

# Bacitracin Differentiation Of Beta-Haemolytic Streptococci Isolated From School Children Living In Uyo, Southern Nigeria

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## Abstract

### Objectives

Bacitracin Susceptibility has traditionally been accepted as a means of presumptive identification/differentiation of Group A Streptococcus (GAS) from other Beta haemolytic Streptococcal (BHS) strains. This means of identification may indeed be faulty as recent studies have shown that other BHS species are also highly susceptible to Bacitracin. These species and especially Group C (GCS) and Group G (GGS) BHS strains have increasingly been shown to play an important role in human streptococcal disease and in some populations carriage rates of GCS/GGS are higher than GAS rates. The continued use of Bacitracin for presumptive identification would lead to an over-estimation of GAS rates particularly in settings where there is a changing epidemiology of BHS infections.

### Design and Participants

This cross-sectional study was done among two hundred and seventy-six primary school children in Uyo, an urban area in Southern Nigeria. The participants were recruited by multi-stage random sampling.

### Methods

Throat swabs taken from participants were cultured overnight on 5% sheep blood agar. Culture plates identified as positive for BHS were sub-cultured for purity and then inoculated with Bacitracin 0.04U discs for presumptive identification/differentiation of GAS. Lancefield Grouping of streptococcal isolates was also done.

### Main Outcome Measures

The main outcome measures were BHS isolate positivity and Bacitracin susceptibility

### Results

Nine BHS isolates were identified, giving a prevalence of 3.3% among the school children with Lancefield grouping identifying GCS in 89% GGS in 11%. No GAS isolates were found. All isolated GCS/GGS however showed susceptibility to Bacitracin.

### Conclusion

The prevalence of BHS throat carriage in the study area was found to be relatively low. Isolates showed 100% susceptibility to Bacitracin.

## INTRODUCTION

Beta-haemolytic streptococci (BHS) are a group of facultative anaerobic Gram-positive organisms that appear in chains or in pairs and are catalase-negative.<sup>1</sup> They are

subdivided into groups by antibodies that recognize their respective surface antigens. These groups may include one or more species. The most important groupable beta-haemolytic streptococci are A, B, C, D, F and G.<sup>1</sup>

Group A Streptococci (GAS) are the most important BHS in humans and is one of the ten leading causes of mortality from infectious diseases.<sup>2</sup> However, research has increasingly shown that Group C streptococci (GCS) and Group G Streptococci (GGS) play an important role in human streptococcal throat infections,<sup>3-4,5-6</sup> and in some studies have been shown to be more prevalent than GAS.<sup>6</sup> School-based studies on BHS throat carriage in asymptomatic school children in the last decade have consistently shown a lack of GAS isolates while GCS/GGS have been consistently isolated.<sup>3-4, 7-8</sup> In one of the studies,<sup>7</sup> there was a positive correlation of ASO titres with BHS carriage despite the fact that there were no GAS isolates. This finding as well as the persistent high rates of rheumatic fever/rheumatic heart disease in the country despite the apparent absence of GAS isolates have prompted some researchers to suggest that other BHS strains could well be rheumatogenic.<sup>8</sup>

Bacitracin is a polypeptide antibiotic produced by bacteria of the licheniformis group of *Bacillus subtilis* var *tracyi*. It was first isolated in 1945 and its major use in humans is in topical antibiotic preparations.<sup>9</sup> Bacitracin is approved for intramuscular use in infants with staphylococcal pneumonia and empyema. This use is however extremely limited due to the nephrotoxicity of the drug.<sup>10</sup> It is also used in the differentiation of GAS from other species of BHS as GAS is highly susceptible to the drug while other BHS are thought to be mostly resistant to it.<sup>11</sup> It may also be used in distinguishing *Haemophilus influenzae*, which is highly resistant to the drug from other respiratory flora.<sup>12</sup>

Bacitracin susceptibility has traditionally been used to identify GAS and differentiate it from other BHS isolates.<sup>13-14</sup> This method of identification of GAS is still widely used in studies on streptococcal throat infections<sup>15-18</sup> despite the fact that streptococcal serotyping kits are widely available. Also, reports have shown that GCS isolates show 90-99% susceptibility to Bacitracin in studies on asymptomatic patients.<sup>14,19</sup> Lower rates of susceptibility, 71% and 57% have been reported for GCS and GGS respectively among patients with acute pharyngitis.<sup>19</sup> A study of 206 BHS from clinical specimens in India found a low specificity of Bacitracin (70%) as a means of differentiating GAS from other BHS species and the study recommended that Bacitracin alone should not be used as a single presumptive test for GAS identification.<sup>20</sup> Hence, the use of Bacitracin as a single presumptive test to identify GAS without further

Lancefield serotyping or molecular identification would inevitably lead to a high rate of reported false positives and would 'muddy the waters' of BHS research especially in the face of evolving epidemiology of BHS throat infections.

This study aimed at establishing the prevalence of GCS/GGS throat carriage in school-aged children in Uyo, Southern Nigeria and the Bacitracin susceptibility profile of GCS/GGS to promote evidence-based decision making in the diagnosis of streptococcal throat infections.

## METHODS

This was a cross sectional study done among pupils aged 6-12 years in primary schools in Uyo, the capital of Akwa Ibom State, Nigeria. The pupils were selected by multi-stage random sampling.

Sample size was determined using Fisher's formula<sup>21</sup> applied to study populations greater than 10,000. The total number of enrolled pupils in primary schools in Uyo was 71,659 making the formula appropriate.

$$N = \frac{Z^2 PQ}{D^2} \quad 21$$

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 (i.e. Calabar Study<sup>4</sup> = 20.6%)

Q = 1-P

Therefore:

$$N = 1.962 \times (0.206 \times 0.794) / 0.052 = 251$$

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

A multi-stage sampling method was used to recruit the pupils for the study. Twelve schools were selected from the six political wards in Uyo metropolis with two schools selected per ward. These schools were selected by simple

random sampling using the Table of Random Numbers. This was the first stage of sampling.

The number of pupils selected from each of the 12 schools and from individual classes of the schools was determined by proportionate sampling in the second and third stages.

In the fourth stage, the first pupil was randomly selected from the list of pupils in that class using the ballot method.<sup>21</sup> All subsequent pupils recruited were then selected, 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 using the method described earlier by Olusanya.<sup>22</sup>

Children who had symptoms of acute pharyngitis, who had used antibiotics in the preceding two weeks and those whose parents did not consent were excluded from the study.

Ethical clearance for the study was obtained from the Institutional Health Research and Ethics Committee of University of Uyo Teaching Hospital, Uyo.

Throat swabs were taken using strict aseptic technique. After wearing a pair of sterile latex gloves, the lead researcher then used sterile swab sticks to take samples from participants.

Subjects were asked to open their mouths wide and say "ah". With the 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 the wooden spatula. Care was taken to ensure that only samples from the throat were taken, and carefully avoiding the saliva from the buccal mucosa. 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>23</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 haemolysis (i.e. beta haemolysis) were identified

and the presence of Streptococcus 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 BHS identified were then sub-cultured for purity and the Lancefield group was then determined from colonies on positive cultures using Oxoid™ Streptococcal Grouping Latex Agglutination Kit, UK, to identify Groups A, B, C, D, F and G streptococci. Identified BHS colonies were sub-cultured for purity and then inoculated with Bacitracin 0.04U discs and antimicrobial susceptibility was determined using the disk diffusion method.<sup>24</sup>

## **DATA ANALYSIS**

Data was recorded in the participant's worksheet and entered into Microsoft Excel 2016 (Microsoft Corporation, USA) and double-checked to ensure accuracy of the entry. The data was then imported to and analysed using the Statistical Package for Social Science for Windows (SPSS Inc Chicago IL, USA), version 20.

The prevalence of GCS/GGS carriage was determined 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 (+/- standard deviation).

Pearson's Chi-square statistical test of significance or the Fisher's exact test (FET) were used as required to determine the relevant associations between throat carriage and the socio-demographic variables. The level of significance was set at  $p < 0.05$ .

## **RESULTS**

Two hundred and seventy-six (276) children aged 6-12 years were recruited into the study. The mean age of subjects was 8.7 years  $\pm$  1.7 years. One hundred and thirty-three (133) were male and one hundred and forty-three (143) were female, giving a male to female (M:F) ratio of 1:1.1. The socio-demographic characteristics of subjects is shown in Table I.

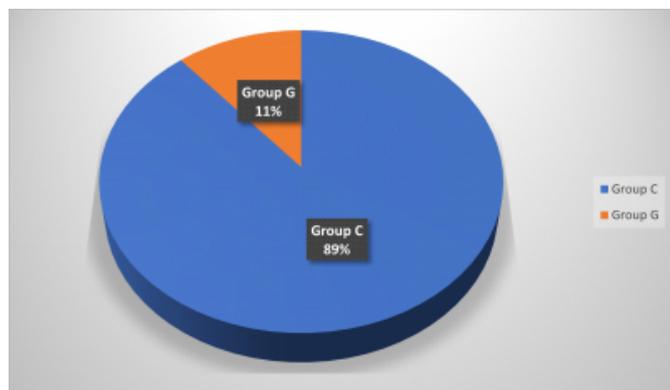
**Table 1**  
Socio-Demographic Characteristics of Study Population

Characteristic	Male (%)	Female (%)	Total (%) (N = 276)
<b>Age Group (years)</b>			
6 – 7	34 (12.3)	36 (13.1)	70 ( 25.4)
8 – 9	49 (17.8)	65 (23.5)	114 ( 41.3)
10 – 12	50 (18.1)	42 (15.2)	92 ( 33.3)
<b>Total</b>	<b>133 (48.2)</b>	<b>143 (51.8)</b>	<b>276 (100.0)</b>
<b>Religion</b>			
Christian	133 (48.2)	141 (51.1)	274 ( 99.3)
Muslim	0 (0.0)	2 ( 0.7)	2 ( 0.7)
<b>Total</b>	<b>133 (48.2)</b>	<b>143 (51.8)</b>	<b>276 (100.0)</b>
<b>Social Class</b>			
Upper Class	45 (16.3)	41 (14.9)	86 ( 31.2)
Middle Class	38 (13.8)	44 (15.9)	82 ( 29.7)
Lower Class	50 (18.1)	58 (21)	108 ( 39.1)
<b>Total</b>	<b>133 (48.2)</b>	<b>143 (51.8)</b>	<b>276 (100.0)</b>

Nine cultures out of 276 were positive for BHS giving an overall prevalence of BHS carriage in the study of 3.3%.

The Lancefield Grouping of BHS Isolates is as shown in Figure 1.

**Figure 1**  
Lancefield Grouping of BHS Isolates



All the GCS and GGS isolated were susceptible when inoculated with Bacitracin 0.04U discs as demonstrated in Table II.

**Table 2**  
Bacitracin Sensitivity of GCS/GGS Isolates

Lancefield Group	Susceptible (%)	Resistant (%)
C	8 (100)	0 (0)
G	1 (100)	0 (0)
<b>Total</b>	<b>9 (100)</b>	<b>0 (0)</b>

## DISCUSSION

The present study demonstrated a prevalence of 3.3% for streptococcal throat carriage with GCS/GGS accounting for all isolates in the study. The absence of GAS isolates is consistent with earlier Nigerian studies over the last decade<sup>3,4,7</sup> which have demonstrated a virtual absence of GAS in studies done among asymptomatic children. In the earliest Nigerian study<sup>25</sup> available, GGS was the commonest isolate and GAS was found in about one-seventh of the subjects. The findings of this study were however in contrast with most other African studies and some studies from the Middle East and South East Asia.<sup>15-18,26</sup> In these studies, GAS was consistently the only or major BHS serotype isolated in asymptomatic carriers. However, these studies either limited their laboratory methods to only the identification of GAS using GAS-specific typing anti-sera.<sup>16,26</sup> while others used Bacitracin susceptibility as definitive proof of GAS<sup>15,17-18</sup> without confirmation with serotyping.

The index study showed that all GCS/GGS isolates were susceptible to Bacitracin. This is in contrast with established knowledge which has offered Bacitracin susceptibility as a means of distinguishing between GAS and other BHS isolates.<sup>13</sup> Some recent studies have however shown consistency with the current study that GCS and GGS isolates show significant susceptibility to Bacitracin.<sup>14,19</sup> This may be especially true for studies among asymptomatic individuals. An Indian study of asymptomatic throat carriers compared favourably with the current study with the GCS isolates showing near-complete susceptibility to Bacitracin.<sup>19</sup>

This finding of near complete susceptibility of non-GAS BHS isolates to Bacitracin in the current study and previous reports.<sup>14,19</sup> calls into question the continued use of Bacitracin susceptibility as a single presumptive means for the diagnosis of GAS as is still being widely practiced in resource-constrained settings.<sup>15,17-18</sup> This practice would indeed lead to an over-estimation of the true GAS burden in

such areas with increased rates of false positives<sup>20</sup> while simultaneously under-estimating the importance of non-GAS streptococci in the causation of human disease.

## CONCLUSION

The study concluded that the overall prevalence of streptococcal throat carriage in school-aged children in the study area was low with GCS/GGS accounting for the burden of streptococcal disease in the study. That none of the isolates proved to be GAS suggests a low burden of GAS diseases in the study area. All GCS/GGS isolates were susceptible to Bacitracin. It is therefore recommended that Streptococcal serotyping, at the minimum rather than Bacitracin susceptibility alone be used as presumptive proof of GAS infection especially in areas with significant non-GAS disease burden.

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