



ISOLATION AND CHARACTERIZATION OF *Streptococcus pyogenes* FROM SPUTUM AND THROAT OF PATIENTS WITH RESPIRATORY TRACT INFECTIONS IN ZARIA, NIGERIA

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ABSTRACT

Streptococcus pyogenes is associated with a wide range of infections including pharyngitis, impetigo, invasive necrotizing fasciitis, streptococcal toxic shock syndrome, puerperal fever, pneumonia and bacteremia. Thus the study was aimed on isolation and characterization of *Streptococcus pyogenes* from sputum and throat swabs collected from patients and analysed employing standard bacteriological methods. Susceptibility was determined using E-Test strips. Out of the 500 samples analysed, a total of 65 organisms were isolated and further assay confirmed 42 (8.4%) of the isolates to be *Streptococcus pyogenes*. In relation to site of sample collection, 12.3% (9/73) of the clinical samples were throat swabs and 7.7% (33/427) were sputum samples and this was statistically significant ($p < 0.001$). Patients from Hajiya Gambo Sawaba Hospital had the highest occurrence of 10% (4/40) while those from Ahmadu Bello University Medical Centre had (29/351) and Zaria Clinic had (9/109) with 8.3% occurrence respectively. This difference was statistically significant ($p < 0.001$). The infection was higher in males (51%) with a prevalence of 8.6% while 49% (243/500) of the females were positive with a prevalence of 8.2% although the difference was not statistically significant ($p = 0.217$). The patients within age group 0-10 years had the highest prevalence (15.8%:12/76) while patients in age group 31-40 years had lowest prevalence (2.4%:2/84). *Streptococcus pyogenes* demonstrated a 50% resistance to penicillin and (11.9%) resistance to ciprofloxacin. The study thus associated *S. pyogenes* with respiratory tract infections in the study population.

Keywords: *Streptococcus pyogenes*, Bacitracin disc, Lancefield, Microgen, pneumonia

INTRODUCTION

Streptococci are a heterogeneous group of gram-positive bacteria (Brooks *et al.*, 2013; Willey *et al.*, 2014; Mahon *et al.*, 2015) belonging to the family Streptococcaceae (Mahon *et al.*, 2015). The classification of streptococci was based on the colony morphology and haemolytic reactions on blood agar, serologic specificity of the cell wall group-specific substance (Lancefield antigens) and other cell wall or capsular antigens, biochemical reactions and resistance to physical and chemical factors, and ecologic features. *Streptococcus pyogenes* is one of the most important bacterial pathogens and the most virulent pathogen in the *Streptococcus* family (Brooks *et al.*, 2013). It is estimated that between 5-15 % of normal individuals harbour *S. pyogenes* usually in the respiratory tract without signs of disease. There is increase in the number of cases of invasive and non-invasive GAS diseases globally since 1980s (Lynskey *et al.*, 2011). An estimated 700 million GAS infection occur worldwide annually with overall

mortality rate of 0.1% while over 650,000 of the cases are invasive and have a mortality rate of 25% (Aziz *et al.*, 2010). The research carried out by Tapia *et al.* (2015) in Mali, Tesfaw *et al.* (2015) in Egypt and Malik *et al.* (2016) in Sudan reported the prevalence of 25.5%, 11.3% and 28.2% respectively. Babaiwa *et al.* (2013) in Benin City, Nigeria and Ajibade *et al.*, (2014) in Ekiti State reported the prevalence of 14% and 47% respectively. Mawak *et al.* (2005) reported 10.45% prevalence of *Streptococcus pyogenes* infections in Jos, Nigeria. Olafemi (2016) also reported a prevalence of 6.7% of GAS infection in Zaria, Nigeria.

Respiratory droplet spread remains the major route of transmission associated with upper respiratory tract infection. Symptoms of strep throat typically appear several days after exposure to the bacteria (CDC, 2015). The most common symptom of a strep infection is a sore throat. Individuals may also have trouble swallowing and the tonsils and lymph nodes may feel swollen.

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Some individuals may experience fever, stomach ache, vomiting, fatigue or headache. A white rash may develop on the tonsils or the throat may have stringy pus (CDC, 2015).

Most *S. pyogenes* are susceptible to penicillin G. Macrolides, such as erythromycin and clindamycin, have often been recommended for penicillin allergic patients and for patients with necrotizing fasciitis. However, resistance to macrolide antibiotics has been increasing in Europe and the United States (Brooks *et al.*, 2013).

The aim of this work therefore is to isolate, characterize and determine the susceptibility of *Streptococcus pyogenes* isolated from sputum and throat of patients with respiratory tract infections in Zaria, Nigeria

MATERIALS AND METHODS

Study Design

The study was a cross-sectional hospital-based, where sputum and throat swab samples were collected from consented patients of all age groups that visited the selected clinics in Zaria, Kaduna State during the study period.

Study Population

The study populations were male and female patients of all age group from all works of life that presented with the symptoms of respiratory tracts infections (RTIs) at the participating hospitals. The hospitals include Ahmadu Bello University Medical Centre (ABUMC), Samaru, Hajiya Gambo Sawaba General Hospital (GSH) and Zaria Clinic and Medical Centre (ZC), Tudun Wada, Zaria, Kaduna State.

Ethical Approval and Consent

Ethical approval was obtained from the Ethical Committee of Kaduna State Ministry of Health and from the various hospitals. A written informed consent was obtained from consented/assented patients.

Inclusion and Exclusion Criteria

Inclusion criteria were patients who gave their consent/assent to be included in the study with symptoms of sore throat and cough. Exclusion criteria were patients who did not give their consent to be included in the study with symptoms of sore throat and cough and patients without symptoms of sore throat and cough.

Collection of Samples and Isolation of *Streptococcus pyogenes*

Sputum and throat swab samples were collected from the participating hospitals. The sputum samples were collected into sterile clean screw-capped containers while the throat swabs were collected using non-toxic sterile swab sticks aseptically with the assistance of experienced clinicians and laboratory scientists and were cultured on chocolate agar. Isolates were

identified by microscopy and biochemical tests. Preliminary assay used include catalase test and sensitivity to bacitracin. Further confirmation was performed using a commercial identification kit (Streptococcus Microgen identification kits Microgen™ Strep ID, U.K) and agglutination tests using Lancefield Microgen kits.

Determination of Antibiotic Sensitivity of the Isolates

The inoculum for primary sensitivity testing was prepared from 24 hours cultured isolates. The turbidity of each of the bacterial suspension was standardized to match 0.5 McFarland standards. A colony from an overnight growth culture of *S. pyogenes* was added to 2ml of sterile physiological saline as suspension medium using a sterile wire loop. The bacterial suspension was adjusted to 0.5 McFarland Standards (1.5×10^8 CFU/ml). Susceptibility of each isolate was analyzed using commercially available E-test strips. Susceptibility tests were performed using Mueller Hinton agar with 5% sheep blood. The following Epsilometer test strips (E- tests strips) were used (Liofilchem MIC Test Strips, U.S.A): Penicillin G (0.016-256 mg/L), Erythromycin (0.016-256 mg/L), and Ciprofloxacin (0.002-32 mg/L). The MIC was read and interpreted according to the criteria developed by Clinical and Laboratory Standard Institute (2017). Results obtained from the laboratory were expressed in percentages and presented in tables and figures which were analyzed statistically. Pearson chi-square analysis was used to determine association between the variables at 95% confidence interval and 0.05 significant levels.

RESULTS

Out of the 500 samples analysed, a total of 65 organisms were isolated and further assay confirmed 42 (8.4%) of the isolates to be *Streptococcus pyogenes* (Table 1). In relation to site of sample collection, 12.3% (9/73) of throat swabs and 7.7% (33/427) of sputum yielded *S. pyogenes* as shown in Table 1. There was statistical significant difference between the sample type and the *Streptococcus pyogenes* infection ($\chi^2 = 184.381$, $df = 1$, $p < 0.001$).

Patients from GSH had the highest occurrence of 10% (4/40) while those from ABUMC had (29/351) and ZC had (9/109) with 8.3% occurrence respectively (Table 2). These difference was statistically significant ($\chi^2 = 400.000$, $df = 2$, $p < 0.001$). The infection was higher in males (51%) with a prevalence of 8.6% while 49% (243/500) of the females were positive with a prevalence of 8.2% (Table 3) although the difference was not statistically significant ($\chi^2 = 1.524$, $df = 1$, $p = 0.217$).

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The patients within age group 0-10 years had the highest prevalence (15.8%:12/76) while patients in age group 31-40 years had lowest prevalence (2.4%:2/84). The result is as

presented in Table 4. *Streptococcus pyogenes* demonstrated a 50% resistance to penicillin and (11.9%) resistance to ciprofloxacin (Table 5).

Table 1: Occurrence of *S. pyogenes* from Patients with Respiratory Tract Infections in Zaria according to the Site of Samples Collection

Variable	No. of Samples Screened	Number positive	Frequency (%)	χ^2 Value	P-value
Sputum	427	33	7.7	184.381	0.001
Throat Swabs	73	9	12.3		
Total	500	42	8.4		

Table 2: Prevalence of *Streptococcus pyogenes* among Patients with Respiratory Tract Infections by Hospital Location in Zaria

Hospital	No. of Samples Screened	No. of Samples Positive	Frequency (%)	χ^2 Value	P-Value
ABUMC	351	29	8.3	400.000	0.001
ZC	109	9	8.3		
GSH	40	4	10.0		
TOTAL	500	42	8.4		

Key: ABUMC: Ahmadu Bello University Medical Centre, GSH: Hajiya Gambo Sawaba Hospital, ZC: Zaria Clinic and Medical Centre

Table 3: Distribution of *Streptococcus pyogenes* among Male and Female Patients with Respiratory Tract Infections in Zaria

Gender	No. of Samples Screened	Number positive	(%) Prevalence	χ^2 Value	P-Value
Male	255	22	8.6	1.524	0.217
Female	245	20	8.2		
Total	500	42	8.4		

Table 4: Distribution of *Streptococcus pyogenes* Isolates from Patients with Respiratory Tract Infections by Age Group in Zaria

Age group (Years)	No. of Samples Screened	No of Samples Positive	Occurrence of <i>S. pyogenes</i> (%)
0 – 10	76	12	15.8
11 – 20	112	6	5.4
21 – 30	143	10	7.0
31 – 40	82	2	2.4
41 – 50	29	4	13.8
> 50	58	8	13.8
Total	500	42	

($\chi^2 = 132.571$, $df=1$, $P = 0.001$)

Table 5: E-Test for Minimum Inhibitory Concentration of *Streptococcus pyogenes* Isolated from Clinical Samples in Zaria on some Standard Antibiotics

E-Test Strips (mg/L)	Number of isolates (%)		
	*Susceptible	* Intermediate	* Resistant
Penicillin (0.016-256)	16 (38.1)	5 (11.9)	21 (50.0)
Erythromycin (0.016-256)	22 (52.4)	5 (11.9)	15 (35.7)
Ciprofloxacin (0.002-32)	34 (81.0)	3 (7.14)	5 (11.9)

*Results are classified according to CLSI (2017)

DISCUSSION

The study established a prevalence of slightly over 8% in the study population. This prevalence is an indication that the organism is active in the area with a potential of causing widespread disease. The prevalence observed in this study was lower than the 14% obtained in Benin City, Nigeria by Babaiwa *et al.* (2013) and 47% in Ekiti State (Ajibade *et al.*, 2014). However, 12% to 30.7% prevalence was reported in India (Sanjeeb *et al.*, 2014; Nirmala *et al.*, 2015) and 29.2 % in Iraq (Ali *et al.* 2015). These differences could be as a result of different cultural practices as well as seasonal variations.

Recovery of *Streptococcus pyogenes* vary significantly with the type of sample collected with throat swabs yielding higher positive outcome (12.3%) than sputum samples (7.7%) in this study. This confirms the report that throat culture is the gold standard diagnosis of Streptococcal pharyngitis (Gerber, 1989). However, a report by Faris (2014) in Jordan indicated higher efficiency of sputum than throat swabs in the detection of *S. pyogenes*.

The high prevalence of *Streptococcus pyogenes* among patients with RTI reporting to Hajiya Gambo Sawaba Hospital compared to other hospitals studied indicates that infections by this organism vary from one locality to another. Hajiya Gambo Sawaba is located in the rural setting with a high population density. This could have accounted for the high patients with RTI. The observation in this study is comparable to work of Sami *et al.* (2015) in Saudi Arabia that reported variation from district to district.

There was no statistically significant association between gender and *Streptococcus pyogenes* infection. This observation disagrees with the reports of Prajapati *et al.* (2012) in Nepal and Sanjeeb *et al.* (2014) in India who reported that males had higher prevalence than females. The finding in this study also differs from the findings of Rijal *et al.* (2009) in Nepal that found 53.4% in females and 46.6% in males and Nirmala *et al.* (2015) in India that reported slightly more in females than males.

The study established a high prevalence among the very young (0-10 years) and elderly (40 and above). The young often present with developing immunity along with activities that expose them to infection. The elderly on the other hand are faced with waning immunity. This report agrees with Babaiwa *et al.* (2013) in Nigeria, Luca-Harari *et al.* (2008) in Romania and Nirmala *et al.* (2015) in India who reported higher prevalence in children of 0-10 years but it contradicts the findings of Prajapati *et al.* (2012) in Nepal and Sanjeeb *et al.* (2014) in India who reported higher prevalence among 10-15 years age bracket.

The *S. pyogenes* isolated from the selected area showed several pattern of resistance. The common factor in resistance has been the issue of abuse of these drugs. Alongside the gross use of these antibacterial in animal feeds which has continued unabated. It has been reported severally that antibiotics are grossly abused in several communities in Nigeria (NAFDAC, 2018). There was high resistance to penicillin (50.0%) in this study, which is the first line of treatment for streptococcal infections and a widely used antibiotic for the treatment of streptococcal infections. The present result contradicts the findings of previous studies of 0% (Nwankwo *et al.* (2016) in Abia State, Nigeria, Camara *et al.* (2013) in Senegal, Sanjeeb *et al.* (2014) in India, Ali *et al.* (2015), Sami *et al.* (2015) in Saudi Arabia, in India by Nirmala *et al.* (2015). However, the result is lower than 87.9% obtained by Faris (2014) in Jordan.

The high resistance to erythromycin (37.5%) seen in this study could be attributed to its high availability, thus access and abuse is possible and being a second line of treatment for streptococcal infections, it is often prescribed. The result is lower than 62% reported by Babaiwa *et al.* (2013) in Benin City, Nigeria and Camara *et al.* (2013) in Senegal that found 0% in their study. The result is however similar to the findings of 38.1% by Sanjeeb *et al.* (2014) in India, and also comparable to the findings of 33.33% by Ali *et al.* (2015) in Iraq, 40.1% reported by Faris (2014) in Jordan.

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Resistance to ciprofloxacin in this study was 11.9%. Ciprofloxacin is a broad spectrum antibiotics often used to treat enteric fever thus its prescription for pneumococcal infection may not be widespread. This finding is comparable to that of Babaiwa *et al.* (2013) in Benin City who reported 15% in their study and with the findings of Faris (2014) in Jordan that found ciprofloxacin resistance to be 14.5% but in contrast to 0% reported by Nwankwo *et al.* (2016) in Abia State, Nigeria and by Prajapati *et al.* (2012) in Nepal, as well as 2.8% reported by Arvand *et al.* (2000) in Berlin.

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CONCLUSION

Based on the finding of this study, the following conclusions were drawn: A prevalence of *S. pyogenes* infection in the study area was 8.4%. Patients in age group 0-10 years had the highest prevalence of streptococcal infection while the lowest incidence was recorded for patients within the age group of 31-40 years. However, it was noted that the prevalence of the infection among male and female patients were similar. The study also established that 50% of the *S. pyogenes* isolated from the patients were resistant to penicillin while 35.7% showed resistance to erythromycin which hitherto were the first line drugs for these organisms.

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