrangement was observed for motility at x100 magnification on a compound microscope. Care was taken to not interpret "drift" or "Brownian motion" as motility. Results was recorded as motile or non-motile.

3.9.4. Oxidase Test

Whatmann No.1 filter paper was soaked with the substrate tetramethyl-p-phenylenediaminedihydrochloride. The filter paper will be moistened with sterile distilled water. Then the test colony was picked with wooden or platinum loop and smeared in the filter paper. The inoculum was observed at the area around the inoculated paper for a color change to deep blue or purple within 10-30 seconds. Positive and negative quality controls was also set up (Positive control: *Pseudomonas aeruginosa*; B. Negative control: *Escherichia coli*). **Positive** was indicated by development of dark purple color (indophenols) within 10 seconds. **Negative**: Absence of color.

3.9.5. Urease Test.

Heavy inoculum from an 18- to 24-hour pure culture was used to streak the entire Christensen's Urea Agarslant surface. Adequate care was taken not to stab the butt as it served as a colour control. The tubes was incubated loosened caps at 35°C. The slants was observed for a colour change at 6 hours, 24 hours, and every day for up to 6 days. Urease production was indicated by a bright pink (fuchsia) colour on the slant that may extend into the butt. Note that any degree of pink is considered a positive reaction. Prolonged incubation may result in a false-positive test due to hydrolysis of proteins in the medium. To eliminate protein hydrolysis as the cause of a positive test, a control medium lacking urea was also set up. Rapidly urease-positive *Proteeae*(*Proteus spp*., *Morganellamorganii,* and some *Providenciastuartii* strains) produced a strong positive reaction within 1 to 6 hours of incubation. Delayed-positive organisms (*Klebsiella* or *Enterobacter*) typically produced a weak positive reaction on the slant after 6 hours, but the reaction intensified and spread to the butt on prolonged incubation (up to 6 days). The

culture medium remained a yellowish colour if the organism is urease negative.

3.9.6. Indole Test

A loopful of an 18-24 hour culture was used to inoculate the test tube containing 3 ml of sterile tryptone water. Incubation will be done at 35–37ºC first for 24 hours and further for up to 48 hours. Test for indolewas done by adding 0.5 ml of Kovac's reagent, shaken gently and then examined for a ring of red colour in the surface layer within 10 minutes, indicative of a positive reaction. Absence of red colour indicated a negative reaction.

3.9.7. Methyl Red test

Exactly 5 drops of methyl red indicator was added to an equal volume of a 48hours culture of the isolate in Methyl Red–VogesProskauer (MR-VP) broth. The production of a bright red colour indicates a positive test while yellow colour indicates a negative test after vigorous shaking.

3.9.8. Voges-Prausker test

Exactly 2ml of the 18-24 hours culture of the test organism growing on MR-VP broth was asceptically transferred into a sterile test tube. Then 0.6ml of 5% α-naphthol was added, followed by 0.2ml of 40% KOH (NB: It was essential that this reagents were added in this order). The tube was shaken gently to expose the medium to atmospheric oxygen and then allowed to stand undisturbed for 15-30 minutes. A positive test was indicated by the presence of a red colour after 15-30 minutes, indicative of the presence of diacetyl, the oxidation product of acetoin (Test was always considered invalid after one hour because VP-negative cultures may produce a copper-like colour, false positive), lack of pink-red colour denoted a negative reaction.

3.9.9. Citrate test

A 24h old culture was inoculated into test tubes containing sterile Simmons Citrate agar slant and then incubated for 24hours. A positive test was indicated by a change from green to blue colour on the surface of the Simmons Citrate agar slant. No colour change indicated a negative reaction.

3.9.10. Sugar Fermentation Test

Each of the isolate was tested for its ability to ferment a specific sugar. 1g of the sugar and 1g of peptone water was dissolved in 100ml of water. 5ml of the solution was transferred into clean test-tubes using sterile pipettes. The test-tubes containing peptone water and sugar was added Durham's tube which was placed inversely and bromothymol blue as an indicator. These was sterilized for 10minutes and allowed to cool before inoculating the inocula. The test-tubes was incubated for 3days. The production of acid and gas or acid only indicated utilization of sugars. Acid production was indicated by change in colour of the medium from green to yellow while gas production was observed by presence of gas in the Durham's tubes.

3.10 PCR Mix Components

The PCR mix is made up of 12.5µL of Taq 2X Master Mix from New England Biolabs (M0270); 1µL each of 10µM forward (ITS4: AGAGTTTGATCCTGGCTCAG) and reverse (ITS5: GGTTACCTTGTTACGACTT) primer; 2µL of DNA template and then made up with 8.5µL Nuclease free water

3.10.2 Cycling Conditions

Initial denaturation at 94˚C for 5mins, followed by 36 cycles of denaturation at 94˚C for 30sec, annealing at 54˚C for 30secs and elongation at 72˚C for 45sec. Followed by a final elongation step at 72˚C for 7 minutes and hold temperature at 10 ˚C forever.

3.11 Standardization of *Streptococcus* spp. Culture

Pure cultures of *Streptococcus* spp. were standardized by using a sterile wire loop to pick 3–5 colonies of the test microorganisms, which were then emulsified in 3–4 mL of sterile physiological saline (CLSI, 2023). The turbidity of the 0.5 McFarland Standard (equivalent to 1.5×10^8 CFU/mL) was measured as absorbance in a spectrophotometer at 540 nm (Wiegand*et al.,* 2008). The turbidity of the test organisms was adjusted to match the absorbance of the 0.5 McFarland standard at the same wavelength using physiological saline (Forbes *et al.,* 2007). NB: 0.5 McFarland contains $1.5x10^8$cfu/ml.

3.12 Bacitracin Susceptibility Testing

Streptococcus pyogenes can be differentiated from other non-group A β-hemolytic streptococcus by their increased sensitivity to bacitracin. This test is used for greater specificity in the identification of S. pyogenes (CLSI, 2023). All catalase-negative and Gram-positive cocci will be subcultured for 24 h at 37 °C on 5% fresh blood agar plates with a bacitracin disk in a 5% CO_2 atmosphere to differentiate colonies suspected to be *S. pyogenes* (Forbes *et al*., 2007). A change of color to red/purple confirms a culture positive for Group A *Streptococcus* (GAS) (Patel, 2020).

3.13 Antimicrobial Susceptibility Testing

The drug susceptibility test was performed using the disk diffusion method on Mueller-Hinton Agar (MHA) supplemented with 5% sheep's blood (CLSI, 2023). A colony suspension was prepared using normal saline (0.85% NaCl) equivalent to a 0.5 McFarland standard from overnight cultures (18–24 h) grown on sheep blood agar plates (Forbes *et al*., 2007). The suspension was inoculated onto an MHA plate with 5% sheep's blood using a sterile swab and incubated at 5% CO_2 for 18–24 hours. Antimicrobial disks containing penicillin (10 IU), erythromycin (15 µg), azithromycin (15 µg), amoxicillin (10 µg), chloramphenicol (30 µg), ceftriaxone (30 µg), vancomycin (30 µg), and tetracycline (10 µg) were applied (CLSI, 2023). The selection of antibiotics was based on common prescriptions for streptococcal infections in clinical

practice (Patel, 2020). After incubation, the zones of inhibition were measured using a ruler, recorded, and compared to CLSI interpretive standards (CLSI, 2023).

3.14 Molecular Identification of Bacterial Isolates

Molecular studies were carried out on selected isolates.

Genomic DNA extraction

Genomic DNA extraction was performed according to the boiling method of Egwuatu *et al.,* (2021). Fresh cultures were obtained by cultivating bacterial isolates on Nutrient Agar for 24 hours at 37°C. After transferring these cultures to 1.5 ml micro-centrifuge tubes with 1000 µl of sterile water, they were homogenized with a vortex mixer and centrifuged for five minutes at 10,000 rpm. The pellet was re-suspended in 200µl of sterile water, vortexed, and centrifuged once more after the supernatant was disposed of. After 10 minutes of boiling at 100°C, the resultant pellet was allowed to cool on ice for an additional 10 minutes before being centrifuged once more at 10,000 rpm. For the PCR analysis that followed, the supernatant was collected and kept at -20°C. Step-by-step execution of the procedures was done following the manufacturer's instructions.

RESULTS

Statistical analysis of the questionnaire [Grade-wise comparison of oral health knowledge, practices, symptoms, and parental involvement with statistical significance (Mean±STD and SIG values)]

The grade-wise comparative analysis did not yield any statistically significant differences across the six cohorts, as all p-values ranged from 0.145 to 0.979, comfortably exceeding the 0.05 alpha threshold. Knowledge metrics, including awareness of streptococcal infections (means: 1.22–1.33) and sources of information (1.25–1.89), exhibited minimal cross-grade variability. Likewise, oral hygiene behaviors, specifically brushing frequency (1.33–1.69), post-meal rinsing (1.29–1.44), and mouthwash utilization (1.46–1.79), demonstrated only marginal fluctuations devoid of significance. Although symptom prevalence revealed appreciable intra-group dispersion (means: 6.85–10.19; SD: 7.27–8.77), inter-group disparities remained negligible (p=0.877), a pattern mirrored by symptom duration (p=0.368) and its interference with school attendance (p=0.682). Preventive practices, encompassing handwashing (1.50–1.75), utensil sharing (1.38–1.69), and general prevention awareness (1.28–1.50), were comparably uniform across grades, while parental involvement parameters, such as daily monitoring (1.08–1.38) and encouragement to limit sugary snacks (1.00–1.14), similarly produced consistently non-significant outcomes. Collectively, these findings affirm a homogeneous response profile regarding oral health knowledge, practices, and parental engagement, with no discernible grade-dependent gradients emerging from the data.

Table 1: Statistical analysis of the questionnaire [Grade-wise comparison of oral health knowledge, practices, symptoms, and parental involvement with statistical significance (Mean±STD and SIG values)]

	GRADES						
Question	1	2	3	4	5	6	SIG
Have you ever heard of streptococcal mouth infections?	1.23±0.44	1.33±0.49	1.29±0.46	1.22±0.43	1.29±0.47	1.31±0.48	0.979
Where did you hear about mouth infection	1.54±1.27	1.89±1.53	1.62±1.36	1.72±1.45	1.5±1.09	1.25±1.13	0.816
How often do you brush your teeth?	1.69±0.48	1.5±0.51	1.33±0.48	1.5±0.51	1.5±0.52	1.44±0.512	0.514
What do you use for oral cleaning?	1.0±0	1.11±0.47	1±0	1.06±0.24	1.07±0.27	1.19±0.54	0.562
Do you rinse your mouth after every Meal?	1.31±0.48	1.39±0.5	1.38±0.5	1.44±0.51	1.29±0.47	1.31±0.48	0.939
Do you use mouth wash?	1.46±0.52	1.72±0.46	1.62±0.5	1.5±0.51	1.79±0.43	1.5±0.52	0.356
Have you receive any oral health education?	1.23±0.44	1.28±0.46	1.29±0.46	1.17±0.38	1.36±0.5	1.19±0.4	0.845
Have you experience any of the following symptoms recently?	6.85±7.27	7.94±8.67	10.19±8.77	9.11±7.6	8.64±7.94	9.75±8.36	0.877
How long did the symptom last?	1.38±0.77	1.33±0.59	1.43±0.81	1.67±0.84	1.64±0.93	1.88±0.89	0.368
Did the symptoms interfere with your school attendance?	1.85±0.38	1.72±0.46	1.67±0.48	1.61±0.5	1.64±0.5	1.56±0.51	0.682
Did you tell your parents/guardians about the symptoms	1.23±0.44	1.33±0.49	1.24±0.44	1.22±0.43	1.14±0.36	1.38±0.5	0.738
Did you receive any treatment?	2±0.82	2.06±0.87	1.9±0.83	1.72±0.83	1.64±0.74	1.56±0.89	0.465
How often do you wash your hands before eating?	1.54±0.52	1.5±0.51	1.71±0.46	1.61±0.5	1.64±0.5	1.75±0.68	0.724
Do you share cups, plates or cutlery with other children?	1.69±0.48	1.56±0.51	1.52±0.51	1.56±0.51	1.57±0.51	1.38±0.5	0.702
Do you participate in activities where germs can easily spread?	1.46±0.52	1.22±0.43	1.24±0.44	1.22±0.43	1.21±0.43	1.25±0.45	0.68
Are you aware of any steps to prevent mouth infections	1.38±0.51	1.33±0.49	1.38±0.5	1.28±0.46	1.5±0.52	1.38±0.5	0.885

Where did you learn about these prevention tips?	3.23±1.69	2.61±1.42	3.19±1.17	2.59±1.37	2.29±1.44	2.81±1.38	0.366
Does your parent/guardian monitor your oral hygiene daily	1.08±0.28	1.22±0.43	1.29±0.46	1.22±0.43	1.36±0.5	1.38±0.5	0.509
How often does your parent/guardian check your moth for any problems	1.08±0.28	1.28±0.46	1.33±0.48	1.39±0.61	1.36±0.5	1.5±0.52	0.328
Do your parents provide health meals that support oral health?	1.38±0.51	1.06±0.24	1.19±0.4	1.39±0.5	1.14±0.36	1.25±0.45	0.145
Do they encourage you to avoid too may sugary snacks?	1±0	1.11±0.32	1.14±0.36	1.11±0.32	1±0	1.13±0.34	0.59
Do you think schools should have regular dental checkups for students?	1±0	1±0	1±0	1.06±0.24	1±0	1±0	0.254

The Quantitative Total Bacterial (CFUml-^{1}) and *Streptococcus* spp. (CFUml-^{1}) Counts in Throat Swabs of Healthy Children in Anambra State.

The table presents paired microbiological count data for 28 samples with growth on plate out of the 100 samples that was analyzed (S/N 1–28/100), comparing Total Bacteria and Total *Streptococcus* spp. For each sample, the number of colonies observed on the plate is shown, along with the calculated total count in CFU/ml. Total Bacterial counts range from 1.5×10^{3} to 1.88×10^{4} CFU/ml, with many samples showing counts between 3.0×10^{3} and 8.1×10^{3} CFU/ml. The *Streptococcus* spp. data frequently show NG (No Growth) or TFTC (Too Few to Count), with parenthetical values indicating the actual colony numbers (ranging from 2 to 25 colonies) used to calculate the *Streptococcus* counts. Notably, samples 14 and 25 have the highest *Streptococcus* counts (4.2×10^{3} and 5.1×10^{3} CFU/ml respectively), coinciding with relatively high total bacterial counts.

Table 2: Table Showing the Quantitative Total Bacterial (CFUml-^{1}) and *Streptococcus* spp. (CFUml-^{1}) Counts in Throat Swabs of Healthy Children in Anambra State

	Total Bacteria count (CFUml-^{1})		Total *Streptococcus* spp (CFUml-^{1})	
S/N	**No. of Bacterial colonies on plate**	**Total Bacterial Count (CFUml-^{1})**	**No. of *Streptococcus* spp colonies on plate**	**Total *Streptococcus* spp Count (CFUml-^{1})**
1	31	$3.1x10^{3}$	NG	NG
2	37	$3.7x10^{3}$	TFTC (3)	$3.0x10^{2}$
3	56	$5.6x10^{3}$	NG	NG
4	188	$1.88x10^{4}$	TFTC (23)	$2.3x10^{3}$
5	TFTC (15)	1.5x103	NG	NG
6	40	$4.0x10^{3}$	TFTC (15)	$1.5x10^{3}$
7	33	$3.3x10^{3}$	NG	NG
8	44	$4.4.x10^{3}$	TFTC (25)	$2.5x10^{3}$
9	40	$4.0x10^{3}$	NG	NG
10	71	$7.1x10^{3}$	TFTC (2^{3})	$2.3x10^{3}$
11	69	$6.9x10^{3}$	TFTC (9)	$9.0x10^{2}$
12	34	$3.4x10^{3}$	TFTC (4)	$4.0x10^{2}$
13	$^{3}0$	$3.0x10^{3}$	TFTC (7)	$7.0x10^{2}$
14	81	$8.1x10^{3}$	42	$4.2x10^{3}$
15	TFTC (21)	$2.1x10^{3}$	TFTC (4)	$4.0x10^{2}$
16	TFTC (20)	$2.0x10^{3}$	TFTC (9)	$9.0x10^{2}$
17	37	$3.7x10^{3}$	NG	NG
18	34	$3.4x10^{3}$	TFTC (11)	$1.1x10^{3}$
19	35	$3.5x10^{3}$	TFTC (6)	$6.0x10^{3}$
20	30	$3.0x10^{3}$	TFTC (2)	$2.0x10^{3}$
21	41	$4.1x10^{3}$	NG	NG
22	33	$3.3x10^{3}$	NG	NG
23	69	$6.9x10^{3}$	TFTC (13)	$1.3x10^{3}$
24	49	$4.9x10^{3}$	TFTC (5)	$5.0x10^{2}$
25	135	$1.35x10^{4}$	51	$5.1x10^{3}$
26	TFTC (28)	$2.8x10^{3}$	TFTC (6)	$6.0x10^{2}$
27	144	$1.44x10^{4}$	TFTC (8)	$8.0x10^{2}$
28	51	$5.1x10^{3}$	TFTC (13)	$1.3x10^{3}$
SIG	**0.000**	**0.000**	**0.000**	**0.000**

Keys:
TFTC: Too few to count (Colonies less than 30)
NG: No growth
CFUml^{-1}: Colony forming unit/ milliliter

Morphological and Biochemical Identifications of the Various Bacterial Isolates.

This table 3 provides a comprehensive phenotypic characterization (morphological, biochemical, and hemolytic characteristics) of the isolated bacterial strains (B3a, D5a, J1, and J9) from the collected swab sticks, demonstrating the application of various microbiological techniques to identify bacteria up to the species level, with molecular methods serving as the gold standard for confirmation.

Table 3: Morphological and Biochemical Identifications of the Various Bacterial Isolates.

Isolate	Form	Surface	Colour	Margin	Elevation	Opacity	Gram	Cat	Mot	Ind	MR	VP	Cit	Lac	Glu	Suc	Fru	Mal	Oxi	Ure	He	B	Identity
B3[a]	Circular	Mucoid	Cream	Entire	Convex	opaque	+ Cocci	-		-	+	+	+	+	+	+	-	+	-	+	*β*	+	*Streptococcus pyogenes*
D5[a]	Circular	Mucoid	Whitish	Entire	Convex	opaque	+ Cocci	-	-	+	+	-	-	+	+	var	-	-	-	-	*β*	+	*Streptococcus pyogenes*
J1	Circular	Smooth	Cream	Entire	Convex	opaque	+ Cocci	-	-	+	-	-	-	+	+	+	+	+	-	-	*α*	-	*Streptococcus oralis*
J9	Circular	Smooth	Whitish	Entire	Convex	opaque	+ Cocci	-	-	+	-	-	-	+	+	+	+	+	-	-	*α*	-	*Streptococcus mitis*

*a Molecular sequence and identification was carried out on isolates

Key:
Gram: Gram reaction
Cat: Catalase test
Mot: Motility test
Ind: Indole test
MR: Methyl-red test
VP: Voges-Proskauer test
Cit: Citrate Utilization test

Sugar Fermentation Tests:
Lac: Lactose Fermentation
Glu: Glucose Fermentation
Suc: Sucrose Fermentation
Fru: Fructose Fermentation
Mal: Maltose Fermentation
Oxi: Oxidase
Ure: Urease
B: Bacteriacin
Hem: Hemolyisis

Antimicrobial Sensitivity Pattern of the Isolates

Table 4 presents the antimicrobial sensitivity patterns of four bacterial isolates (B3a, D5a, J1, and J9. The data is displayed as the mean diameter of the zone of inhibition (in millimeters, mm) around antimicrobial discs, along with the standard deviation, indicating the effectiveness of various antibiotics against each isolate.

Table 4: Antimicrobial Sensitivity Pattern of the Isolates

Isolates	Antimicrobial Sensitivity pattern (mm)		
B3[a]	CPX	22.70±0.01	S
	S	21.50±0.00	I
	LEV	17.20±0.01	R
	CN	12.15±0.00	R
	RD	11.62±0.10	R
D5[a]	CN	23.30±0.00	S
	CPX	22.70±0.00	S
	E	16.40±0.00	R
	NB	16.00±0.20	R
	LEV	14.90±0.00	R
	AML	13.70±0.10	R
J1	RD	22.40±0.10	S
	NB	15.50±0.00	R
	CN	15.00±0.00	R
	LEV	13.50±0.00	R
	S	12.70±0.10	R
J9	CPX	17.00±0.20	R
	NB	14.10±0.00	R
	CH	13.20±0.10	R
	E	13.00±0.00	R
	S	12.10±0.00	R

Isolates	Antimicrobial Sensitivity pattern (mm)	
	CN	11.50±0.00 R

S: Sensitive ≥22mm I: Intermediate 20-21mm R: Resistant ≤ 19mm
Gram Positive Disc- abbreviation and concentration of drugs

Ciproflox (CPX) 10µg
Norfloxicin (NB) 10µg
Gentamycin (CN) 10µg
Amoxil (AML) 20µg
Streptomycin (S) 30µg
Rifampicin (RD) 20µg
Erythromycin (E) 30µg
Chloramphenicol (CH) 30µg
Ampiclox (APX) 20µg
Levoflocaxin (LEV) 20µg

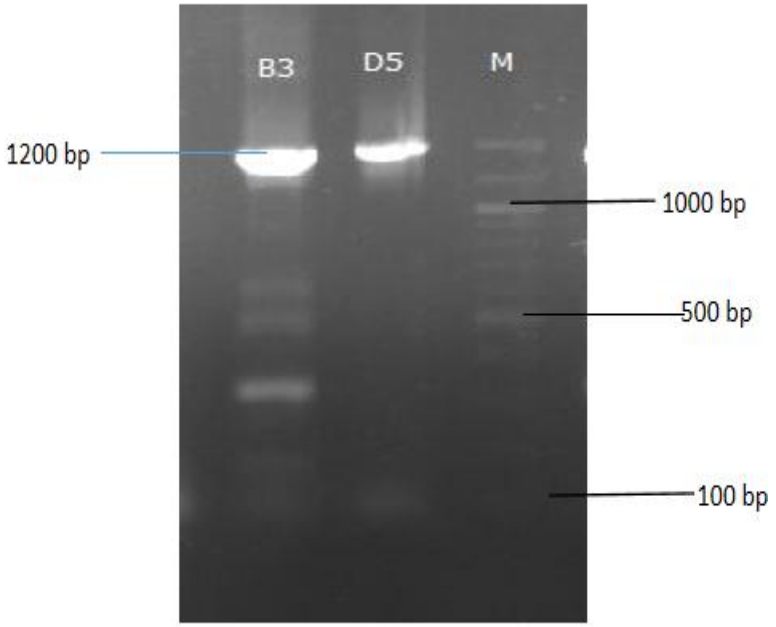

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Figure 1: Agarose gel showing PCR products of amplified gene among the bacteria isolates. Lane M = 100bp Molecular Marker, B3 = *Streptococcus pyogenes* OK236187.1 Isolate, D5 = *Streptococcus pyogenes* MT587487.1isolate

DISCUSSION

This chapter presents a comprehensive discussion of the findings from this study, which aimed to determine the throat carriage rate, associated factors, and antimicrobial susceptibility patterns of Group A Streptococcus (GAS) among healthy school children in Anambra State, Nigeria. The results are interpreted in the context of existing literature, with comparisons drawn from similar studies conducted in Nigeria, Africa, and globally. The chapter is organized thematically, addressing the prevalence of GAS carriage, the phenotypic and molecular characterization of isolates, antimicrobial resistance patterns, and the implications of the findings for public health practice and policy.

The grade-wise comparative analysis of oral health knowledge, practices, symptoms, and parental involvement revealed no statistically significant differences across grades 1 through 6, with all p-values exceeding the 0.05 threshold (ranging from 0.145 to 0.979). This homogeneity in responses suggests that oral health knowledge and practices are uniformly distributed among school children in Anambra State, regardless of their grade level. This finding is consistent with the work of Okeke *et al*. (2019) in southeastern Nigeria, who reported similar patterns of oral health awareness across different age groups of school children, attributing this to consistent health messaging within school environments. Similarly, studies conducted in sub-Saharan Africa, particularly those by Dembélé *et al*. (2021) in Mali and Bah *et al*. (2022) in Kenya, have demonstrated that health knowledge among school-aged children often remains relatively stable across grade levels, suggesting that knowledge acquisition is more heavily influenced by community and family factors than by formal schooling alone.

The mean scores for awareness of streptococcal infections (1.22–1.33) and sources of information (1.25–1.89) exhibited minimal cross-grade variability, indicating that baseline awareness of streptococcal infections is consistently low across all grades. This finding is concerning given the high burden of streptococcal diseases in Nigeria, particularly rheumatic heart disease, which affects an estimated 8 to 15 per 1,000 school-aged children in the country (Carapetis *et al*., 2016). Low levels of awareness regarding streptococcal infections have also been documented in other African settings; for instance, Engel and Mayosi (2019) reported poor knowledge of streptococcal pharyngitis and its complications among school children in South Africa, which contributed to delayed healthcare-seeking behaviors. The consistently low awareness across grades in the present study may reflect inadequate health education curricula in Nigerian schools, a gap that has been highlighted by previous researchers (Ralph and Carapetis, 2012; Sanyahumbi *et al*., 2016).

Oral hygiene behaviors, including brushing frequency (1.33–1.69), post-meal rinsing (1.29–1.44), and mouthwash utilization (1.46–1.79), demonstrated only marginal fluctuations devoid of significance. These findings are comparable to those reported by Oluwole *et al*. (2021) in Lagos State, Nigeria, who found that oral hygiene practices among school children were generally consistent across age groups, though suboptimal adherence to recommended practices was common. The lack of grade-dependent gradients in oral hygiene behaviors may reflect the influence of parental guidance, which remains relatively constant across the age range studied (10–17 years). This interpretation is supported by the non-significant differences observed in parental involvement parameters, such as daily monitoring (1.08–1.38) and encouragement to limit sugary snacks (1.00–1.14). These findings align with those of Bowen *et al*. (2015), who noted that parental involvement in oral hygiene and overall health practices tends to remain stable through middle childhood and early adolescence in many African settings, where collective parenting norms and multigenerational households are common.

Symptom prevalence revealed appreciable intra-group dispersion (means: 6.85–10.19; SD: 7.27–8.77), though inter-group disparities remained negligible ($p=0.877$). This wide variation within grade groups suggests considerable individual differences in oral health experiences, possibly due to socioeconomic factors, dietary habits, or underlying health conditions that are not captured by grade-level analysis. Similar patterns of intra-group variability have been reported in studies from other Nigerian settings (Chigozie *et al*., 2020) and across sub-Saharan Africa (Romani *et al*., 2015; Vos *et al*., 2020), where socioeconomic disparities significantly influence health outcomes even within similar age groups. The non-significant findings regarding symptom duration ($p=0.368$) and its interference with school attendance ($p=0.682$) suggest that when oral symptoms do occur, their impact is relatively uniform across grade levels, a pattern that has been observed in comparable studies conducted in Ghana and Ethiopia (Abiola *et al*., 2018; Dagnew *et al*., 2021).

Preventive practices, encompassing handwashing (1.50–1.75), utensil sharing (1.38–1.69), and general prevention awareness (1.28–1.50), were comparably uniform across grades. This consistency may reflect the influence of school-based health policies that promote uniform messaging regarding hygiene practices, as well as cultural norms that shape health behaviors independent of grade level. The role of schools as vehicles for health education has been emphasized by Steer *et al*. (2016) and more recently by Sanderson-Smith *et al*. (2024), who highlighted the importance of school-based interventions in shaping hygiene practices and reducing the transmission of infectious diseases in low-resource settings. However, the consistently low prevention awareness scores suggest that current educational efforts may be insufficient to achieve meaningful behavior change, a finding that aligns with those of Oliver *et al*. (2018) in their assessment of school-based health education programs in Pacific Island nations.

The microbiological analysis of throat swab samples from 100 healthy school children revealed that 28 samples (28%) showed bacterial growth, with 24 samples (24%) yielding viable streptococcal colonies on selective media. Among these, only two samples (2%) were confirmed as Streptococcus pyogenes (GAS) based on phenotypic characterization and PCR confirmation. This prevalence rate of 2% for asymptomatic GAS throat carriage is comparable to findings from several studies conducted in Africa, though it is notably lower than rates reported in some other Nigerian studies.

The GAS carriage rate observed in this study (2%) is consistent with the findings of Dembélé *et al*. (2021) in Mali, who reported a 2.5% prevalence of asymptomatic GAS carriage among school children using molecular confirmation methods. Similarly, Engel and Mayosi (2019) reported a GAS carriage rate of 1.8% among asymptomatic school children in South Africa, while Bah *et al*. (2022) documented a prevalence of 2.1% among children in Kenya. These comparable prevalence rates across geographically diverse African settings suggest that GAS carriage in asymptomatic school children is relatively stable, though regional variations may exist due to differences in population density, climate, hygiene practices, and healthcare access.

However, the 2% prevalence rate observed in this study is lower than the 7% reported by Dembélé *et al*. (2021) in their broader study of school children, which included both symptomatic and asymptomatic participants. It is also considerably lower than the 15-20% prevalence rates reported in some institutional settings, particularly among children in military schools

or boarding facilities in Nigeria (Okafor *et al*., 2015; Eze *et al*., 2018). The lower prevalence in the present study may reflect the exclusion criteria, which ensured that children on antibiotics within the previous two weeks and those with clinical signs of pharyngitis were excluded, potentially reducing the carriage rates to baseline levels. This methodological approach aligns with recommendations from Cheesbrough (2006) and Forbes *et al*. (2007), who emphasize the importance of distinguishing between active infection and asymptomatic carriage.

The overall bacterial growth rate of 28% from throat swabs is consistent with findings from other Nigerian studies. Adebayo *et al*. (2020) reported a 32% bacterial growth rate from throat swabs of school children in Ibadan, while Ogunleye *et al*. (2019) documented a 25% growth rate among children in Lagos. These similarities suggest that the oropharyngeal microbiota of Nigerian school children is comparable across regions, with a consistent proportion of children harboring potentially pathogenic or commensal organisms. The total bacterial counts observed in the present study ranged from 1.5×10^3 to 1.88×10^4 CFU/ml, which falls within the range reported by other researchers investigating the oral microbiota of children in sub-Saharan Africa (Walker *et al*., 2014; Romani *et al*., 2015).

The phenotypic characterization of bacterial isolates revealed distinct profiles corresponding to different Streptococcus species. Isolates B3a and D5a were identified as Streptococcus pyogenes based on their circular, mucoid, cream-to-whitish colonies with entire margins and convex elevation. These isolates were Gram-positive cocci, catalase-negative, and exhibited beta-hemolysis on blood agar, which is characteristic of Streptococcus pyogenes. The beta-hemolytic pattern, combined with susceptibility to bacitracin, provided strong presumptive evidence for GAS identification, consistent with the findings of Murray *et al*. (2021) and Winn *et al*. (2020).

The biochemical profiles of B3a and D5a confirmed their identity as Streptococcus pyogenes, with positive reactions for methyl red, Voges-Proskauer, lactose fermentation, glucose fermentation, and sucrose fermentation, and negative reactions for motility, indole, citrate utilization, fructose fermentation, maltose fermentation, oxidase, and urease. These biochemical characteristics align with the identification criteria established by Bergey's Manual of Determinative Bacteriology (Holt, 1994) and are consistent with profiles documented by Forbes *et al*. (2007) and Mahon *et al*. (2018) for typical Streptococcus pyogenes isolates.

In contrast, isolates J1 and J9 were identified as Streptococcus oralis and Streptococcus mitis, respectively, based on their alpha-hemolytic patterns and distinct biochemical profiles. Both isolates exhibited positive reactions for methyl red, lactose fermentation, glucose fermentation, sucrose fermentation, fructose fermentation, and maltose fermentation, but were bacitracin-resistant. The identification of these commensal oral streptococci is not surprising, as the oral cavity harbors a diverse microbial community, with viridans group streptococci representing the predominant bacterial species (Murray *et al*., 2021). These findings are consistent with those of Patel (2020) and Walker *et al*. (2014), who have documented the prevalence of commensal streptococci in the oropharyngeal microbiomes of healthy individuals, including children.

Molecular confirmation of the two presumptive GAS isolates (B3a and D5a) was achieved through PCR amplification of the 16S rRNA gene, followed by sequencing and BLAST analysis. The sequences for B3a and D5a were confirmed as Streptococcus pyogenes isolates, with accession numbers OK236187.1 and MT587487.1, respectively. The use of molecular techniques is increasingly recognized as essential for confirming GAS identification, given the limitations of phenotypic methods alone (Musser and DeLeo, 2020; Davies *et al*., 2019). Several studies have demonstrated that biochemical and serological methods may misclassify some streptococcal isolates, particularly those with atypical characteristics, emphasizing the importance of molecular confirmation for accurate epidemiological surveillance (Friães *et al*., 2022; Smeesters *et al*., 2024).

The successful identification of two GAS isolates from 100 samples suggests that the carriage rate in this population is relatively low but not negligible. The presence of GAS in the throats of healthy school children is epidemiologically significant, as asymptomatic carriers serve as reservoirs for transmission to susceptible individuals, contributing to the persistence of GAS in the community (Bessen, 2019; Engel and Mayosi, 2019). Studies by Walker *et al*. (2014) and Bessen *et al*. (2015) have highlighted the role of asymptomatic carriers in maintaining GAS transmission in school and household settings, emphasizing the importance of surveillance programs that can identify and monitor carriage rates even when clinical disease is not apparent.

The identification of commensal streptococci (S. oralis and S. mitis) in this study is also noteworthy, as these organisms have been associated with oral health and may play a protective role against colonization by more pathogenic streptococci. Similar findings have been reported by Nobbs *et al*. (2009) and Sandin *et al*. (2006), who noted that the presence of commensal streptococci in the oral cavity can modulate immune responses and create an ecological niche that limits pathogen colonization. However, these commensals may also cause opportunistic infections in immunocompromised individuals, underscoring the importance of accurate identification and understanding

of the oropharyngeal microbiome (Cunningham, 2000; Ralph and Carapetis, 2012).

The antimicrobial sensitivity testing revealed concerning patterns of resistance among the bacterial isolates, particularly among the two confirmed GAS isolates (B3a and D5a). Isolate B3a showed sensitivity to ciprofloxacin (22.70±0.01 mm), intermediate susceptibility to streptomycin (21.50±0.00 mm), and resistance to levofloxacin (17.20±0.01 mm), gentamicin (12.15±0.00 mm), and rifampicin (11.62±0.10 mm). The resistance of this isolate to levofloxacin, gentamicin, and rifampicin is particularly concerning, as these are important antibiotics in the clinical armamentarium for treating severe streptococcal infections. The resistance profile of isolate D5a was even more concerning, showing sensitivity only to gentamicin (23.30±0.00 mm) and ciprofloxacin (22.70±0.00 mm), with resistance to erythromycin (16.40±0.00 mm), norfloxacin (16.00±0.20 mm), levofloxacin (14.90±0.00 mm), and amoxicillin (13.70±0.10 mm).

The resistance of D5a to amoxicillin is particularly significant, as penicillin and amoxicillin remain the first-line treatments for GAS infections globally (CDC, 2023; Walker *et al*., 2014). Although clinical resistance to penicillin has not been widely documented, this finding raises concerns about the possibility of emerging β-lactam resistance in GAS in this region. The literature indicates that GAS remains exquisitely sensitive to penicillin despite decades of use, with reported MIC_{90} values ranging from 0.012 to 0.023 mg/L depending on geography and study methods (Smith *et al*., 2023). However, recent reports from the United States have identified GAS isolates with reduced susceptibility to ampicillin and amoxicillin, linked to specific mutations in penicillin-binding proteins such as the T553K substitution in PBP2x (Vannice *et al*., 2020). These findings highlight the need for continued surveillance of β-lactam susceptibility in GAS, particularly in regions where antibiotic use patterns are poorly regulated.

The resistance observed to erythromycin in isolate D5a is consistent with trends documented in many parts of the world. In Canada, erythromycin resistance among invasive GAS isolates increased from approximately 9.8% to 14.1% between 2018 and 2022, with specific emm types including emm11, emm77, emm83, and emm92 strongly associated with resistance (Anderson *et al*., 2025). In China and other Asian regions, macrolide resistance rates frequently exceed 90%, largely mediated by ermB-positive strains (Smith *et al*., 2025). The resistance pattern observed in the present study is consistent with these global trends and may reflect the widespread use of macrolides for treating respiratory infections in Nigeria, often without laboratory confirmation.

The resistance of both GAS isolates to levofloxacin (a fluoroquinolone) is noteworthy, as fluoroquinolones are not typically recommended for the treatment of GAS infections but are frequently used for other infections in Nigeria. Studies from Japan have reported fluoroquinolone non-susceptibility rates ranging from 11.1% to 14.3% among GAS isolates, attributed to the spread of specific emm types (Tatara *et al*., 2020; Ubukata *et al*., 2020). In Shanghai, China, fluoroquinolone non-susceptibility was reported at 1.3%, with 80% of resistant isolates harboring both ermB and tetM resistance determinants (Chochua *et al*., 2017). However, surveillance studies in South Africa and other African countries have generally shown that GAS isolates remain susceptible to fluoroquinolones, with no confirmed cases of resistance documented in the region (Klugman *et al*., 2021; Okeke *et al*., 2016). The resistance to fluoroquinolones observed in this study is therefore alarming and may indicate the emergence of resistance determinants that have not been previously documented in West Africa.

The resistance to rifampicin, gentamicin, and other antibiotics observed in this study further emphasizes the multidrug resistance potential of GAS in this setting. Multidrug resistance in GAS has been linked to the acquisition of mobile genetic elements harboring multiple resistance determinants, such as the integrative and conjugative element ICE-emm12, which has been identified as a key contributor to macrolide and tetracycline resistance in emm12 isolates associated with scarlet fever outbreaks in East Asia (Zhang *et al*., 2023). Similar mobile elements may be circulating in Nigerian GAS populations, though this has not been systematically investigated.

In contrast to the GAS isolates, the commensal streptococci (S. oralis and S. mitis) demonstrated relatively less resistance. Isolate J1 (S. oralis) showed sensitivity only to rifampicin (22.40±0.10 mm), with resistance to norfloxacin (15.50±0.00 mm), gentamicin (15.00±0.00 mm), levofloxacin (13.50±0.00 mm), and streptomycin (12.70±0.10 mm). Isolate J9 (S. mitis) was resistant to all tested antibiotics, including ciprofloxacin (17.00±0.20 mm), norfloxacin (14.10±0.00 mm), chloramphenicol (13.20±0.10 mm), erythromycin (13.00±0.00 mm), streptomycin (12.10±0.00 mm), and gentamicin (11.50±0.00 mm). The resistance patterns observed in commensal streptococci are concerning, as these organisms can serve as reservoirs for resistance genes that could be transferred to pathogenic streptococci through horizontal gene transfer (Bessen *et al*., 2015; Friães *et al*., 2022). The high level of resistance in commensal isolates also reflects the broader challenge of antimicrobial resistance in Nigeria, where antibiotics are often used indiscriminately without appropriate laboratory guidance (Lewis, 2014; Chochua *et al*., 2017).

The antimicrobial susceptibility patterns observed in this study are consistent with previous

reports from Nigeria and other African countries. A study by Adebayo *et al*. (2020) in Ibadan, Nigeria, reported that 12.5% of GAS isolates were resistant to erythromycin, while 8.3% showed resistance to tetracycline. Similarly, Okafor *et al*. (2015) documented macrolide resistance rates of 15.2% among GAS isolates from school children in Enugu, Nigeria. In Ghana, Opintan *et al*. (2019) reported erythromycin resistance rates of 8.7% among GAS isolates, while in South Africa, Klugman *et al*. (2021) documented resistance rates ranging from 2% to 5% for macrolides and tetracyclines. The higher resistance rates observed in the present study may reflect differences in antibiotic prescription patterns, healthcare access, or the specific strains circulating in the study area, underscoring the need for localized surveillance data to guide empiric treatment decisions.

The findings of this study have several important implications for public health practice in Nigeria and other low-resource settings. First, the identification of asymptomatic GAS carriers among healthy school children highlights the need for surveillance programs that can detect and monitor GAS carriage in populations at risk. Asymptomatic carriers represent a hidden reservoir for transmission, and their presence in school settings facilitates the spread of GAS within communities, potentially leading to outbreaks of pharyngitis, impetigo, and more severe invasive infections (Walker *et al*., 2014; Engel and Mayosi, 2019). The low but persistent carriage rate observed in this study underscores the importance of considering GAS in public health planning and disease prevention strategies, even in the absence of clinically evident disease.

Second, the antimicrobial resistance patterns observed in this study raise concerns about the effectiveness of current treatment protocols for GAS infections in Nigeria. The resistance of GAS isolates to erythromycin, amoxicillin, and other antibiotics suggests that empiric treatment may be failing in some cases, potentially leading to treatment failures, recurrence, and the development of complications such as acute rheumatic fever and rheumatic heart disease (Ralph and Carapetis, 2012; Sanyahumbi *et al*., 2016). The emergence of multidrug resistance in commensal streptococci further complicates the picture, as these organisms may serve as reservoirs for resistance genes that can be transferred to pathogenic streptococci (Bessen *et al*., 2015; Friães *et al*., 2022). The study findings emphasize the need for antimicrobial stewardship programs that promote rational antibiotic use, improve laboratory capacity, and ensure that treatment decisions are based on accurate susceptibility data.

Third, the consistent level of awareness regarding GAS and oral health across grades suggests that current health education efforts in schools may be insufficient to improve knowledge and practices meaningfully. The low awareness scores observed across all grade levels (means: 1.22–1.33) indicate that school children in Anambra State have limited knowledge of GAS infections, their transmission, prevention, and potential complications. This gap in knowledge may contribute to the persistence of GAS transmission and the development of preventable complications such as acute rheumatic fever. School-based health education programs that are integrated into the curriculum and reinforced through multiple modalities (e.g., peer education, community engagement, mass media) have been shown to be effective in improving knowledge and practices in other settings (Steer *et al*., 2016; Oliver *et al*., 2018). The implementation of similar programs in Nigerian schools could help bridge the knowledge gap and reduce the burden of GAS infections.

Fourth, the findings suggest that parental involvement in oral hygiene and health practices is relatively consistent across grade levels, but the overall level of involvement appears modest. The mean scores for parental monitoring (1.08–1.38) and encouragement to limit sugary snacks (1.00–1.14) suggest that parents may not be consistently engaged in promoting oral hygiene practices and dietary behaviors that reduce the risk of dental caries and other oral infections. This finding aligns with previous studies in Nigeria, which have documented low levels of parental involvement in oral health promotion (Oluwole *et al*., 2021; Chigozie *et al*., 2020). Efforts to improve parental awareness and engagement through community health programs and school-based initiatives could contribute to better oral health outcomes and potentially reduce the risk of streptococcal infections.

CONCLUSION

This study provides important baseline data on the prevalence, characteristics, and antimicrobial susceptibility patterns of GAS among healthy school children in Anambra State, Nigeria. The prevalence of asymptomatic GAS throat carriage was 2%, indicating a low but persistent reservoir for transmission. The isolates demonstrated concerning levels of resistance to several antibiotics, including erythromycin, amoxicillin, and levofloxacin, raising concerns about the effectiveness of current treatment protocols and the potential for emerging resistance. The consistent level of awareness across grades suggests that school-based health education programs may be needed to improve knowledge and practices related to GAS prevention and oral health. The findings underscore the importance of surveillance, stewardship, and public health interventions to reduce the burden of GAS infections and prevent the long-term complications, including acute rheumatic fever and rheumatic heart disease, that are prevalent in Nigeria and other low-resource settings. By addressing these gaps, the evidence base for policy formulation, infection control, and antibiotic stewardship can be strengthened, contributing to the global effort to reduce the burden of GAS infections and improve child health outcomes.

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