



# Streptococcal Throat Carriage among Primary School Children Living in Uyo, Southern Nigeria

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## Abstract

Surveillance of the carrier state for  $\beta$ -hemolytic streptococcal (BHS) throat infections remains essential for disease control. Recent published works from Sub-Saharan Africa have suggested a changing epidemiology in the burden of BHS throat infections. The objective of the present study was therefore to determine the prevalence and pattern of BHS throat carriage in school-aged children in Uyo, Akwa Ibom State. This was a prospective cross-sectional study of 276 primary school children in Uyo. Subjects were recruited by multistage random sampling. Obtained throat swabs were cultured on 5% sheep blood agar. Lancefield grouping on positive cultures was done by using the Oxoid Streptococcal Grouping Latex Agglutination Kit, United Kingdom. Antimicrobial susceptibility testing was done with the disk diffusion method. Associations were tested with Fischer's exact test. The prevalence of BHS carriage was 3.3%. Group C Streptococcus was identified in 89% of isolates and Group G Streptococcus in 11%. Younger age and larger household size were associated with asymptomatic streptococcal throat infections. Antimicrobial susceptibility was highest with cefuroxime and clindamycin (89% of isolates each), while 78% of isolates were susceptible to penicillin. None of the tested isolates was susceptible to co-trimoxazole. The prevalence of streptococcal throat carriage in the study area was low. There were no Group A Streptococcus isolates suggesting an evolving epidemiology of BHS disease in the study area.

## Keywords

- ▶  $\beta$ -hemolytic streptococci
- ▶ group A streptococci
- ▶ throat carriage
- ▶ surveillance

## Introduction

Group A Streptococcus (GAS) is an important cause of infectious disease mortality, accounting for up to half a million deaths annually.<sup>1–3</sup> It is the most commonly isolated bacterial agent in acute pharyngitis and accounts for 15 to 30% of all throat infections.<sup>3–6</sup> However,  $\beta$  hemolytic streptococci (BHS) have also been isolated from asymptomatic individuals, who serve as reservoirs of infection in the community.<sup>7,8</sup> Active surveillance of BHS disease may thus be achieved by periodic evaluation of the prevalence of streptococcal throat carriage in a community.<sup>8–10</sup>

Streptococcal throat infection may be complicated by early suppurative as well as medium and long-term non-

suppurative sequelae such as acute rheumatic fever and post-streptococcal glomerulonephritis, with high morbidity and mortality.<sup>11,12</sup> Rheumatic heart disease affects over 15 million persons worldwide, causing about a quarter of a million deaths annually, with highest prevalence recorded in Sub-Saharan Africa.<sup>13,14</sup> While rheumatic fever is described in carriers, recurrent rheumatic fever episodes, with higher risk of rheumatic heart disease, have also been reported in some throat carriers.<sup>15,16</sup> Some authors have also stated an immune response in association with asymptomatic infections.<sup>17,18</sup>

Worldwide estimates for asymptomatic streptococcal throat infections show wide variation. In general, when well children attending outpatient clinics are considered,

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the rate of BHS carriage is between 2 and 5%.<sup>4</sup> However, school studies have been known to show a much wider range with BHS carriage rates between 8 and 40%.<sup>4</sup> A meta-analysis of literature published over the past 70 years by Oliver et al puts the GAS throat carriage prevalence rate at 5.9% (4.3–8.1%) in low- and middle-income countries, whereas a higher rate of 10.5% (8.4–12.9%) has been documented in the developed world.<sup>19</sup>

Studies from Sub-Saharan Africa on streptococcal throat carriage remain relatively rare. In Nigeria, in the last decade, three studies reported rates varying between 4 and 28.9% for streptococcal throat carriage in children.<sup>9,18,20</sup> Furthermore, these Nigerian studies have all demonstrated an absence of GAS in asymptomatic carriers despite purportedly high rates of rheumatic fever/rheumatic heart disease in the region. This increase in non-GAS BHS throat carriage has also been reflected in another African study, as well as studies from other parts of the world.<sup>7,21–28</sup>

Streptococcal throat infections are usually treated with a 10-day course of oral penicillin V or amoxicillin. Erythromycin or azithromycin may be used for penicillin-allergic individuals.<sup>29–31</sup> Though macrolide resistance has been widely reported especially with erythromycin and bacteriologic failure occasionally with penicillin, no large community or hospital-based studies have demonstrated *in vitro* resistance of GAS throat isolates to penicillin.<sup>30–35</sup>

The current study sets out to establish the prevalence of asymptomatic streptococcal throat infection and antimicrobial susceptibility patterns in school-aged children in Uyo, Southern Nigeria.

## Methods

The current study was performed in Uyo, a city with six political wards located in the Nigerian rainforest at an altitude of 70 m above sea level and with average annual rainfall of 2,509 mm.<sup>36</sup>

Written permission to carry out the study was obtained from the Akwa Ibom State Ministry of Education. Ethical clearance for the study was obtained from the Institutional Health Research and Ethics Committee of University of Uyo Teaching Hospital. Written/informed consent was obtained from all parent(s)/guardian(s) before recruiting their children into the study.

The study was performed between September 2017 and February 2018, which would correspond to the late rainy season and dry season in the study area.

Sample size was determined by using Fisher's formula applied to study populations greater than 10,000.<sup>37</sup> The total number of enrolled pupils in primary schools in Uyo was 71,659 making the formula appropriate.

$$Z^2 PQ/D^2$$

Where:

N = minimum sample size

Z = 1.96

D = total width of the expected confidence interval (set at 0.05)

P = prevalence from similar study at a nearby location<sup>18</sup> = 20.6%

Q = 1-P

Therefore:

$$N = 1.96^2 \times (0.206 \times 0.794)/0.05^2 = 251$$

An attrition rate of 10% was assumed, and thus, the total number of subjects enrolled in the study was 276.

Primary school students aged 6 to 12 years, whose parents gave consent, were enrolled in the study. Children with symptoms of acute pharyngitis or those who had used antibiotics in 2 weeks preceding the study were excluded.

## Sampling Method

A multistage sampling method was used to recruit for the study. Overall, 12 schools, that is, two per political ward were selected from the 55 schools (15 public and 40 private) in the Uyo metropolis. Four public and eight private schools were selected to achieve proportionate representation from the schools. These schools were selected by simple random sampling using the table of random numbers.

The number of pupils selected from each of the 12 schools was determined by proportionate sampling in the second stage. The school sample size was determined by using the formula<sup>9</sup>:

$$N2 = a \times N1/n$$

Where:

N2 = number of pupils recruited from a given primary school

a = population of index school

n = total number of pupils in all the selected primary schools

N1 = total sample size

In each school, one arm of each class was selected randomly for the study by using the ballot method.<sup>38</sup> The number of pupils obtained from the chosen arm in a given school was calculated by using the class population (all arms), the school population, and the school sample size as calculated. The calculated sample size of each school (N2) was then distributed proportionately among the classes by using the formula<sup>9</sup>:

$$N3 = a \times N2/b$$

Where:

N3 = class sample size

a = class population (all arms)

b = population of all classes in the school

N2 = school sample size

The first pupil was randomly selected from the list of pupils in that class by using the ballot method.<sup>38</sup> All subsequent pupils recruited were then selected by using a systematic sampling method. The list of pupils in the class was

arranged alphabetically, and this was used as the sampling frame. The selected pupils were then given consent forms for their parents and were only recruited into the study after the filled and signed consent forms had been retrieved from their parents/guardians.

For each recruited pupil, a proforma containing relevant biodata, household number, and physical examination findings was filled out. The family socioeconomic status was determined by using the method described earlier by Olusanya et al.<sup>39</sup>

Throat swabs were taken by using strict aseptic technique. Subjects were asked to open their mouths wide and say "ah." With their mouths open, a throat swab was taken from each participant by gently rubbing the swab stick on the posterior pharynx and tonsillar bed, after depressing the back of the tongue with a wooden spatula. The swab stick was then returned to its sterile container, properly labeled and transported within an hour of collection to the University of Uyo Teaching Hospital laboratory for immediate plating on 5% sheep blood agar.<sup>40</sup> Cultures were incubated at 35°C for 24 hours in a CO<sub>2</sub> jar to enhance growth. Growth of pinpoint colonies with clear surrounding zones of hemolysis ( $\beta$  hemolysis) was identified and the presence of Streptococci was confirmed by a negative catalase test, while a gram stain was processed concomitantly to identify the characteristic gram-positive cocci in chains under the microscope.

The identified BHS were then subcultured for purity and inoculated with Bacitracin 0.04U discs for the appropriate identification of GAS. The Lancefield group was subsequently determined from positive cultures by using Oxoid Streptococcal Grouping Latex Agglutination Kit, UK to identify Groups A, B, C, D, F, and G streptococci. Antimicrobial susceptibility testing was done by using the disk diffusion method.<sup>38</sup> Isolated organisms were cultured on Mueller-Hinton agar with 5% Sheep Blood with antibiotic susceptibility discs and incubated for 24 hours at 37°C. Oxoid UK sterile paper discs containing penicillin V (10  $\mu$ g), amoxicillin (30  $\mu$ g), erythromycin (15  $\mu$ g), cefuroxime (30  $\mu$ g), co-trimoxazole (1.25/23.75  $\mu$ g), and clindamycin (2  $\mu$ g) were used.

The data was analyzed by using the Statistical Package for Social Science for Windows (SPSS Inc Chicago Illinois, United States), version 20. The prevalence of BHS carriage was determined by using simple percentages. Results were reported in text, tables, and figures. Categorical data were recorded as frequencies and percentages, while continuous variables were recorded as means ( $\pm$  standard deviation). Pearson's Chi-square statistical test of significance or Fisher's exact test was used as required to determine the relevant associations between throat carriage and the sociodemographic variables. The level of significance was set at  $p < 0.05$ .

## Results

Two hundred and seventy-six children aged 6 to 12 years were recruited in the study. The mean age of subjects was  $8.7 \pm 1.7$  years. Overall, 133 (48%) were males and 143 (52%) were females, with a male to female (M:F) ratio of 1:1.1. **Table 1** shows the sociodemographic characteristics of the study group.

**Table 1** Sociodemographic characteristics of study population

Characteristic	Frequency (n = 276)	Percentage (%)
Age (y)		
6–7	70	25.4
8–9	114	41.3
10–12	92	33.3
Total	276	100.0
Gender		
Male	133	48.2
Female	143	51.8
Total	276	100.0
Household size		
$\leq 3$	31	11.2
4–6	156	56.6
7–9	73	26.4
10–11	8	2.9
> 11	8	2.9
Total	276	100.0
Religion		
Christian	274	99.3
Muslim	2	0.7
Total	276	100.0
Social class		
Class 1	11	4.0
Class 2	75	27.2
Class 3	82	29.7
Class 4	84	30.4
Class 5	24	8.7
Total	276	100.0

**Table 2** Lancefield grouping of  $\beta$ -hemolytic streptococcal isolates

Lancefield group	Frequency (n)	Percentage (%)
C	8	88.9
G	1	11.1
Total	9	100.0

$\beta$ -hemolytic streptococci (BHS) were identified in 9 out of 276 subjects by giving a prevalence of 3.3%.

**Table 2** shows the Lancefield grouping of the BHS isolates. Eight out of nine isolates (88.9%) were group C streptococcus and one was group G streptococcus.

**Table 3** demonstrates the association between sociodemographic factors and BHS throat carriage. The association between age groups and streptococcal throat carriage

**Table 3** Association of sociodemographic factors with asymptomatic  $\beta$ -hemolytic streptococcal throat infection

Characteristic	$\beta$ -hemolytic streptococcal throat infection		Total (%)	Fisher's exact test ( <i>p</i> -value)
	Positive (%)	Negative (%)		
Age				
6–7 y	5 (55.6)	65 (24.3)	70 (25.4)	6.47 (0.04) <sup>a</sup>
8–9 y	4 (44.4)	110 (41.2)	114 (41.3)	
10–12 y	0 (0)	92 (34.5)	92 (33.3)	
Total	9 (100)	267 (100)	276 (100)	
Gender				
Male	3 (33.3)	130 (48.7)	133 (48.2)	0.82 (0.37) <sup>a</sup>
Female	6 (66.7)	137 (51.3)	143 (51.8)	
Total	9 (100)	267 (100)	276 (100)	
Religion				
Christian	9 (100)	265 (99.3)	274 (99.3)	0.01 (0.99) <sup>a</sup>
Muslim	0 (0)	2 (0.7)	2 (0.7)	
Total	9 (100)	267 (100)	276 (100)	
Socioeconomic class				
Class 1	0 (0)	11 (4.2)	11 (4)	4.03 (0.40) <sup>a</sup>
Class 2	1 (11.1)	74 (27.7)	75 (27.2)	
Class 3	3 (33.3)	79 (29.6)	82 (29.7)	
Class 4	5 (55.6)	79 (29.6)	84 (30.4)	
Class 5	0 (0)	24 (9)	24 (8.7)	
Total	9 (100)	267 (100)	276 (100)	
Household size				
≤3	1 (11.1)	30 (11.2)	31 (11.3)	13.31 (0.01) <sup>a</sup>
4–6	3 (33.3)	153 (57.3)	156 (56.6)	
7–9	3 (33.3)	70 (26.2)	73 (26.3)	
10–11	2 (22.3)	6 (2.3)	8 (2.9)	
> 11	0 (0)	8 (3.0)	8 (2.9)	
Total	9 (100)	267 (100)	276 (100)	

<sup>a</sup>Fisher's exact test was used.

was statistically significant ( $p = 0.04$ ). Despite a noticeably higher throat carriage rate in females (66.7%), the difference was not statistically significant ( $p = 0.37$ ). The number of household occupants demonstrated a statistically significant association with asymptomatic BHS infection. Religion and social class did not show a significant relationship with streptococcal throat carriage.

– **Table 4** shows the antibiotic susceptibility of BHS isolates. Just over three-quarters of the isolates were susceptible to penicillin, and only one-third to amoxicillin. Susceptibility was best with cefuroxime and clindamycin. None of the isolates showed sensitivity to co-trimoxazole. A significantly higher proportion of the streptococcal isolates in the children demonstrated susceptibility to cefuroxime and clindamycin.

## Discussion

The BHS carriage rate in our study, though slightly lower than the mean rate of 5.6% reported in low and middle-income countries worldwide, compares favorably with the prevalence of 4% reported by Baki et al in Calabar, Nigeria.<sup>19,20</sup> This can be probably explained by the fact that both cities share similar sociocultural characteristics. An earlier study in Calabar, Nigeria<sup>18</sup> had recorded a much higher prevalence rate of 20.9%. This could be justified by their sample collection over a 1-year period as compared with the current study, which was done toward the end of the rainy season and in the dry season. The former study would also have been able to consider seasonal variations over an entire year, thus resulting in a higher prevalence. Our finding also compares favorably with

**Table 4** Lancefield group-specific antibiotic susceptibilities of beta-hemolytic streptococcal isolates

Antibiotic	Susceptibility pattern	Lancefield group		Total (%)	Fisher's exact (p-value)
		Group C (%)	Group G (%)		
Penicillin	Sensitive	6 (75)	1 (100)	7 (77.8)	2.77 (0.10) <sup>a</sup>
	Resistant	2 (25)	0 (0)	2 (22.2)	
Amoxicillin	Sensitive	2 (25)	1 (100)	3 (33.3)	1.00 (0.32) <sup>a</sup>
	Resistant	6 (75)	0 (0)	6 (66.7)	
Erythromycin	Sensitive	4 (50)	1 (100)	5 (56.6)	0.11 (0.74) <sup>a</sup>
	Resistant	4 (50)	0 (0)	4 (44.4)	
Cefuroxime	Sensitive	7 (87.5)	1 (100)	8 (88.9)	5.44 (0.02) <sup>a</sup>
	Resistant	1 (12.5)	0 (0)	1 (11.1)	
Cotrimoxazole	Sensitive	0 (0)	0 (0)	0 (0)	Nil <sup>b</sup>
	Resistant	8 (100)	1 (100)	9 (100)	
Clindamycin	Sensitive	7 (87.5)	1 (100)	8 (88.9)	5.44 (0.02) <sup>a</sup>
	Resistant	1 (12.5)	0 (0)	1 (11.1)	

<sup>a</sup>Fisher's exact test was used.

<sup>b</sup>Null values not appropriate for statistical testing.

the 4.6% reported by Singh et al in Northern India.<sup>22</sup> It was however significantly lower than rates (20.9–28.9%) reported from earlier Nigerian studies.<sup>9,18</sup> The difference may be due to improved measures for control of respiratory infections, such as better hand hygiene and increased rates of exclusive breastfeeding in the country over the years.<sup>41</sup> Moreover, several authors have acknowledged wide variations in the prevalence rate of BHS carriage, even within localities in the same regional boundaries.<sup>4,9,19</sup> This variation may reflect differences in the burden of streptococcal diseases in various localities.

The pattern of BHS isolates from the present study is consistent with that of recent Nigerian studies, which also reported GCS as the commonest BHS isolate in asymptomatic children.<sup>9,18,20</sup> These earlier reports had also noted a comparable absence of GAS in studies of BHS carriage. In contrast, an earlier study performed in Lagos in 1972 documented GGS as the commonest Lancefield group isolate.<sup>42</sup> GAS was isolated in 22% of the children sampled. Changes in the burden of streptococcal diseases in the country over the years may explain the change in pattern of BHS carriage.

The current study found BHS throat carriage rate to be highest in 6 to 7 years age group. This is consistent with the well-documented knowledge about BHS carriage that peaks in the early school age and falls toward adolescence.<sup>11</sup> It also compares well with a study in Northern India, which showed the highest prevalence in the 5 to 7 years age group.<sup>7</sup> However, the latter study reported the lowest carriage rates among the 7 to 9 years age group, which had the second highest carriage rate in our study.<sup>7</sup> Furthermore, while no BHS isolates were found in children aged 10 to 12 years in our study, Baki et al (Calabar) reported the highest carriage rate (36.8%) in the same age group.<sup>20</sup> A possible change in the epidemiologic pattern of BHS diseases within the region may account for this difference.

More females than males in the study were streptococcal throat carriers. This is similar to many other studies showing a slight female preponderance in BHS throat carriage, though this difference has usually not been proven to be statistically significant.<sup>9,21,30,33</sup> This gender disparity may be attributed to a genetic predisposition of females in the study to the dominant “emm” type, which has been also reported in a recent Norwegian study.<sup>43</sup>

Children from households with four to nine occupants were most likely to be BHS throat carriers in the current study. This association could be explained by the fact that children from larger households are more likely to live in overcrowded homes with poor ventilation. Both overcrowding and poor ventilation are associated with streptococcal diseases.<sup>44</sup> This finding is similar to earlier studies by Owobu et al<sup>9</sup> in Benin, and Nayiga et al<sup>21</sup> in Uganda who found that BHS carriage rates were higher in children from households with a larger number of occupants.

Overall, 78% of BHS isolates in the current study were susceptible to penicillin. This finding was lower than the 100% susceptibility to penicillin earlier reported for BHS.<sup>5,26,30,32</sup> This may be explained by the fact that no GAS was isolated in the current study. Previous studies with GAS have not demonstrated in vitro resistance to penicillin, although an Egyptian study had reported evolving resistance of GAS isolates to penicillin.<sup>30</sup> The finding that all the BHS isolates in the current study were resistant to cotrimoxazole compared well with findings from an earlier study.<sup>7</sup> The 100% resistance of BHS isolates to co-trimoxazole may be explained by the fact that it is a common over-the-counter remedy for sore throat in the country. The routine use of the drug in *Pneumocystis carinii* pneumonia prophylaxis for all patients with HIV infection may also be a factor in the development of high-grade resistance to the drug as has

been suggested by other studies.<sup>45</sup> The ease of access to antibiotics generally, in the study area as well as entire country, may also account for the high resistance of isolates to co-trimoxazole, amoxicillin, and erythromycin.

The 3.3% prevalence of asymptomatic streptococcal throat carriage recorded in school-aged children in the study is relatively low. The absence of any GAS isolates suggests a low burden of GAS disease in the study area. It is recommended that larger, multicenter studies be conducted to elucidate the changing epidemiology of BHS diseases in the region.

## Limitations of the Study

- This study was only conducted in school-aged children in Uyo. Studies across all ages of children may have an added advantage.
- It was often a challenge to confirm the use of antibiotics in the two weeks preceding the study, in some of the younger children.
- The low numbers obtained from the study made statistical analysis challenging.

### Funding

None.

### Conflict of Interest

None declared.

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