Original Research Article

Antibiotic susceptibility pattern of group A beta-haemolytic streptococcal throat isolates of primary school children in Rivers State, Nigeria.

ABSTRACT:

Group A beta-haemolytic streptococcus (GABHS) pharyngitis when untreated can cause non-suppurative sequelae such as acute rheumatic fever and post-streptococcal acute glomerulonephritis. Prompt treatment of infected individuals with appropriate antibiotics that the organism is susceptible to, is important in preventing these untoward complications.

This study intends to identify the antibiotic susceptibility of GABHS in Rivers State, Nigeria, and to guide health care workers in choosing effective antibiotic for the treatment of GABHS infections in children.

Methodology

Throat swabs were taken from primary school pupils aged 6 to 12 years in Rivers State between May and July 2015. The throat swabs were cultured using 5% sheep blood agar. Antibiotic susceptibility was carried out on the Group A beta-haemolytic streptococcal isolates. Data was analyzed using the Statistical Package for Social Sciences (SPSS) version 23.0 using descriptive statistics. Chi square tests of significance were used with a 95% confidence interval ($P < 0.05$).

Results

A total of 18 out of 456 pupils had a positive streptococcal throat culture for GABHS giving a prevalence rate of 3.9%. The isolated GABHS were most susceptible to azithromycin (87%) followed by penicillin (81.5%) and ampicillin (64.8%). The pathogens were least susceptible to tetracycline (37%) and co-trimoxazole (18.5%).

Conclusion

The prevalence of GABHS was low and it was mostly susceptible to Azithromycin and penicillin. These could be used as the drug of choice in the empirical treatment of GABHS throat infection in the locality.

Keywords: Group A beta-haemolytic streptococcus, Throat isolates, Antibiotic susceptibility**,** Drug of choice, Rivers State.

INTRODUCTION

Group A Beta-Haemolytic Streptococcus (GABHS) in the respiratory tract of humans is responsible for streptococcal pharyngitis and the non-suppurative post-streptococcal sequelae of Acute Rheumatic Fever (ARF) and Post Streptococcal Acute Glomerulonephritis (PSAGN).[1] The high prevalence of ARF plays a key role in the high burden of rheumatic heart disease (RHD) reported from developing countries.[1,2] Rheumatic heart disease, the commonest acquired heart disease in Nigerian children is reportedly responsible for about 35.8-58% of cases of acquired heart disease among them.[3-6] Up to two-thirds of patients with RHD had a history of sore throat about-one to four weeks before the onset of the disease.[2] It is reported that 32-66.6% of cases of PSAGN, a common cause of renal disorders in Nigerian children follow acute GABHS pharyngitis.[7-8] The estimated burden of severe GABHS disease globally is 18.1million

as at 2005 with 1.78 million new cases each year. It is estimated that about 517,000 deaths occur globally, each year, from ARF, RHD, PSAGN and GABHS-associated invasive diseases.[1,9]

GABHS pharyngitis is self-limiting.[2,3] However, reasons adduced for prompt antibiotic treatment include acute relief of symptoms, by shortening the duration of symptoms by about 12 to 24 hours, prevention of suppurative and non-suppurative complications, as well as reducing the risk of communicability.[2-4] Based on cost considerations, narrow spectrum of activity, safety and effectiveness, penicillin V has remained the first drug of choice by the American Academy of Pediatrics and the World Health Organization (WHO) for the treatment of streptococcal pharyngitis.[2,3,10] Ten days of oral antibiotic therapy is standard, but a single dose of intramuscular penicillin G is equally useful in patients who are unlikely to complete the entire course of oral antibiotics.[2,3,10] For those who are allergic to penicillin V or G, the macrolides like erythromycin or azithromycin are effective alternatives.[2,3,10] Amoxicillin, a synthetic amino-penicillin, is often substituted for penicillin V especially for the paediatric patient because it tastes better, is notably cheap and available as chewable tablets.[2,3,10,11]

The prognosis for appropriately treated GABHS pharyngitis is reportedly excellent and complete recovery is the rule.[2,3,10,11] In view of the relatively long latent period of ARF (one to four weeks), treatment instituted within nine days of disease onset is virtually 100% successful in preventing ARF/RHD.[2,3,10,11]

Although WHO recommended the use of penicillin for the treatment of GABHS, studies have shown that the antibiotic sensitivity pattern to the organism is variable in different localities and over time in the same locality.[15-17]

No studies have been carried out in Rivers Sate, Nigeria, to determine the pattern of antibiotic susceptibility to GABHS pharyngitis. This study is therefore aimed at identifying the antibiotic susceptibility of GABHS in our locality.

MATERIALS AND METHODS

Study design

This was cross-sectional school-based study in Emohua Local government Area (EMOLGA), in Rivers State, Nigeria, involving pupils in public and private primary schools.

Study site

Emohua Local Government Area is located in the rural area and has a land area of 831km² with a total population of 201,057 (2006 census) and 238,310 population projection for 2011. Most of the inhabitants of EMOLGA are peasant farmers, hunters and fishermen. Generally, industries and basic infrastructure such as potable pipe borne water, electricity and good roads are lacking in EMOLGA. There were 54 approved primary schools in the local Government Area (LGA) as at 2010/2011. These comprised 51 public schools and three private schools. The LGA has 14 political wards in two constituencies. Constituency one has 27 approved public and 3 private schools, while constituency two has 24 approved public schools only.

Sampling Method

The study population was made of all pupils attending both private and public primary schools in EMOLGA in Rivers State, Nigeria. All the primary schools were first stratified into public and private schools. The sample size was calculated based on the formula[18] $n = \frac{z^2(pq)}{e^2}$, where: n =

sample size, e = margin of sample error tolerated at 5%, z = 1.96 at 95% confidence limit, p = prevalence rate of positive throat culture of BHS among primary school children in South-South Nigeria, which was 32.7%,[19] and q = 100-p. Therefore, n = 3.8416 x 0.33 x 0.67/ 0.0025= 339.8 ≈ 340. Allowing for a 20% attrition rate, 20% of sample size = 68. The final minimum sample size = 68 + 340 = 408.

The stratified multi-stage sampling method was used to select the 456 pupils for the study. The list of government-approved primary schools in Emohua LGA was used as the sampling frame. The 54 approved primary schools were first stratified into the two constituencies. Constituency one had 30 schools, while constituency two had 24 schools, giving a ratio of 5:4. The schools in each constituency were further stratified into public and private schools. Constituency one had 27 public and 3 private schools while constituency two had 24 public schools. Based on the ratio of schools in each constituency, a total of nine schools were selected, five from constituency one (four public and one private) and four public schools from constituency two by simple balloting. Seven out of the eight selected public schools had recently been renovated and the number of classrooms increased. Each school was stratified into six classes (primary one to six). In schools with more than one arm of a class, an arm was chosen by balloting to represent others, while in those with only one arm, that arm was chosen. Ten pupils were then randomly selected from each class, using the class register as the sampling frame.

The pupils registered in the primary schools whose parents gave written informed consent, had not taken antibiotics 2 weeks before the study and did not use any mouth wash on the day of the study were included in the study. On the other hand, pupils whose parents did not give consent,

had taken any form of antibiotics 2 weeks before the study or used mouth wash on the day of the study were excluded from the study.

Study procedure

Questionnaires were used to assess the socio-demographic characteristics of the parents and pupils, and presence or absence of overcrowding at home and school. The researcher and trained research assistants then examined the pupils for presence or absence of fever, cervical lymphadenopathy, and enlarged/hyperaemic tonsils. This was then followed by collection of throat swabs, storage and transportation of the samples to the University of Port Harcourt Microbiology laboratory using the Amies transport swabs, (lot pvmeoo, batch d03g41) (*Copan Transystem, Brescia, Italy*). The samples were plated on 5% sheep blood agar and standard microbiology techniques, including the catalase test, were used to identify BHS.[2,12,13] The BHS organisms were further classified as Lancefield Group A, B, C, D, F and G using the Immulex TM streptococcus group kit, R (EF 73265, Lot group TL3, expiration date 2017-08-01), produced by *Statens Serum Institute Diagnostica, Hillerod, Denmark*. Antibiotic susceptibility testing was carried out using the disc diffusion method using antibiotics-impregnated discs (for azithromycin, penicillin, ampicillin, amoxicillin, ceftazidine, cefuroxime, tetracycline and co-trimoxazole) from *Oxoid laboratories, Hampshire, United Kingdom*. The doses of antibiotics used were penicillin G 10IU (lot/chB 1494308, expiration 2017/05), 25mcg of amoxicillin (Lot/chB 1330498, expiration 2016/04), 10mcg of ampicillin lot/chB 1448534, expiration 2017/01), 30mcg of cefuroxime (Lot/chB 1421765, expiration 2016/11), 30mcg of ceftazidime (lot/chB 1597596, expiration 2017/12), 15mcg of azithromycin (lot/chB 1560284,expiration

2017/10), 30ug of tetracycline (lot/chB 1569670, expiration 2017/10), and 25mcg of cotrimoxazole (lot/chB 1517104, expiration 2017/07).

Ethical approval

Ethical approval was obtained from the University of Port Harcourt Teaching Hospital Ethical Committee and permission granted by the Rivers State Ministry of Education. Written informed consent was also obtained from the parents of the pupils.

Statistical analysis

Data from the study were collated and analyzed using the Statistical Package for Social Sciences (SPSS) version 23. Test of significance was set at *P* value < 0.05 at 95% confidence interval.

RESULTS

A total of 456 pupils aged 6-12 years with a mean age of 9.40 (SD ±1.83) years participated in the study. They included 221 (48.5%) males and 235(51.5%) females giving a male female ratio of 1:1.1.

Group A Beta Haemolytic Streptococcus (GABHS) was isolated from the throat swab of 18 pupils, giving a prevalence rate of 3.9%. They included 9 male and 9 female pupils, 10 (55.6%) were aged 6-9 years and 8 (44.4%), 10-12 years. The median age of the pupils with positive GABHS culture was 8.5 years, and the mean 9.06 (SD ±2.13) years. Primary school education 8 (44.4%) was the most attained educational level among the fathers and mothers of the pupils. Petty trading 12 (66.7%) was the commonest occupation among the mothers compared to 6 (33.3%) of the fathers that were petty traders, Table 1. Nine (50%) of these children belonged to

socioeconomic class 3, while the remaining 9 (50%) were in socioeconomic class 4. Of the 18 pupils with GABHS, 10 (55.6%), 6 (33.3%) and 2 ((11.1%) belonged to families with < 5, 5-10 and > 10 persons respectively. Majority, 14 (77.8%) lived in overcrowded accommodation.

Table 1: Educational level and occupation of parents of the pupils with GABHS

Parameters of Parents	Frequency	Percentage
Educational level of Fathers		
Tertiary	2	11.1
Secondary	5	27.8
Primary	8	44.4
None	3	16.7
Educational Level of Mothers		
Tertiary	1	5.6
Secondary	2	11.1
Primary	9	50
None	6	33.3
Occupation of Fathers		
Professionals/Senior public servants/Businessmen	1	5.6
Non-academic professionals/Intermediate public servants	1	5.6
Skilled workers/Junior civil servants.	4	22.2
Petty traders/ Labourers	6	33.3
Unemployed/ Subsistence farmers	6	33.3

Occupation of Mothers		
Professionals/Senior public servants	0	0
Non-academic professionals/Intermediate public servants	0	0
Skilled workers/Junior civil servants.	0	0
Petty traders	12	66.7
Housewives/Subsistence farmers	6	33.3

Clinical features of children with GABHS

None of the children with GABHS isolates had fever, however 2 pupils had cough, 2 had catarrh but 8 (44.4%) had enlarged and hyperaemic tonsils, Table 2.

Table 2: Clinical features of pupils with GABHS

Clinical features	Frequency	Percent
Enlarged and hyperaemic tonsils	8	44.4
Cervical lymph node enlargement	7	38.9
Cough	2	11.1
Catarrh	2	11.1
Sore throat	1	5.6 no changes done as some children had more than one symptom and total is >100%

Antibiotic susceptibility pattern of GABHS

The GABHS was most susceptible to azithromycin 16 (88.9%), followed by penicillin 15 (83.3%). The least antibiotic it was susceptible to was co-trimoxazole 3 (16.7%), Table 3.

Table 3: Antibiotic susceptibility pattern of GABHS

Antibiotics	Frequency	Percentage (%)
Azithromycin	16	88.9
Penicillin	15	83.3
Ampicillin	13	72.2
Amoxicillin	11	61.1
Ceftazidine	10	55.6
Cefuroxime	7	38.9
Tetracycline	4	22.2
Co-trimoxazole	3	16.7

Association between GABHS infection with demographic and clinical factors

GABHS was more prevalent among children aged 6-9 years (OR 1.600, 95% CI 0.619-4.140), among females (OR 1.060 95% CI 0.414-2.737) and among those living in overcrowded houses (OR 1.870, 95% CI 0.608-5.807), although these were not statistically significant, Table 4. GABHS was also more prevalent among children with enlarged and hyperaemic tonsils (OR 1.600, 95% CI 0.619-4.140), Table 5.

Table 4: Association between GABHS infection with demographic factors

Demographic Factors	GABHS infection		Odds Ratio	95% CI	P Value
	Yes	No			

	N (%)	N (%)			
Age (Years)					
6-9	10 (55.6)	204 (46.6)	1.600	0.619, 4.140	0.232
10-12	8 (44.4)	234 (53.4)			
Gender					
Female	9 (50)	226 (51.6)	1.066	0.415, 2.737	0.542
Male	9 (50)	212 (48.4)			
Overcrowded accommodation					
No	4 (22.2)	153 (34.9)	1.870	0.608, 5.807	0.197
Yes	14 (77.8)	285 (65.1)			
Socio-economic status					
Low	9 (50)	268 (61.2)	1.576	0.614, 4.051	0.238
Middle	9 (50)	170 (38.8)			

Table 5: Association between GABHS infection with clinical factors

Clinical factors	**GABHS Infection**		**Odds Ratio**	**95% CI**	**P value**
	Yes	**No**			
	N (%)	**N (%)**			
Enlarged and hyperaemic tonsils					
No	10 (55.6)	292 (66.7)	1.600	0.619, 4.140	0.232
Yes	8 (44.4)	146 (33.3)			

Cervical lymph node enlargement					
No	7 (38.9)	166 (37.9)	0.959	0.365, 2.523	0.197
Yes	11 (61.1)	272 (62.9)			

DISCUSSION

The prevalence of GABHS among primary school children in the present study was identified as 3.9%. This was much lower than the 7.2% in Nepal,[17] 32.7% in Calabar,[19] and 66.7% in Abeokuta.[20] The study in Calabar although carried out among children attending primary schools, was done earlier than this study. Besides, the indiscriminate use of antibiotics in Nigeria may have contributed to the low prevalence of GABHS in our study as most antibiotics can now be obtained over the counter without a prescription. In addition, seasonality of GABHS infection may have affected the prevalence since the study was not carried out all year round, hence may have been during a low infection season. The higher prevalence in Abeokuta may be due to the fact that all the children had symptomatic pharyngitis in addition to the small sample size of 30. The study population in Abeokuta and Pokhara valley in Nepal involved children aged less than 6 years and those older than 12 years which may also affect the prevalence.

With regard to the susceptibility pattern of GABHS isolates in the present study, the isolates were evidently most sensitive to azithromycin, followed by penicillin and ampicillin. A similar remarkable sensitivity to Azithromycin was also reported by Sadoh *et* al[15] in Benin city, Nigeria, in Senegal,[16] and Pokhara, Nepal.[17] This is hardly unexpected, probably because of the low

"prescription pressure," a lower likelihood of self-prescription as well as over the counter abuse due to the high cost of Azithromycin compared to penicillin and amoxicillin. Azithromycin being a macrolide is usually recommended for patients who are allergic to penicillin. [2,3,10] Looking at its high susceptibility in this study, it may as well be prescribed as a first drug of choice and not just for those who are sensitive to penicillin.

The present study showed a high susceptibility of GABHS to Penicillin although it was second to azithromycin. Penicillin has been the drug of choice in the treatment of GABHS with 100% sensitivity in several studies.[16,17,21,22] However, some studies,[23,24] have shown a less than 100% sensitivity just as the present study. The possible reason for this reduced sensitivity could be as result of the emergence of penicillin-resistant strains of GABHS, probably by B-lactamase production or co-pathogen interference and alteration of microbial balance.[23]

The present study also shows a high sensitivity of the GABHS to ampicillin unlike that reported by Sadoh et al[15] which indeed identified a relatively high level of resistance of the pathogen to ampicillin. The reason for this difference could be due to the inevitable abuse of ampicillin being one of the earliest available antibiotic in the country. However, with the availability of more antibiotics now, the prescription pressure may have shifted from ampicillin, thereby improving its sensitivity.

It is also noteworthy that the current study showed only 48% sensitivity to amoxicillin, a finding which is at variance with other reports in Nigeria,[15] Senegal[16] France,[21] Nepal[17] and Yemeni.[25] The reduced sensitivity to amoxicillin may be a consequence of the increasing overuse, inappropriate use and misuse of this relatively cheap, readily procured over the counter antibiotic which occur across geographical, economic and social boundaries.[26] The temporal changes in

antimicrobial use and differences in usage pressure that might have accounted for the current findings is underscored by the findings in a more recent study by Babaiwa *et al*[27] in Benin city, Nigeria, where GABHS was also resistant to amoxicillin and amoxicillin-clavulanic acid. Furthermore, Uzodimma et al[20] in a recent study in Ogun State, Nigeria, reported that only 5.8% of GABHS throat isolates were susceptible to amoxicillin.

Cefuroxime and ceftazidine are cephalosporins which are often prescribed empirically for treatment of sore throat by physicians.[28] In this study the susceptibility of GABHS to these cephalosporins was between 38-55%. In a meta-analysis by Casey and Pichlero, cephalosporins were found to have a better bacteriological and clinical cure than penicillin.[29]

Resistance to tetracycline was noted in this study, an observation that was similar to the findings in a comparable series from Dakar,[16] and Ethiopia.[30] This relatively cheap antimicrobial, which was used without prescriptions for treatment of many ailments (including diarrhoea and sore throat), would have been easily abused with the consequent development of resistance.[26]

CONCLUSION

The streptococcal throat isolates in the present study, were most sensitive to azithromycin, penicillin and ampicillin, in that order, while the least sensitivity of the isolates was to cotrimoxazole and tetracycline.

RECOMMENDATIONS

In view of the cost and the need to avoid indiscriminate use and abuse, penicillin should remain the first line antimicrobial in children with streptococcal pharyngitis, azithromycin may be best

reserved as a second line antimicrobial for children and adolescents, with no evidence of clinical improvement after 48-72hours of treatment, with or without microbiological corroboration. More research work is needed on antibiotic susceptibility of GABHS in the locality.

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Competing interests

The authors declare no conflicts of interest

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