STREPTOCOCCUS PHARYNGITIS IN SCHOOL CHILDREN IN ORAUKWU, SOUTH-EAST NIGERIA: A RISK FACTOR FOR DEVELOPMENT OF RHEUMATIC FEVER.

Ochiabuto O. M. T. B.[1]*, Ogbachalu O. M.[1], Obi C. M.[1], Ekekwe E. N.[1] and Uneze B. C.[2]

[1]Microbiology Unit, Department of Medical Laboratory Sciences, Faculty of Health Sciences and Technology, Nnamdi Azikiwe University, Awka. Nnewi Campus. Nigeria.
[2]Department of Physiology, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Awka. Nnewi Campus.

*Author for Correspondence: Ochiabuto O. M. T. B.
Microbiology Unit, Department of Medical Laboratory Sciences, Faculty of Health Sciences and Technology, Nnamdi Azikiwe University, Awka. Nnewi Campus. Nigeria.

ABSTRACT
Adult Endocarditic and Heart related diseases are preventable by early screening. A prevalence study on Streptococcus pharyngitis in school children and adolescent as a risk factor for the development of acute rheumatic fever was carried out in Oraukwu town in Nigeria. A total of 87 Students of both sexes aged between 5-25 years were recruited. Anti-streptolysin O and C-reactive protein screening were carried out using Slide-agglutination and Immunochromatographic techniques. Throat swab culture and biochemical tests were carried out using Standard cultural methods. Hematological indices – ESR, White cell and Differential counts were done using Manual counting and Westergreen methods. Blood pressure was measured using mercury Sphingomanometre method. Susceptibility testing was done using Standard disc - diffusion method. Out of the 87 (100%) students studied, 36(41.4%) were sero positive for ASO consisting of 20(22.98%) males and 16(18.39%) female, at a ratio of 1. 23: 1. No significant difference existed between positive and negative cases (p>0.05). Sex was not a risk factor for infection. (p>0,05). S. pyogenes was more frequently isolated from throat culture 7(8.1%) than enteric organisms- E. coli 1(1.1%) and Klebsiella 1(1.1%). Statistical difference existed between total number of positive isolates n=9 and negative cases n=78, (p<0.05). 12(13.8%) subjects were positive for C-reactive protein irrespective of positive ASO screening with a male 6(68%) to female 6(68%) ratio of 1:1. Significant difference existed in positive cases compared to negative cases.(p<0.05). Positive ASO and C-reactive protein cases were more prevalent in the age bracket 10-14. Socio -demographic variables showed that sharing of cups was the main source of infection among the children with history of sore throat, with fever the most presenting symptom. Hematological parameters were unaffected. S. pyogenes isolates were more susceptible to Levofloxacin (100%) and resistant to Norbactin.(100%). Improved drinking water habits in Children can reduce the infection rate in the town.

KEYWORDS: Streptococcus, Pharyngitis, Children, Adolescents, Oraukwu, Rhumatic fever.

INTRODUCTION

Brooks *et al*. (2010) described rheumatic fever as one of the 2 non–suppurative complications of group A beta hemolytic streptococcal infection in humans. Unlike glomerulonephritis (the other complication), acute rheumatic fever development follows infection of pharyngo-tonsillar tissues, but not the skin (Nester *et al*., 2004), and occurs only in predispose individual. Clinically, acute rheumatic fever is manifested by inflammatory conditions involving the joints (polyarthritis), the heart (carditis), skin (erythema marginatum) and subcutaneous nodular formation (Anneclor, 2006). Of these associated symptoms, carditis with subsequent damage to heart valve (valvular heart disease) can become a chronic condition leading to

congestive heart failure, strokes, endocarditis and death (Carapetis *et al*., 2005).

Robertson *et al*. (2006) stated that unlike Ischemic heart disease, Valvular heart disease due to rheumatic fever causes severe disability and premature death mainly in children and young adult during their year of greatest social and economic productivity.

Acute Rheumatic fever disease is believed to be caused by antibody cross-reactivity that can involve the heart, joint, skin as well as the brain tissue. The link between acute rheumatic fever and group A beta hemolytic streptococcal (GABHS) pharyngitis has been established firmly by observational and experimental data. Research has shown that 3% of GABHS pharyngitis lead to

rheumatic fever if left untreated, but the risk increase from 3% to 75% after a recurrent GABHS pharyngitis (Taranta *et al.,*1964).

As stated by Mbewu, (2006) the incidence of the disease is declining in the developed countries of the world, but this is not true for the less privileged countries like Nigeria. This is attributed to a number of factors - lack of resources for disease control, political-social and economic instability, poverty, overcrowding and malnutrition (WHO, 2005). Humans are the natural reservoir for *streptococcus pyogenes* and infection are spread from person to person via contact with infected object.

A diagnostic guide based on the clinical manifestation of the disease was first published by Dr. T. Ducket Jones in the USA in 1944 to help overcome the challenges in the diagnostic of rheumatic fever (Jones, 1944). This diagnostic guide has since undergone modification and revision in an attempt to improve specificity in establishing the initial attack to avoid over diagnosis (Ferrieri, 2002). Diagnosis is made when there is an evidence of two of the major manifestation (Polyarthritis, Subcutaneous nodules, Erythema marginatum, Carditis, Sydenham's Chorea) or one major manifestation with any two of the minor manifestations of the disease (Leucocytosis, raised ESR, raised c-reactive protein, electrocardiograph showing heart block, also abdominal pain and nose bleed), along with evidence of streptococcal infection (elevated antistreptolysin O titre (Jones, 1944).

Approaches to reduce the impact of acute rheumatic fever include primary prevention through the recognition of GABHS pharyngitis and treatment with antibiotics, with management of cardiovascular complications and secondary antibiotic prophylaxis to prevent recurrence (Omokhodion, 2000). Preventive measures includes the establishment of surveillance programme to measure disease burden, advocacy to increase resources allocation for the treatment of affected children and young adults, and the implementation of primary and secondary prevention schemes in all states of the federation (Mayosi, 2006).

OBJECTIVES OF THE STUDY
1. Determine the prevalence of *Streptococcus* pharingitis in children and Adults in Oraukwu, Anambra State, Nigeria.
2. Find out the sex and age range mostly affected.
3. Find the risk factors associated with being infected in the study groups.

MATERIALS AND METHOD
Research design
The study is a cross sectional study designed to assess the incidence of streptococcal pharyngitis in students at Oraukwu and and those at risk of developing complications (cardiovascular complication).

Area of study
The research was carried out at Oraukwu. The town is located in Idemili North Local Government Area of Anambra State, Nigeria and lies approximately 40 kilometers east of Onitsha along the old Enugu-Onitsha trunk road. Oraukwu is bounded by Adazi-ani in the East, Abatete in the West, Aro in the North and Nkwo-Ozu in the south. There are eight villages in the town - Otta, Ezenaja-amada, Nkwelle, Irrukwelleko, Amaeze, Dim-ubana, Uru-amada, and Ibenabo. Oraukwu has a population of about 150,000 including indigenes and non indigenes. Their occupation includes farming, carpentry, palm wine tapping, trading and other few white collar jobs. There are good social amenities and infrastructures which include 7 kindergarten, 5 primary school, 3 secondary school, hospitals including a maternity house, one laboratory and a commercial bank. Also are good water supply (stream and borehole), good power supply and accessible roads. The inhabitants are mostly Christians with few traditionalists. Ruler ship is via the 'Igwe' and his cabinet, the Obi's and Ichies.

SELECTION OF SUBJECTS AND SAMPLE
Primary, secondary and post secondary school students between the age of 5 and 25 years were included in the study. A total of 87 subjects consisting of 46 male and 41 female were selected. Subjects on antibiotics and herbal treatment were excluded from the study group. The subject's biodata of age, sex, socio-economic status (from parents occupation) were recorded using questions. Also other relevant information as related to the research (disease history, predisposing factors, signs and symptoms of disease present) were recorded.

Sample collection: Throat swab and blood samples were collected from each subject. Collection of throat swab was done in an open environment with sterile swab stick according to method described by Ochei,(2000) while blood sample was collected according to Cheesbrough, (2000).

Throat culture: The swab stick was introduced into a well exposed mouth with the tongue depressed by a wooden spatula where necessary, and the pharyngeal wall was swabbed. Care was taken not to collect saliva. The swab stick was carefully removed from the mouth and replaced into its container, and was transported to the laboratory in a pack of ice. In the Laboratory, crystal violet blood agar (CVBA), Blood agar, Chocholate agar and Mac Conkey agar were inoculated. Incubation was done at 37°c for 48 hours. Visible colonies were identified by routine biochemical tests according to Cheesbrough,(2000). Antimicrobial susceptibility testing was done using Kirby-beur disc diffusion method with Optune diagnostics discs(Nigeria). (Cheesbrough,2005) with Standard drug concentrations according to NCCS Specifications.

Bloob sample: 5ml of blood sample was collected by venepuncture from each subject. Thin blood film was

prepared and stained with Leishman staining technique. Differential counting of cells was done with light microscope with 100x objective lens. ESR of each sample was determined using Westergreen method, while total white cell count was done with Turk's fluid and counted with Improved Neubauer counting chamber as described in Daci and Lewis,(2012). The blood was allowed to clot and serum used for Antistreptolysin O titre and C-reactive protein screening were performed using slide agglutination procedures (). In- built positive and negative controls were used as instructed by Manufacturers. All samples were analyzed immediately within 1 hour.

RESULT
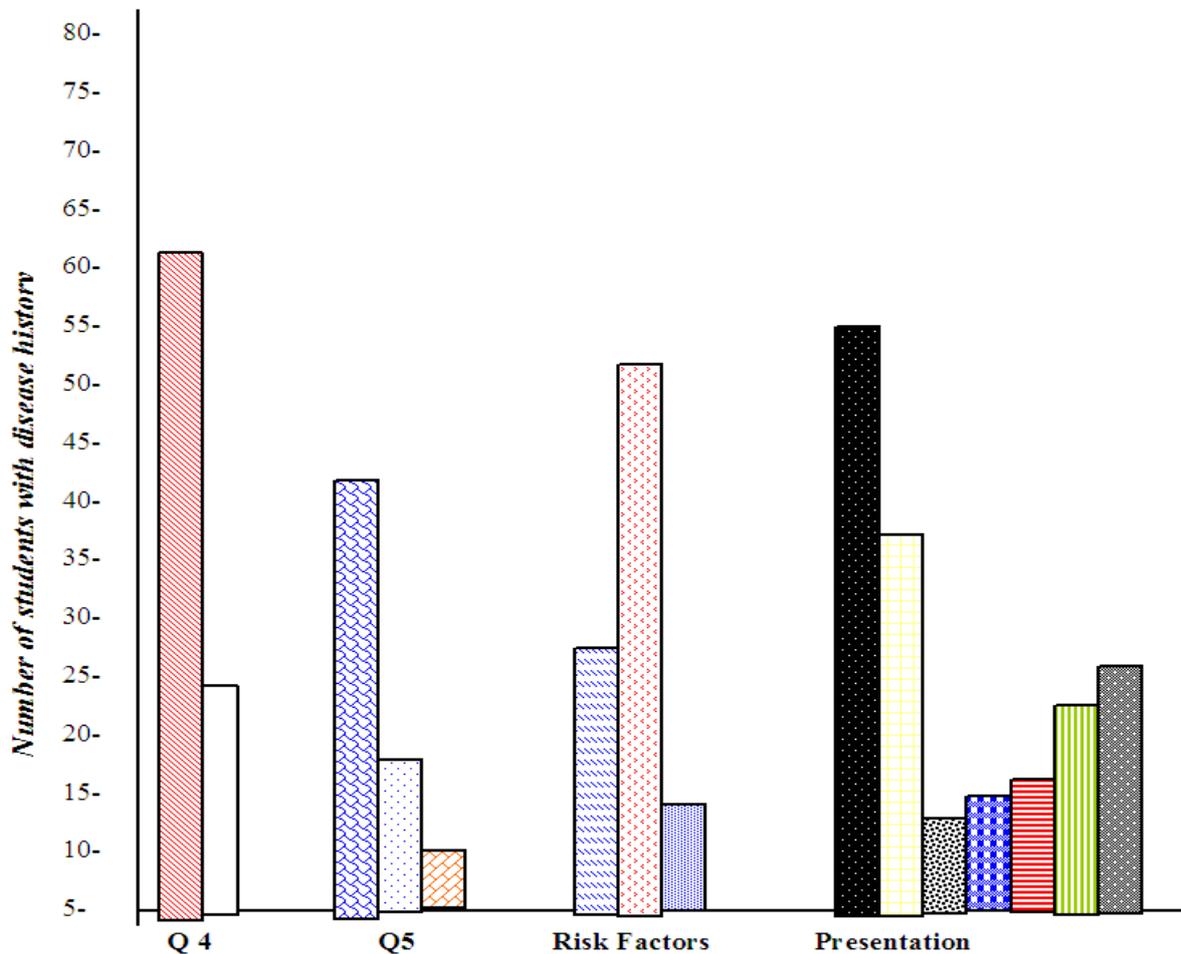
Key:
- History of sore-throat
- No disease history
- Recent pharyngitis
- Childhood pharyngitis
- Sharing of cup
- Crowded condition
- Close contact
- Dysphagea
- Nose bleed
- Dypnoea
- Chest pain
- Joint pain
- Chorea
- Fever

Figure 1: A bar chart showing the frequency of student with sore throat in relation to exposure to environmental risk factor, and their clinical manifestations.

Table 1: Table showing the total sero-prevalence of positive antistreptolysin O in relation to sex.

Sex	Positive		Negative		Total
	N	%	N	%	
Male	20	22.98	26	29.88	46
Female	16	18.39	25	28.73	41
Total	36	41.4	51	58.6	87

X^2 = 2.586, p = 0.108 (P> 0.05)
Key: N = Number of students
     % = Percentage

Table 2: Frequency table of total bacteria isolates from throat swab culture.

Isolate s	Frequency	Male	Female	No isolate	Percentage
S. pyogenes	7	5	2		8.1
E.coli	1		1		1.1
Klebsiella	1	1			1.1
No isolate	-	40	38	78	89.6
Total	9	46	3	78	100

X^2 = 129. 931, p = 0.000 (p< 0.05)

Table 3: Table showing the susceptibility pattern of the isolates.

	Sensitive (S)		Moderately Sensitive (M)		Resistance	
	N	%	N	%	N	%
Ciproflox (CPX)	2	22.2	3	33.3	4	44.4
Norfloxacin (NB)	0	0	0	0	9	100
Gentamicin (GN)	0	0	2	22.2	7	77.7
Amoxil (AML)	5	55.5	2	22.2	2	22.2
Streptomycin (5)	0	0	4	44.4	5	22.2
Rifampicin (RD)	0	0	0	0	9	56.6
Erythromycin (E)	8	88.8	1	11.1	1	11.1
Chloramphenicol (CH)	3	33.3	3	33.3	3	33.3
Ampiclox (APX)	4	44.4	5	55.5	0	0
Levofloxacin (LEN)	9	100	0	0	0	0
Tarivid (TR)	2	22.2	0	0	7	77.7

Key: N = Number of isolate sensitive to the drug
     % = Percentage

Table 4: Frequency table of positive C - reactive protein screening in relation to sex.

Sex	Positive		Negative		Total
	N	%	N	%	
Male	6	6.89	40	45.9	46
Female	6	6.89	35	40.3	41
Total	12	13.8	75	86.2	87

X^2 = 45.621, p = 0.00 (p> 0.05)

Table 5: Frequency table comparing positive ASO test, with positive Culture and C-reactive protein.

Age bracket In years	+ ASO	+CRP	+Culture	+CRPvs Culture	ASO vs CRP	ASO Culture
5-9	10	2	2	0	3	1
10-14	17	6	4	2	3	4
15-19	5	2	1	1	2	1
20-25	4	1	2	0	0	1
total	36	12	9	8	7	3

P = 0.036*   p = 0.056   p = 0.199
R = 0.205   r = 0.205   r = 0.139
Key*: Significant
R = correlation coefficient *

Table 6: Discriptive Statistics of the hematological parameters of the students.

Parameter	Mean	Standard deviation
ESR mm	8.6	4.27
TOTAL WBCx10^3	5.808	1.6229
NEUTROPHILS%	58.79	8.166
LYMPHOCYTES%	35.66	7.825
MONOCYTES%	5.24	3.959
EOSINOPHIL%	1.85	1.149
BASOPHILS%	0	0

Figure 1 above showed that out of the total number of student sampled by questionnaire, (n=87), 60 (68.9%) consisting of 30% male and 30 female had history of sore throat given a male to female ratio of 1:1 and they presented with diferent signs and symtoms. 27 (31.1%) of the students had no disease history. 41 (68.33%) of those student with disease history suffered pharyngitis during childhood. A total of 52 (59.0%) students shared cup while drinking while 29 (29.7%) had close contact with relative/friend with sore throat. Student that live in crowded condition were 10 (11.3%). Fever n = 53 followed by difficulty in swallowing n =33 and chorea (blepherospasm) = 33 were the main presenting clinical

signs. Also chest pain n = 15, nose bleed n = 13, dypnoea n = 14 and skin rash n= 2 were recorded.

Table 1 showed the sero-prevalence of positive anti streptolysin O in relation to sex. Out of the 87(100%) student screened 51(58.6%) were negative while 36 (41.4%) were sero-positive out of which out of which male were 20 (55.5%) and female were 16 (44.4%) given a male and female ratio of 1.25:1. There was no statistical difference between positive ASO in the student with pharyngitis and sex of the student.

Table 2 shows the frequency table of total bacterial isolate from throat culture. Result shows that 78 (89.6%) of the student studied had no growth while a total of 9 (n =9) had growth of which *s. pyogenes* n = 7, *E.coli* n = 1 and *klebsiella* n = 1. 5 male (n=5) and 2 (n =2) had positive growth for *streptococcus pyogenes*. Test of significance showed that there was a significant difference in positive throat culture in relation to those with no growth.

Table 3 showed that the isolates were most sensitive to Levofloxacin (n =9) (100%) followed by Erythromycin n =8 (88.8%) and Amoxil n = 5 (55.5%). No organism isolated showed sensitivity to Rifampicin and Norbactin n= 9 (100%), 7 (77.7%) of isolate were resistant to Gentamicin. Table 4 showed that of the 12 (13.8%) that were positive for C- reactive protein, 6 (6.89) were male while female were 6 (6.89%) given a male to female ratio of 1:1, p- value = 0.00 (p< 0.05) showing a significant difference in subjects with positive screening to those with negative screening n = 75 (86.2).

In table 5, positive ASO was compared with positive culture and positive CRP, and positive CRP was compared with positive culture. Result showed that 7 (n =7) of the 36 student with positive ASO are also positive for throat culture with 4 student (n =4) in the age bracket 10-14 years. Test of significance showed no difference (p>0.05) in positive ASO and throat culture. Of the 36 student with positive ASO, 8 were also positive for CRP with the same 10-14 age bracket recording high value.

Also of the 12 (n=12) with positive CRP, 3(n=3) are also positive for culture consisting of 2 (n =2) and 1 (n=1) student in the age bracket 10-14 and 15-19 respectively. There was a significant difference in positive CRP and positive throat culture (p< 0.05) of the student as related to their age.

In table 6, mean values of the heamatological parameter of the students examined was presented. There was no much changes as indicated by their mean values.

DISCUSSION
Acute rheumatic fever continues to contribute greatly to the burden of cardiovascular disease in sub-Saharan Africa despite advance in medical care (Akintude & Oladimeji, 2009). There is -therefore the need to emphasis routine screening for at - risk subjects and to appropriately institute adequate therapy and preventive measures to avert the late presentation of the disease.

In keeping with other reports, the study group here includes children (5 – 25 years) at risk of rheumatic fever. From the result, 60 (68. 9%) out of the 87 student examined have had history of sore throat out of which 41 (68.33%) had current pharyngitis, as of the time of the study with a male to female ratio of 1:1.

Although some throat and difficulty in swallowing were the major symptoms of pharyngitis presented by the students, less than half of the student 39 (44.81) presented with these complaints. This is because a children seldomly complain of sore throat or difficulty in swallowing (Benzhoff et al., 2002).

The uses of individual clinical features as predictors of possible pharyngitis were poor as shown by then low sensitivity in table 1. Fever was a common finding 52 (59.7%) among the student followed by dysphagea 39 (44.8%) and then blepherospasm 32 (36.78%). These has always been associated with infection with *S.pyogenes* as reported by results from other studies (Sador *et al*., 2008).

The fact that these children are susceptible to rheumatic fever development is exemplified by recurrent chest pain & joint present in15 (17.2%) and 26 (29.8%) of the student respectively. The unusual isolation of Gram negative organisms *(klebsiella* and *E.coli)* may also explain the common findings of fever and chest pain in these individual. This is because these organisms can enter the blood causing septiceamia with pyrexia of unknown origin (PUO) (Nester *et al.*, 2004).

This study also revealed that sharing of cups 52 (29.7%) and contact with close relative with sore throat 29(29.7%) are the major risk of transmission of the etiologic agent (*S. pyogenes*) among the students. Crowded living condition contributed only 10 (11.3%) which is contrary with the report by other studies, which recorded crowded living condition as the main factor for the disease.

This can be explained by the fact that the children still practice the use of common cup for drinking in there respective classes. Crowded living conditions contributed less because greater population of the study group indigenes are living in good housing facililties.

However, the study showed that there was no significant difference (P > 0.05) in the sero-prevalance of antibodies to the *S.pyogenes* organism (ASO) to the presence of infection in the study group. This could be as a result of past infection with persistent antibody titer in the individual. Anti-streptolysin O is formed in serum of an individual exposed to streptococcal species irrespective of the strain, and remains for long periods (months) before clearing from the circulation (Brooks et al., 2010).

Sex was not a factor as both male and female were equally exposed in the environment. There was a statistical significance (P<0.05) in positive C- reactive protien screening to the negetive result. This is concorrent with other findins that there is always an inflammatory condition in cases of pharyngitis. It also reflects the high frequencies of positive ASO screening.

Isolates of *S. pyogenes* from the throat swab of the subjects was statistically significant. This is in line with the report that the organism is always present in acute stage of pharyngitis (Bisno, 2001). high frequency of positive isolate was recorded in male students because male are more active in the environment. The unusual isolation of *E. coli* and *Klebsiella* was noted and this could have been transfered from the anal region to the throat following poor hygiene.

This study also showed that *S. pyogenes, E coli* and *Klebsiella* isolates had 100% sensitivity to Levofloxacin, followed by Erythromycin. Levofloxacin is a new antibiotic and most organism both Gram positive and negetive are succeptible to it. Sensitivity of *S.pyogenes* to Erythromycin is in line with other reports that Erythromycin could be use as second choice antibiotic in patient with penicillin allergies in the treatment of *S. pyogenes* pharyngitis.

The mean heamatological parameters of the students (white cell count, ESR) were normal for all the students. This has always been seen in asymptomatic children with subclinical infection ,besides ESR is a non-specific test. Heamodynamic changes was not recorded in the students although their mean blood pressure was a little toward the upper range value 130/85 mmHg.

REFERENCES

1. Akintunde, A. A. and Oladimeji, G.O. (2009). Late presentation of rheumatic fever disease: a justification for renewal of preventive methods. *PanAfrican Medical Journal,* 3: 22-24.
2. Banzhoff, N., Beck, C., Meyer, F., Werner, J A. And Baum, E.(2002).Clinical findings in patient with sorethroat. A study on intra observa reliability. *Family Practice*, 19: 466-468.
3. Bisno, A.L. (2001). Acute pharyngitis. *New English Journal of Medicine,* 344: 205-211.
4. Brooks, G. F., Butel, J. S. and Morsse, S.A.(2010). The streptococci. *Jawetz, melnick & Adelbergs* Medical Microbiology, 23rd edition. Mc Graw Hill companies Inc, Asia, 197-202.
5. Carapetis, J.R., Steer, A.C. and Mulholland, E.K. (2005).The global burden of group A streptococcal disease. *Lancet Infections Disease,* 5: 685-694.
6. Cheesbrough, M. (2000). District laboratory practice in tropical countries. Part 2. 2nd edition. Cembridge University Press; New York, USA, 159-162.
7. Jones, T.D. (1944).Diagnosis of rheumatic fever. *Journal of the American Medical Association,* 126: 481-484.
8. Mayosi, B. (2006). A proposal for the eradication of rheumatic fever in our life timeœ. *Soutè African Medical Journal,* 96(3): 229-230.
9. Nester, E. W., Denise, G.A., Evans Robert, G., Nancy, N.P. and Martha T.N. (2004). Microbiology: a human perspective. 4th edition McGraw Hill Higher education, 565-568.
10. Ochei, J. and Kolhatkar, A. (2008). Medical Laboratory Science, Theory and Practice. New Delhi, Tata. McGraw Hill publishing company Limited, 665-672.
11. Omokhodion, S.I. (2006). Management of patient with rheumatic fever and rheumatic heart diseases in Nigeria: need for a national system of primary, secondary and tertiary prevention. *South African Medical Journal,* 96(3): 237-239.
12. Sador, W. E., Sador, A.E., Adegbonre, O. O. and Olusola, O. O. (2008). Bacterial isolates of Tonsilitis and Pharyngitis in a paediatric casualty setting. Journal of medical and biomedical research ressearc, 7(1): 37-44.
13. Sally, A. (2006). Frequency of rheuma heart disease in the catchment's areas of guaterg Hospitals. *South African Medical Journal,* 96(3 pt 2): 233-237.
14. Taranta, A., wood, H.F. and Feinstein, A.R. (1964). Rheumatic fever in children and adolescent: Relationship of recurrence rate per Streptococcal Infection to the titers of Streptococcus antibodies. *Annals of Internal Medicine*, 60(5): 47-57.
15. World Health Organisation Expert Consultation on Rheumatic Fever and Rheumatic Heart Disease (2001). Geneva.