PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY OF GROUP A BETA HAEMOLYTIC STREPTOCOCCAL ISOLATES AND ASSESSMENT OF THE SENSITIVITY OF SELECTED CLINICAL PREDICTIVE RULES IN CHILDREN PRESENTING WITH ACUTE PHARYNGITIS TO THE UNIVERSITY TEACHING HOSPITAL, LUSAKA, ZAMBIA.

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A dissertation submitted in Partial fulfilment of the requirement for the award of the degree of Masters of Medicine in Paediatrics and Child Health.

(School of Medicine)

THE UNIVERSITY OF ZAMBIA

2012

DECLARATION

I hereby declare that this dissertation represents my own work and has not been presented either wholly or in part for a degree at the University of Zambia or any other University.

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Approval

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ABSTRACT

Background: Group A beta haemolytic streptococcus(GABHS) associated pharyngitis is an important infection in children because of its potential to complicate into rheumatic fever and rheumatic heart disease – these are preventable with timely and appropriate antibiotic treatment. Physicians in resource poor settings such as Zambia have largely had to rely on unvalidated clinical scoring systems to decide on whether to administer antibiotics when presented with a child who has evidence of pharyngitis. The objective of this study was to determine the prevalence and antibiotic susceptibility of GABHS isolates as well as to assess the sensitivity of three clinical diagnostic criteria in children presenting with acute pharyngitis to the paediatric department.

Methods: This was a descriptive cross sectional study that was carried out over a period of six months (April- September 2011) at the paediatric outpatient department of the University Teaching Hospital in Lusaka, Zambia, involving children 3- 15 years of age presenting with pharyngitis. The enrolled study participants all underwent a standard clinical assessment during which their respective clinical findings were recorded using data sheets. In addition, all the recruited participants had laboratory work out that included a throat swab for culture and subsequent typing as well as antibiotic sensitivity testing of respective isolated organisms. Data analysis with regard to prevalence and antibiotic susceptibility of GABHS isolates as well as assessment of the sensitivity of three clinical diagnostic criteria (Zambia treatment guideline criteria; The WHO ARI management criteria; The modified Centor score) was done using Epi Info version 3.3.2 and Open Epi version 2.3.

Results: A total of 146 children were recruited. Of these, 22 had GABHS pharyngitis, accounting for a prevalence of 15.1%. All the GABHS isolates were susceptible to penicillin while 5(19%) had reduced susceptibility to erythromycin. None of the parameters on the Zambia treatment guideline criteria ,when used individually ,was found to have sensitivity to detect GABHS pharyngitis; Pharyngeal exudates had a sensitivity of 4.5%, 40% for painful enlarged tonsils, 18% for both fever and tender cervical lymph nodes and 36% for the absence of viral signs. The WHO ARI management criteria were not assessed because none of the children with GABHS pharyngitis displayed any of the signs required by the criteria. The modified

Centor score had a sensitivity of only 9.1% while the area under the Reciever Operator Curve (ROC) was 0.51.

Conclusion: The prevalence of GABHS pharyngitis in children presenting with acute pharyngitis at UTH is 15.1%. Penicillin remains the suitable drug of choice for treatment and both tested criteria have poor sensitivity to identify GABHS pharyngitis. Further study in a primary care setting is needed to develop and adapt more sensitive criteria for the diagnosis of GABHS pharyngitis suitable for a high rheumatic heart disease endemic area with limited resources like Zambia.

DEDICATION

To my two constant encouragers - Mum and Dad- who have always believed in me.

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CHAPTER ONE

1.0 BACKGROUND

Group A beta haemolytic streptococcus (GABHS), also known as *Streptococcus Pyogenes*, is the most important bacterial cause of pharyngitis because of the associated post infectious immune-mediated complications of Rheumatic fever and Rheumatic heart disease[1]. There is an estimated prevalence of 15.3 million cases of RHD in the world with the highest prevalence reported in sub Saharan Africa at 5.7 cases per thousand[52] . Rheumatic heart disease accounts for a large proportion (77%) of morbidity due to cardiac diseases in children at the University Teaching Hospital (UTH)[2]. Adequate antibiotic treatment of the pharyngitis is known to prevent these complications[1].

To our knowledge prior to this present study there was no data on the prevalence of GABHS pharyngitis at the UTH nor was the antibiotic susceptibility of the organism known. The circulating GABHS serotypes associated with pharyngitis also remain unknown. One of the most important reasons for this lack of data is the fact that throat cultures are not routinely done at UTH, probably due to the expenses involved. The Zambian treatment guidelines advocate for the empirical treatment of GABHS pharyngitis based on a set of presenting clinical features in the patients. However no clinical scoring system has ever been validated for use in the diagnosis of GABHS pharyngitis in Zambian children.

This study was thus aimed at determining the prevalence of GABHS associated pharyngitis as well as establishing the antibiotic susceptibility of the isolates. We further tested, in Zambian children at UTH, the sensitivity and specificity of three clinical predictive rules developed specifically for low resource settings for the diagnosis of GABHS pharyngitis. The benefit of the results of this study includes baseline data that is now available for use in deciding the type of antibiotics to use in the empirical treatment of suspected bacterial pharyngitis.This study has also provided a basis for further evaluations and adaption for general clinical use, of the

clinical predictive rules in a bid to create a more sensitive criterion for the diagnosis of GABHS pharyngitis.

1.1 STATEMENT OF THE PROBLEM

Acute pharyngitis is a commonly seen paediatric condition whose most serious complication, rheumatic heart disease, is a major cause of paediatric cardiac morbidity in Zambia. Appropriate and timely treatment of acute GABHS pharyngitis is known to prevent this complication. To our knowledge no data on the prevalence and antimicrobial susceptibility of GABHS in Zambia was available prior to this study.

Most Zambians receive care in primary health facilities. These often do not have the laboratory facilities to diagnose GABHS. Yet clinical scoring systems do exist that elsewhere are utilized to predict which children are likely to have GABHS pharyngitis and therefore benefit from antibiotics. The current study has for the first time evaluated some of the existing clinical scoring systems.

1.2 STUDY JUSTIFICATION

The prevalence of rheumatic heart disease in Zambia is estimated to be 12.6 per 100,000 children, one of the highest in the world[3]. Yet no specific study had ever been done in Zambia to determine the prevalence GABHS pharyngitis. This study has probably provided baseline data on the magnitude of the problem which may assist policy makers to plan and assign resources for both diagnosis and treatment.

Timely and appropriate treatment of GABHS can prevent complications. At the current moment there is no surveillance data on the antimicrobial sensitivity patterns of GABHS .Studies in the west indicate an increasing resistance to erythromicin[4-7]. In Zambia a ten day course with either penicillin or a macrolide has remained the main stay of empirical treatment for over thirty years[8]. This study investigates the current antimicrobial susceptibility of GABHS isolates.

To measure the prevalence of group A beta haemolytic Streptococcal throat isolates in children presenting with pharyngitis to the University Teaching Hospital and to assess the sensitivity of selected clinical predictive rules for diagnosis of GABHS pharnygitis.

1.5.2 Specific objectives

The specific objectives of the study were as follows:

1. Determine prevalence of GABHS in children presenting to UTH with pharyngitis
2. Determine antimicrobial sensitivity of the pharyngeal GABHS isolates
3. Determine the clinical characteristics associated to GABHS pharyngitis
4. Assess the sensitivity of selected clinical predictive rules for empirical diagnosis of GABHS pharyngitis in children presenting to UTH.

Like other Gram positive organisms streptococcus pyogenes, possess a peptidoglycan cell wall. This cell wall contains group specific and type specific antigens[19]. The group specific carbohydrate is called the lancefield A antigen, and is used to differentiate GABHS from other streptococcal groups.

The type antigens however, are protein in nature. The most important of these is the "M" protein, a trans membrane protein, which is found on the organisms cell surface and fimbriae. The M protein is a virulence factor.

Over 100 serotypes have been designated based on the use of agglutination reactions with M specific antibodies[19]. The anti sera required for these reactions are only made in highly specialized laboratories which are found in developed countries. Consequently it is commercially unavailable such that this typing system is only used for surveillance purposes or in epidemiological studies. Additionally the M serotying system is fraught with problems such as, limited availability of M typing sera, newly encountered M types(therefore non typable), and difficulty in interpretation of the reactions.

The particular serotypes responsible for causing pharyngitis tend to be region dependant[20] . In the united states of America prevalent serotypes include M1, M2, M12, M28, M3 ,M4 , and M6 [10,21]. Surveillance in Canada showed M types M1 (26.4%), M12 (9.8%), M28 (8.9%), M3 (6.8%), M4 (6.2%), M11 (4.8%), M89 (3.1%), M6 (3.0%), M2 (2.6%), and M77 (1.9%). Non-typeable isolates accounted for 15.4% of the collection[22]. Thus similar serotypes to those reported in the USA. A study done in Ethiopia however, found that with M serotyping; only 45.7% of the strains were M typeable[23] .

A newer system of sub typing based on the sequencing of the emm gene – the gene encoding the M protein- has improved the ability to accurately classify subtypes. The emm method of typing involves DNA sequencing and allows for the rapid direct deduction of the sequence of up to 500 bases of the 5' end of emm genes. This system relies upon the use of the two highly conserved primers to amplify a large portion of the *emm* gene. The hyper variable sequence encoding M serospecificity lies adjacent to one of the amplifying primer sequences, allowing for direct sequencing. This is much more specific and reliable than serologic M typing[24].As mentioned earlier as many as 45 % of Ethiopian GAS isolates were not typable using

the M system. Using the emm system, further analysis revealed completely new strains with the conclusion that the emm types of GAS in Ethiopia were different from those reported in temperate climates[25].This clearly has implications for vaccine development as Steer AC *et al.* demonstrated, in a review article in which they found that as many as 45 % of African GAS emm types are not included in the experimental multivalent vaccine[26]. No study to elucidate the prevalent emm types had ever been carried out in Zambia. Further complicating this situation is the fact that the serotypes found in any particular population are not static but rather tends to be dynamic[26,27]. Hence the need for continuous surveillance as already exists in countries such as the USA and Canada. No such surveillance is currently being done in Zambia.

2.2 Clinical manifestations

The clinical features of Streptococcal pharyngitis generally develop 2 to 4 days after exposure to the pathogen and include sore throat, fever, malaise, headache, dysphagia among many symptoms. There is erythema of the tonsillo-pharynx often with exudates and cervical lymphadenopathy. These presenting features however, are not pathognomic for GABHS pharyngitis and so it is still difficult based on clinical features alone to differentiate between viral and streptococcal pharyngitis[1,19].

Several studies have been conducted to try and correlate these various clinical features with the probability of isolating GABHS in cases of pharyngitis. Nandi *et al.* in India, noted significant association between isolation of GABHS from throat swabs when enlarged tonsils, erythematous throat, sore throat and tender cervical lymphadenopathy were present[13]. In addition,Bassili *et al.* noted that the highest prevalence of GABHS pharyngitis tended to be in the spring. They further reported significant predictors of GABHS pharyngitis as age 10-15 years, the presence of dysphagia, vomiting, pharyngeal exudate, and scarlatiniform rash[16].

Although acute GABHS pharyngitis often follows a benign course it can result in complications particularly if left untreated. Some of the suppurative complications are peritonsillar and retropharyngeal abscess. The non suppurative complications of

only 90% such that recommendations are that all negative specimens be sent for culture[1].

As has been seen, the laboratory diagnostic methods for streptococcal pharyngitis are expensive[29,30] and for health care systems in the developing world are not always available. Additionally most patients are seen in the out patient setting and therefore asking them to come back after 48 hours for the culture results is often not feasible. Consequently several attempts have been made to correlate the clinical features of pharyngitis to the likelihood of getting a positive throat culture for GABHS. Numerous clinical criteria have been formulated. Examples of such criteria include the WHO clinical predictive rule, the Centor score, which has only been validated in adults and the modified Centor scoring system which has been validated even in children.

Table 1: Modified Centor Score for GAS Pharyngitis

Patient Age Range (not valid for <2)	3-14 years old 15-44 years old ≥ 45 years old	+1 0 -1
Exudate or Swelling on Tonsils?	Yes	+1
Fever? (T > 38° C, 100.4° F)	Yes	+1
Tonsillar swelling or exudates	Yes	+1
Absence of cough	Yes	+1

Source: Empirical Validation of Guidelines for the Management of Pharyngitis in Children and Adults[31]

In the modified Centor score,one point is assigned to each of the 4 criteria: temperature >38 ^{0} C; absence of cough; swollen,tender anterior cervical nodes; and

tonsillar exudate.The higher the background prevalence of GABHS pharyngitis is in a given population the higher the probability that the presence of any of the four criteria will, be associated with a positive culture[1]. As the table below shows this score still requires the use of laboratory facilities which as we have said are not always available in low resource settings.

Table 2: Decision rule based on the modified Centor score

MODIFIED CENTOR SCORE	SUGGESTED MANAGEMENT
0	NO FUTHER TESTING OR ANTIBIOTIC
1	NO FUTHER TESTING OR ANTIBIOTIC
2	CULTURE ALL, ANTIBIOTIC IF POSITIVE
3	CULTURE ALL, ANTIBIOTIC IF POSITIVE
4	TREAT EMPIRICALLY WITH ANTIBIOTIC AND/OR CULTURE

Source: Empirical Validation of Guidelines for the Management of Pharyngitis in Children and Adults[31]

The Centor score while reducing the proportion of children requiring throat cultures still requires the limited availability of laboratory support which is not always the case in primary health care settings in Zambia.

The Zambian empirical treatment guidelines for acute pharyngitis recommends that a child be treated for GABHS pharyngitis if they have any one of the following: high fever, white pharyngeal exudates, tender enlarged anterior cervical lymph nodes, grossly enlarged painful tonsils which are asymmetrical or absence of signs suggestive of viral pharyngitis. All these features are given equal weight and no

The objective of this study was to develop a clinical decision rule that allows for the reduction of empirical antibiotic therapy for children with pharyngitis in low-resources settings by identifying non–group A streptococcus pharyngitis[35]. The Brazilian scoring system places an emphasis on identification of true negatives (specificity) so as to reduce the inappropriate prescription of antibiotics by clinicians. Such a scoring system was considered inappropriate for our region which is highly endemic for rheumatic heart disease and where the emphasis is on establishing criteria that picks up all true positives. For this reason we felt it would be inappropriate to test the sensitivity of the criteria.

In view of the aforementioned lack of documentation of aetiological patterns of acute pharyngitis (AP) in children in our country and the region, in this study we sought to assess the prevalence of GABHS isolates and establish the emm types in children presenting with acute pharyngitis at UTH. In addition we sought to assess and validate some of the above selected clinical predictive rules including the WHO predictive rule and the Modified Centor score in comparison to the Zambian empirical treatment guidelines for the diagnosis of acute pharyngitis in our setting.

2.4 Management

Early treatment of GABHS pharyngitis within the first nine days is known to be effective as both primary and secondary prevention against the two major complications ; rheumatic fever and rheumatic heart disease [36,37]. And so for years the Zambian standard treatment guidelines have advocated for the use of an oral penicillin as first line and the use of a macrolide in penicillin allergic individuals who are suspected to have GABHS pharyngitis[9] . Over the years many studies have continued to report susceptibility of GABHS isolates to penicillin[5,6,7,8,14,338,39]. Similarly Bassilli *et al.* in Egypt reported low resistance to the conventionally used penicillin and erythromycin. In contrast several studies from different regions have shown an increase in resistance of GABHS isolates to macrolides[5,6,7,8,38,39]. Of note though are two randomized, single-blind, multicenter antibiotic efficacy trials in children using recommended doses of either oral penicillin V or intramuscular BPG for treatment of acute-onset pharyngitis which showed that thirty-five percent of

284 evaluable patients treated with oral penicillin V and 37% of Benzyl Penicillin G treated patients were microbiologic treatment failures at either 10 to 14 or 29 to 31 days[33,36]. This has significant implications for a country like, Zambia were penicillin and macrolides for ten days have remained the main stay of empirical treatment for over twenty years. The current antimicrobial susceptibility of GABHS pharyngeal isolates in Zambia is currently not known.

3.2.2. Inclusion criteria

Inclusion criteria included:

- Age 3 years to 15 years
- Child meeting the criteria for case definition
- Written consent

3.2.3. Exclusion criteria

The exclusion criteria included:

- Children who may have received antibiotics in the preceding 7 days
- Children who were known to have a Previous history of Rheumatic fever

3.3. Sample size calculation and sampling method

An initial sample size of 246 was calculated using EpiInfo statcalc version 6 at a confidence interval of 95% with a P-value of 0.05 and an estimated prevalence of 15% assuming that the study would be carried out over a 12 month period in hopes of documenting the effect of season on the prevalence. However by the end of the second month only 15 children had been recruited into the study and the study period had had to be reduced to six months.

In view of the above the confidence interval was adjusted to 90% and with a P-value of 0.05, an estimated prevalence of 15% at a power of 80% and allowing a rate of 5% loss to follow up, a sample size of 140 was arrived at using EpiInfo statcalc,version 6.

The choice of a prevalence estimate of 15% was based on findings in similar studies in Egypt and India.

Because of the scarcity of patients, the recruitment of children into the study was done consecutively.

3.4. Clinical Assessment

Children presenting to the UTH outpatient department were screened by the investigator, who is a medical doctor. Some of the children were referred to the study for screening by other doctors working in the out patient department. Those considered to be eligible by the study physician, were identified and if they met the inclusion criteria were subsequently enrolled into the study. The purpose of the study and study procedures were then explained to the care giver. Once the consent was obtained the investigator then obtained a standard medical history and then proceeded to perform a standard medical examination. The demographic data and the presenting signs and symptoms of each child were recorded on a standardized clinical case record form (Refer to history and examination assessment forms in appendix III and IV).

The investigator then performed a throat swab and stored it in Amie's transport media. Each child was assigned a unique study number which was inscribed on each of the data sheets as well as the laboratory request form. The specimen was then dispatched to the UTH microbiology laboratory within 24 hours.

3.5. Laboratory Assessment

The main UTH microbiology laboratory was used to process the specimens and various laboratory workouts are as detailed below:

3.5. 1 Specimen collection

The sampled and enrolled children after clinical assessment had a sterile throat swab taken using a sterile throat culture cotton swab probe (Sterilin ryon tipped bud in Amie's transport media). With the patient's head tilted back and the throat well illuminated, the tongue was then depressed so that the back of the throat could be seen. The swab was then rubbed up and down the back of the throat and against any white patches in the tonsillar area taking care to avoid the tongue and the cheeks. The swab was then placed in Amie's transport media tubes and transferred to the laboratory within 24 hours of collection.

3.5.2 Sample Processing

3.5.2.1 Isolation of beta haemolytic Streptococcus

The swabs were inoculated onto blood agar. The specimen was then incubated at 37°C in aerobic conditions. *Streptococcus pyogenes* was identified by having a clear zone of haemolysis around colonies on sheep's blood agar. Bacitracin disks were then added[1].

3.5.2.2 Lancfield grouping

Lancefield grouping was performed using a latex immunoagglutination test.

Streptex(remel)

3.5.2.3 Antibiotic Susceptibility Testing

All the GABHS isolates were assessed for antimicrobial sensitivity to various antibiotics including penicillin and Erythromicin. Antibiotic susceptibility was determined using the Kirby-Bauer disk diffusion method.

3.5.2.4 Documentation of Laboratory Results

Each result was entered into the study laboratory log book as well as the microbiology electronic data base. Later the results were recorded onto an excel spread sheet as the primary study data base.

3.5.2.5 Disposal of Isolates

All isolates from the study were assigned unique laboratory serial numbers and stored in the microbiology freezer for further analysis using molecular biological techniques which are not available at present.

3.6.0 Data Management and Analysis

3.6.1. *Variables*

Dependent (outcome) variables:

The study dependant variables included the following:

- Culture results
- emm typing results
- Antibiotic susceptibility

Independent (predictor) variables

The study independent variables included the following:

- Age
- Month
- Number of people in the household
- Number of shared rooms
- Fever- this will be defined as $\geq$ 38.0°C
- Exudate: yellow/white matter seen on the pharynx or tonsils
- A tender node: tenderness of an anterior cervical lymph node on palpation, confirmed
- either by a statement from the child or by his or her facial expression
- Large node: an anterior cervical lymph node 1.5 cm in diameter
- Pain on swallowing: obtained from the patient history.

- Enlarged tonsils

3.6.2 Data management and analysis

A secondary data base prior to analysis was created on Epi Data. The baseline information was obtained from the history, physical examination and the laboratory result data sheets. The data was then analysed using EpiInfo version 3.3.2 and OpenEpi version 2.3.The Body Mass Index (BMI) was not analysed because this information was incomplete for most of the children.

Simple proportions were used to calculate the prevalence of GABHS pharyngitis in the study population as well as to calculate the antibiotic susceptibility of the GABHS isolates. Odds ratios were used to determine the association of the different independent variables to GABHS pharyngitis. This was achieved by carrying out univariate and multivariate analysis of the independent variables there by determining the odds ratio and adjusted odds ratio respectively. Open Epi version 2.3 was used to calculate the sensitivity, specificity, positive predictive ratio and the negative predictive ratio of the various clinical predictive rules. Open Epi version 2.3 was used to construct a Receiver Operator Curve for the Centor score by determining the individual sensitivity of each of the four levels of the score.

3.7. Ethical Approval

Ethical approval was sought from the institutional review committee of the University of Zambia, School of Medicine. All participants gave informed consent which was sought from the care givers and where appropriate assent was sought from the children after the purpose of the study had been explained. All decisions about treatment were those that are currently recommended for best practice by the health institution and also according to the clinical judgment of the investigator. No penalty was imposed on any child whose parents declined permission to consent. The findings of the study were made available to the participants and the health institution where necessary.

CHAPTER FOUR

4.0 RESULTS

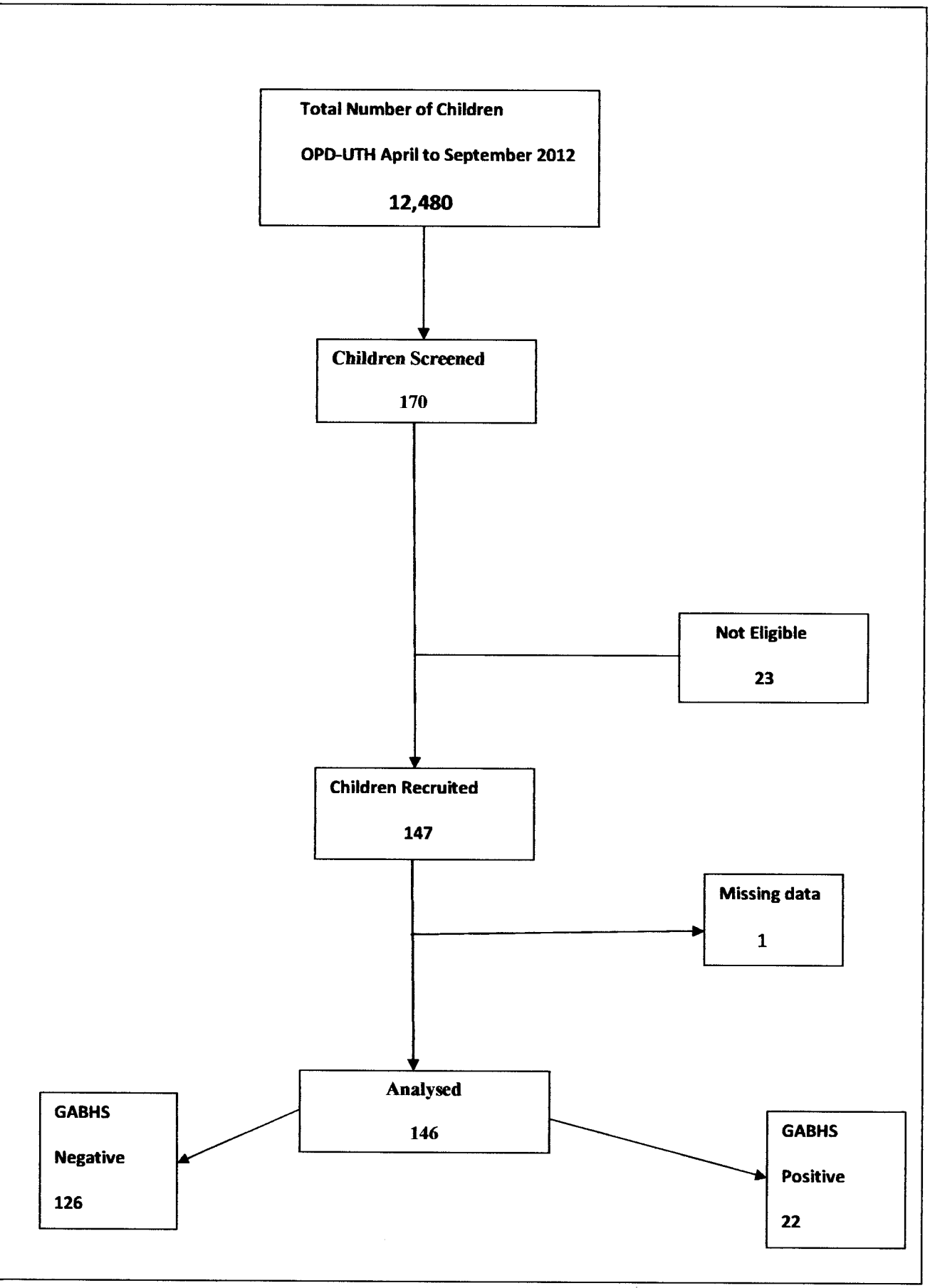
4.1. Socio-demographic and Baseline Characteristics of the participants

The number of children seen and screened in the paediatric outpatient department between April and September 2011 was 12480. A total number of 170 were approached to join the study by the study physician but only 147 were recruited (figure 1).

Table 4 summarizes the socio-demographic and baseline characteristics of the enrolled participants which was eventually analysed. Most of the 146 children recruited, 55% (79), were between the age of 5 and 10 years old while 24% (34) were less than 5 years old but older than 3 years . The mean age of the children was 7.4 years with a standard deviation of 3.38. Girls predominated in the study accounting for 56% (80). Most of the participants 87% (102) came from households with between 5 and 10 people and most 57% (70) lived in homes that had between 3 to 5 rooms.

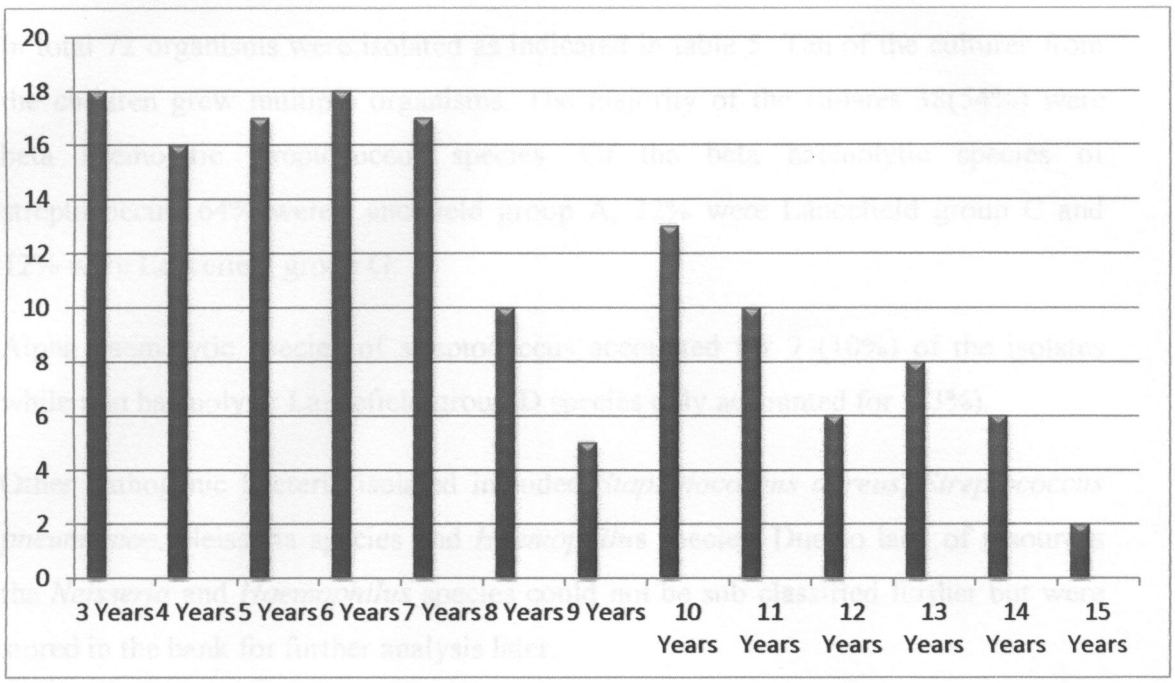
The majority 65% (66) of the children were reported as having experienced fever at some point during their illness. Of note though is the fact that only 12.6% (18) were actually found to have temperature greater than 38°C on examination. Of these 7% (9) had temp greater than 39° C. Other symptoms experienced by the children include headache in 42% (56), abdominal pain in 24% (33) and sore throat in 58% (83).The signs noted in the children include rhinitis in 58% (85), conjunctivitis in 9.7% (14), scarletiniform rash in 10% (15), exudates in 13.7% (20) and tender cervical lymphadenopathy in 28.9% (14).

Figure1. Flow chart showing the recruitment of study participants



Characteristic N=146	Frequency (%)	Crude OR(90% CI)	P Value	Adjusted OR(90% CI)	P Value
a. Age Category					
< 5 years	34(24)	1.00	0.0	1.00	
5 – 10 years	79(55)	2.90(0.96-8.69)	0.11	3.77(0.99-14.3)	0.10
>10 years	30(21)	0.86(0.21-3.49)	1.4(0.2-7.4)	1.4(0.2-7.4)	0.73
b. Sex					
Male	64(44)	1.00	0.0	1.00	
Female	80(56)	1.1(0.51-2.41)	0.82	1.01(0.39-2.61)	0.98
c. Household Crowding					
<5 people	32(7)	1.0	0.00	1.00	
5-10 people	102(87)	1.85(0.62-5.46)	0.40	1.35(0.40-4.54)	0.68
d. Household Ventilation					
<3 rooms	26(7)	1.00	0.00	1.00	
3-5 room	70(57)	1.64(0.53-5.12)	0.47	4.00(0.88-18)	0.13
> 5 rooms	45(36)	1.05(0.30-3.60)	0.95	2.00(0.43-9.7)	0.44
e. Symptoms and signs					
History of fever	66(65)	0.91(0.78-1.07)	0.37	0.93(0.79-1.12)	0.51
Headache	56(42)	1.18(0.47-2. 93)	0.72	0.63(0.06-5.98)	0.69
Abdominal pain	33(23)	1.18(0.47-2.93)	0.72	0.60(0.06-5.38)	0.64
Sore throat	83(58)	1.12(0.51-2.40)	0.81	0.79(0.28-1.81)	0.55
Fever(>38°C)	18(12.6)	1.81(0.65-5.01)	0.33	2.05(0.57-7.30)	0.35
Cervical lymphadenopathy	44(30.1)	2.14(0.81-5.61)	0.19	3.42(1.02-11.3)	0.09
Rhinitis	85(58)	0.76(0.34-1.68)	0.58	0.48(0.16-1.39)	0.25
Conjunctivitis	14(9.7)	2.34(0.40-1.35)	0.42	2.54(0.35-18.1)	0.43
Scarletiform rash	15(10)	2.67(0.46-15.3)	0.35	2.69(0.40-18.0)	0.39
Exudates	20(13.7)	3.80(0.67-2.14)	0.20	3.0(0.45-20.6)	0.33
Tender Cervical lymph node	14(28.9)	0.11(0.11-1.21)	0.07	0.10(0.01-1.25)	0.07

Figure2. Chart showing age distribution of the participants



4.2. Association of Various baseline characteristics with GABHS pharyngitis

Being between aged between 5 -10 years was associated with GABHS pharyngitis (adjusted OR 3.77,CI 0.99-14.3, P Value 0.10) as was living in a home with between 3 to 5 rooms (adjusted OR 4.00(CI 0.88-18), P Value 0.14).

Among the clinical features, the presence of cervical lymphadenopathy (adjusted OR 3.4,CI 1.02-11.3, P Value0.09), exudates (adjusted OR 3.0, CI 0.45-20.6,P Value 0.33), and the presence of fever (adjusted OR 2,CI 0.57-7.30 ,P Value 0.35), the presence of conjunctivitis (adjusted OR 2.54,CI 0.35-18.1, P Value 0.44) and the presence of scarletiform rash(adjusted OR 2.69, CI 0.40-18.0, P Value 0.39)were associated to GABHS pharyngitis. None of these associations were statistically significant however.

4.3. Spectrum of Organisms Cultured and Prevalence of GABHS pharngitis

In total 72 organisms were isolated as indicated in table 5. Ten of the cultures from the children grew multiple organisms. The majority of the isolates 38(54%) were beta haemolytic streptococcus species. Of the beta haemolytic species of streptococcus, 64% were Lancefield group A, 32% were Lancefield group C and 12% were Lancefield group G.

Alpha haemolytic species of streptococcus accounted for 7 (10%) of the isolates while non haemolytic Lancefield group D species only accounted for 2(3%).

Other pathogenic bacteria isolated included *Staphylococcus aureus*, *Streptococcus pneumoniae*, Neisseria species and *Haemophilus* species. Due to lack of resources the *Neisseria* and *Haemophilus* species could not be sub classified further but were stored in the bank for further analysis later.

Two of the children that presented with a scarletiform rash and pharyngitis were later found to have Rubella on analysis of serum immunoglobulin M titres.

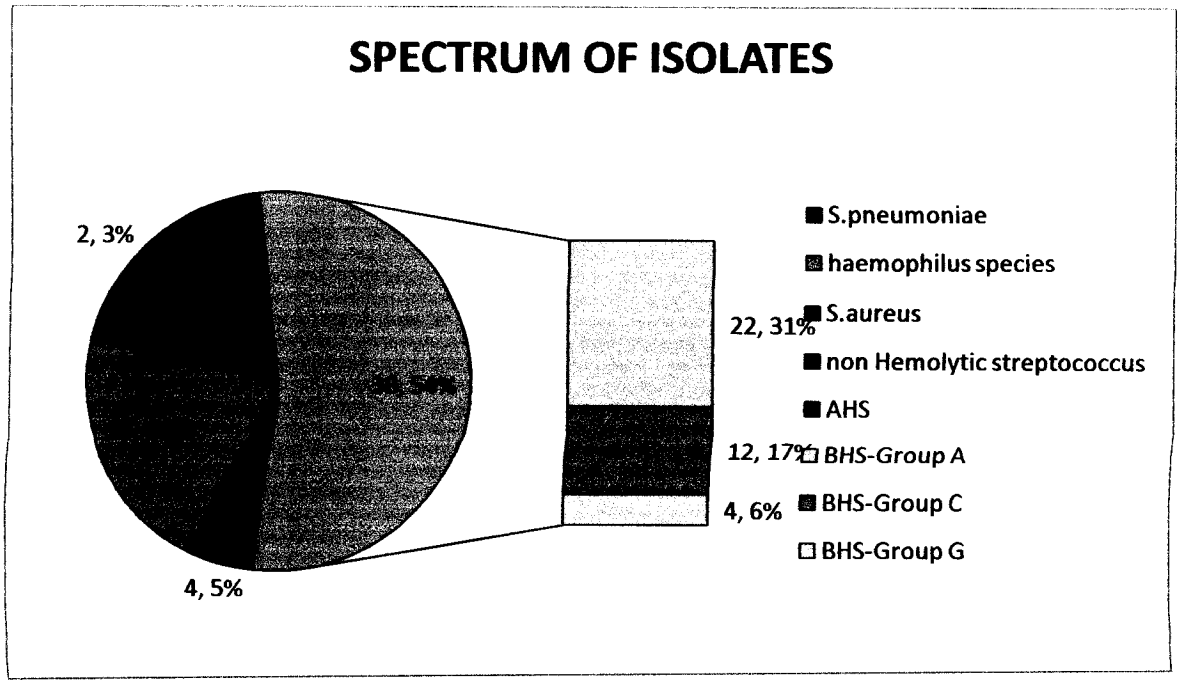
Of a total of 147 children who were recruited only 22 had GABHS cultured from their throats accounting for a prevalence of GABHS pharnygitis of 15.1%.

Table 5 Spectrum of Organisms Isolated

Organism	**Frequency (%) *N=72**
STREPTOCOCCUS PNEUMONIAE	4 (5%)
HAEMOPHILUS SPECIES	15 (20%)
STAPHYLOCOCCUS AUREUS	5 (7%)
NEISERRIA SPECIES	5 (7%)
NON HAEMOLYTIC STREPTOCOCCUS	
GROUP D	2 (3%)
ALPHA HEMOLYTIC STREPTOCOCCUS	7 (10%)
BETA HAEMOLYTIC STREPTOCOCCUS (54%)	38
GROUP A	22
C	12
G	4

***10 specimens grew out more than 1 organism**

Figure3. Chart showing the spectrum of organisms isolated



BHS- beta haemolytic streptococcus, AHS- alpha haemolytic streptococcus

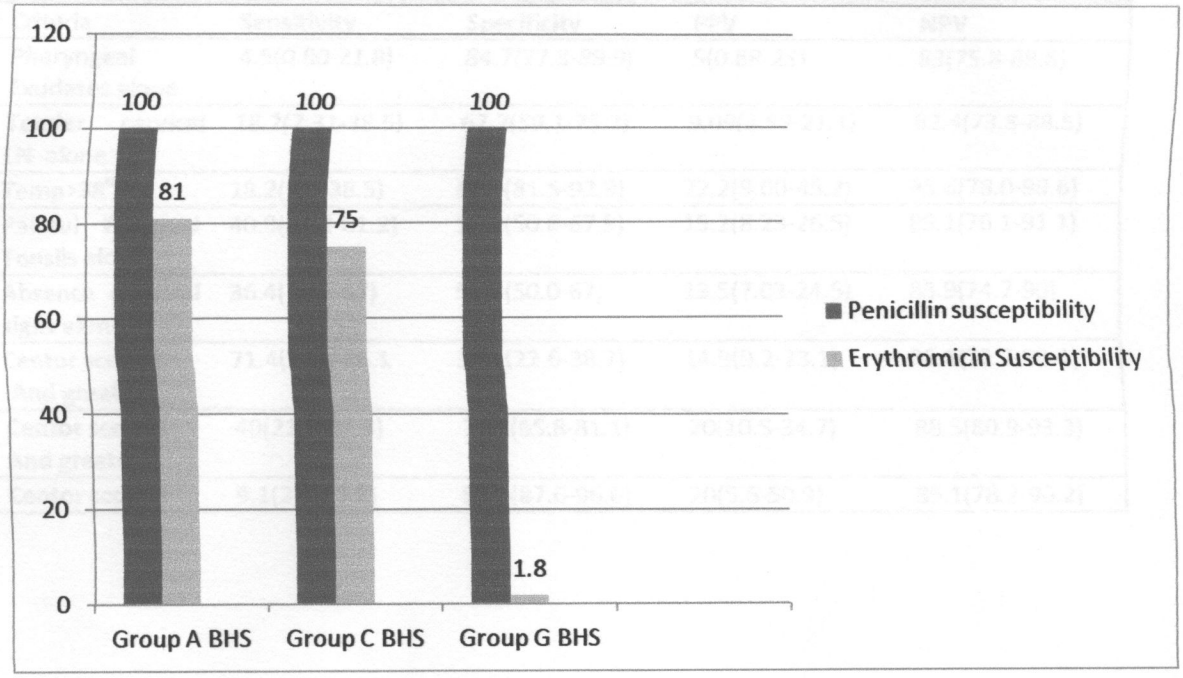
4.4. Antibiotic Susceptibility of the beta haemolytic Streptococcal isolates

All 38(100%) the beta haemolytic Streptococcal isolates were susceptible to Penicillin.

Of the 22 Lancefield group A isolates 17 (81%) were susceptible, 3(14%) had intermediate susceptibility while only 1(5%) were resistant to erythromycin.

Among the 12 Lancefield group C isolates susceptibility to erythromycin was full in 9 (75%), intermediate in 2(17%) and resistant in 1(8%).All Lancefield group G (4) and D (2) isolates, were susceptible to erythromycin.

Figure4. Chart showing the antibiotic susceptibility of BHS isolates



BHS- beta haemolytic streptococcus

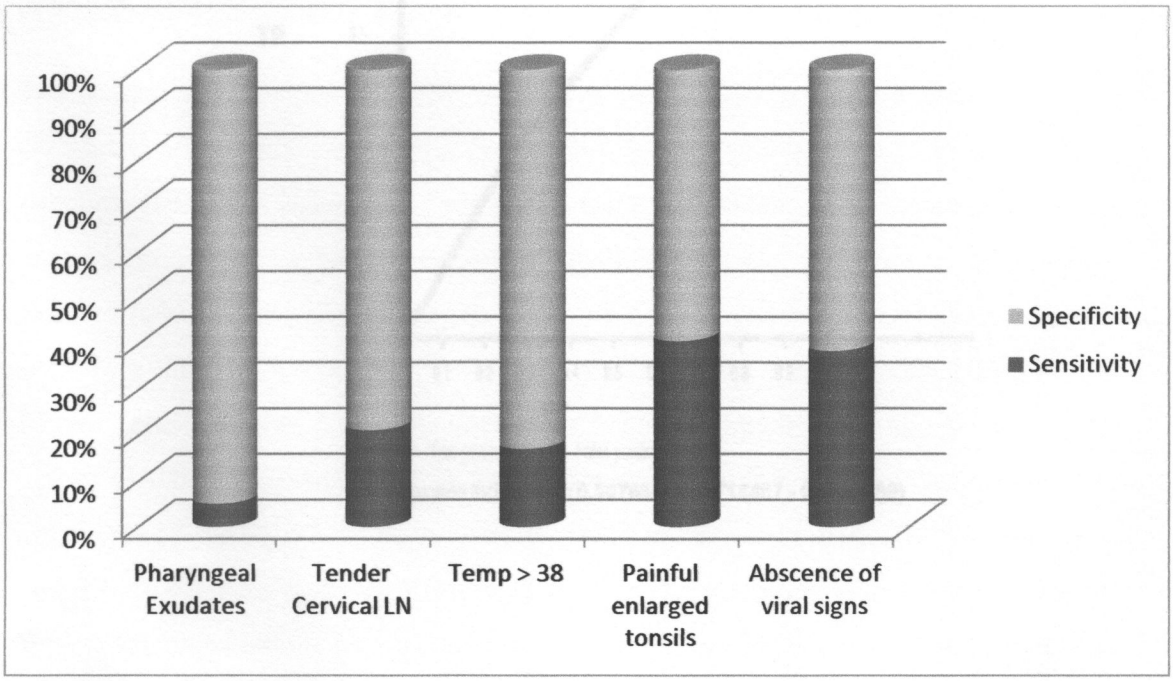
4.5. Performance of Various Scoring Systems

None of the five individual parameters on the Zambian Treatment Guidelines criteria when used on their own was found to have sensitivity to predict a positive culture of GABHS; pharyngeal exudates (Sensitivity 4.5%), painful enlarged tonsils (sensitivity 40%), fever (18%), and tender cervical lymph nodes (sensitivity 18%) and absence of viral signs (36%).

None of the children with GABHS pharyngitis on cultur were found to have a combination of all the five parameters together therefore making it impossible to assess the sensitivity of a combination of all four parameters occurring together.

Table 7. Sensitivity, Specificity and predictive values for GAS pharyngitis using various parameters of the various predictive rules				
Criteria	**Sensitivity**	**Specificity**	**PPV**	**NPV**
Pharyngeal Exudates alone	4.5(0.80-21.8)	84.7(77.3-89.9)	5(0.88-23)	83(75.8-88.8)
Tender cervical LN alone	18.2(7.31-38.5)	67.7(59.1-75.3)	9.09(3.59-21.1)	82.4(73.8-88.5)
Temp>38°C	18.2(7.3-38.5)	88.4(81.5-92.9)	22.2(9.00-45.2)	85.6(78.0-90.6)
Painful Enlarged Tonsils alone	40.9(23.2-61.2)	59.7(50.8-67.9)	15.2(8.23-26.5)	85.1(76.1-91.1)
Absence of Viral signs alone	36.4(19.7-57)	58.2(50.0-67)	13.5(7.03-24.5)	83.9(74.7-90)
Centor score 2 And greater	71.4(50.0-86.1	30.1(22.6-38.7)	14.9(9.2-23.1)	86.1(72.2-93.4)
Centor score 3 And greater	40(21.8-61.3)	74.1(65.8-81.1)	20(10.5-34.7)	88.5(80.9-93.3)
Centor score 4	9.1(2.5-27.8)	93.5(87.6-96.6)	20(5.6-50.9)	85.1(78.2-90.2)

Figure 4 Chart showing the sensitivity and specificity of various parameters on the Zambian treatment criteria

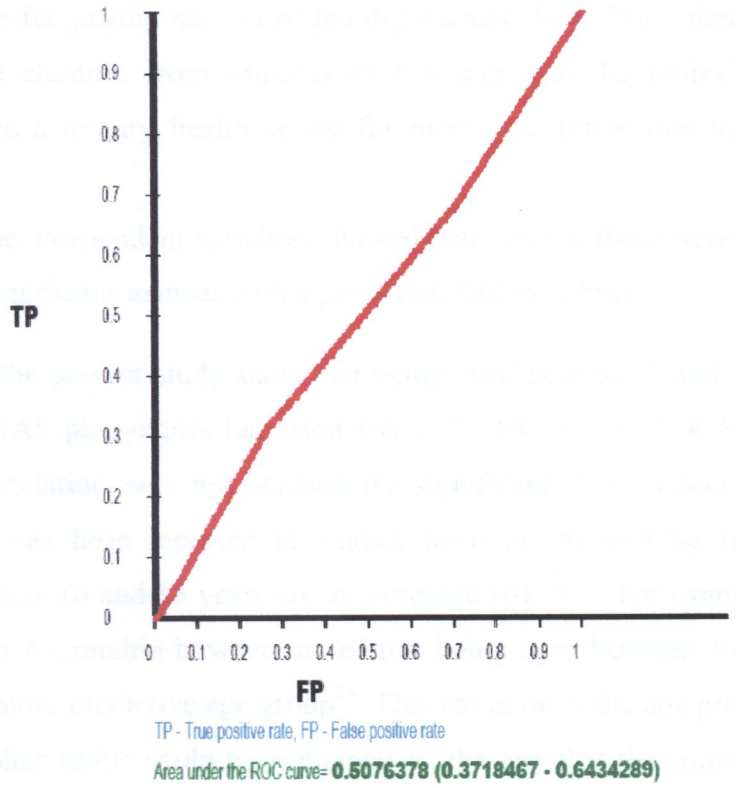


The WHO ARI programme criterion was found to be completely invalid in the sense that none of the GABHS positive children met the criterion. In this case all the

clinically suspected GABHS pharyngitis children picked by the WHO criterion were found to have a negative culture.

The modified Centor score had a sensitivity of 9.1% and a specifity of 85.1%. A Receiver Operator Curve for the modified Centor score was constructed as shown below and the calculated area under the curve was 0.51.

Figure 6. Receiver Operator Curve for the Modified Centor Score



CHAPTER FIVE

5.0. DISCUSSION

5.1. Characteristics of the participants and the association of various baseline characteristics with GAS pharyngitis

The results show that most of the children seeking treatment for pharyngitis were those between the age of five and ten years. This is consistent with what has been reported in other literature. Not surprisingly the expected association of GABHS pharyngitis with crowding and limited living space were not illustrated in this study. We attribute this to the fact that most of the children enrolled could be considered as coming from middle class homes since the majority of the patients in this study were recruited from the fee paying section of the department. In addition this may have occurred because children from impoverished homes may be unlikely to seek medical help from a tertiary health centre for mere sore throat due to economic constraints[49].

The analysis of the independent variables showed that none of them were associated in a statistically significant manner with a positive GABHS culture.

The results from the present study show that being aged between 5 and 10 years is associated with GAS pharyngitis (adjusted OR 3.77, CI 0.99-14.3, P Value 0.10) although this association was not statistically significant. This association is in contrast to what has been reported in studies from the developing world were children between age 10 and 15 years are at increased risk[13,16] . For example Basilli *et al.* in a study in Alexandria however noted that being aged between 10 years and 15 years was the more predictive age group[23]. This variation in the age group at most risk of GABHS pharyngitis could be explained by the fact that the clinical features associated to GABHS tend to vary from region to region as described by Rimoin *et al*[15]. Based on their findings, Rimoin *et al.*, therefore strongly recommended that all clinical scoring systems be validated in the populations in which they were meant to be used before being adopted

The analysis also showed that children living in homes with between 3-5 shared rooms were more likely to have GAS pharyngitis (adjusted OR 4.00,CI 0.88-18, P Value 0.14) than children living in homes with less than 3 shared rooms. This again

is just a reflection of the fact that most the participants (57%) enrolled in the present study came from homes with between 3 to 5 shared rooms.

Of note is the fact that the mere report of fever (temp > 38 °C) having had been present at some point during the illness was poorly associated with a positive GAS culture. In contrast the presence of fever at the time of examination was noted to be associated though not in a statistically significant manner (adjusted OR 2, CI 0.57-7.30, P Value 0.35). This is similar to the study by Basilli *et al.* which found that the presence of fever was a non specific predictor of GABHS pharyngitis.

The presence of conjunctivitis (adjusted OR 2.54, CI 0.35-18.1, P Value 0.44) and the presence of scarletiform rash (adjusted OR 2.69, CI 0.40-18.0, P Value 0.39) were associated to GABHS pharyngitis though not in a statistically significant manner. The present study showed a non significant association between a positive GABHS culture and cervical lymphadenopathy (adjusted OR 3.4, P Value0.09) as well as the presence of exudates (adjusted OR 3.0, P Value 0.33). This association is similar to the findings of Nandi et al. in a study conducted in the Chandigah area of india.

5.2. Pattern of bacterial isolates in children with pharyngitis

Our results show that b-haemolytic streptococcus accounted for 54% of the bacterial isolates, which is consistent with what has been reported in other studies [40,41,42]. This illustrates the large proportion of pharyngitis that is caused by bacterial pathogens in this population of children. The proportion of GABHS (22 isolates) among the b-haemolytic streptococcal isolates was 64% while the most prevalent non-GABHS streptococcal isolates were group C and group G accounting for 32% and 12% respectively. This pattern is similar to what has earlier been reported in Egypt[23]. This is in contrast to the much lower proportion of non -GAS streptococcal isolates of 6.5% reported in Pittsburgh, USA[18]. In fact some carriage studies done in Africa have noted a predominance of the two streptococcal groups and have suggested that group C and G streptococcus are far more important throat pathogens in the tropics. This has led to increasing speculation by workers in this field on whether group G and/or C may play a role in the pathogenesis of acute rheumatic fever contrary to

what has previously been believed [43] . Of note also is the fact that no group B streptococcus was isolated. This is in contrast to the findings by tiemiestra *et al.* who reported a prevalence of 6%.

The prevalence of GAS pharyngitis was found to be 15.1%. This is similar to figures of 17% reported on the Indian subcontinent and in Alexandria Egypt[23]. The prevalence of GAS pharyngitis in another Egyptian study in Cairo by Basilli *et al.* however revealed a significantly higher prevalence of 24 %[32]. The hospital were the Egyptian study was carried out was not a tertiary health centre like the hospital were the present study was carried out. This perhaps accounts for this discrepancy.

Of interest is the fact that the only throat commensal haemophilus species isolated were Haemophilus *para- phrophilus* and *Haemophilus aphrophilus* . These probably reflect throat carriage. The glaring absence of Haemophilus influenza among the isolates may be explained by the fact that many of these children had received the conjugate Hib vaccine. It is known that Hib vaccination does reduce Hib pharyngeal carriage rates in the vaccinees[44,45].

5.3. Antibiotic susceptibility of the beta haemolytic streptococcal isolates

All the GAS isolates were found susceptible to penicillin as expected from literature. However up to 19% of the GAS isolates had either intermediate susceptibility or complete resistance to erythromycin. This phenomenon has also been reported in GAS isolates in Europe and the far east although the reported prevalence of resistance was much higher[6,7,8] . These studies showed that various M types of GAS displayed resistance. The Greek study reported that all the isolates that were resistant to erythromycin were also resistant to Azithromycin and Clarythromycin. The Greek study demonstrated that the mechanism of resistance involved the possession of mef (A), erm (A) [subclass erm (TR)] or erm (B) genes. The present study did not test sensitivity to other macrolides nor were the molecular techniques necessary to ascertain the mechanism of resistance carried out on the isolates either. This means that children with GAS pharyngitis treated with erythromycin may still remain at risk of developing rheumatic fever and consequently rheumatic heart disease. And as such the preferred drug for the treatment of suspected GAS pharngitis ought to be

penicillin. Further antimicrobial testing with other classes of drugs as well as other macrolides will be carried out on the isolates to better provide advice on the course of action in the case of penicillin allergic patients.

All the Lancefield group D and G isolates were susceptible to both penicillin and erythromycin while only 75 % of the group C isolates however were susceptible to erythromycin.

5.5. Performance of Various Scoring Systems

The Zambian treatment guideline for the management of acute pharyngitis has a list of symptoms and signs that when present in a patient are considered suggestive of GABHS pharyngitis. The guideline however does not suggest how many parameters should be present to make the diagnosis. The present study found that the sensitivity and specificity of the various individual parameters was as follows : pharyngeal exudates (Sensitivity 4.5%, sensitivity 84%), painful enlarged tonsils (sensitivity 40%, specificity 59.7%), fever (sensitivity18%, specificity 88.8%), and tender cervical lymph nodes (sensitivity 18%, 67.7%) and absence of viral signs (sensitivity 36%, specificity 58.8 %).This may be an indication that none of these signs when used individually can be considered as a sensitive predictor of GABHS pharyngitis. Of interest is the fact that only 11(7.5%) of the children enrolled into this study displayed both exudates and cervical lymphadenopathy. None of these children had GABHS culture positivity. In addition none of the children with GABHS pharyngitis had a combination of all the four parameters together therefore making it impossible to assess the sensitivity of a combination of all four parameters occurring together. This could be due to the relatively smaller number of children recruited into the study. However the study by Steinhoff et *al.* in Egypt which had enrolled three times more children than we did, recorded a similarly low number of children displaying a combination of these signs. For example only 7.5% of their enrolled children displayed both pharyngeal exudates and tender cervical lymphadenopathy. Clearly then this brings into question the utility of clinical scoring systems that use symptom combinations which rarely occur together.

The WHO Global Acute Respiratory Infections (WHO ARI) treatment programme criterion was found to correlate poorly to the gold standard of throat culture. This is because none of the children with GABHS pharyngitis were detected by this tool. The WHO criteria suggests that, in the absence of other guidelines for children under five, acute streptococcal pharyngitis should be suspected and presumptively treated with penicillin when pharyngeal exudate plus tender, enlarged cervical lymph nodes are found[48]. A similar attempt was made to prospectively validate these criteria by Steinoff *et al.*in Egypt who found that the sensitivity and specificity of the criteria were 12% and 93% respectively. They thus concluded that if followed the WHO guideline left up to 88 % of susceptible children at risk of rheumatic heart disease[32].In addition Rimoin *et al.* in a study done in three countries found that in children aged 5 or older, sensitivity of the WHO clinical predictive rule, ranged from 3.8% in Egypt to 10.8% in Brazil[46].

The modified Centor score recommends that children with scores of 1 need no further assessment while those with scores of 3 and 4 require cultures to guide therapy while those with a score of 4 can be treated with antibiotics empirically. The results in this present study indicate that a score of 4 is indeed highly specific but has a low sensitivity. The area under the ROC curve was 0.50 indicating that the Centor score is a poor discriminator between children who have GABHS pharyngitis and those who do not. This would act as a major draw back to the use of the Centor score in an area like ours which has a high prevalence of rheumatic heart disease.

These results bring into question the prudence of reliance on clinical features wheather individually or as part of a predictive rule to make a diagnosis. Indeed Shaikh *et al.* in a systematic review of clinical predictive rule conclude that symptoms and signs, either individually or combined into prediction rules, cannot be used to definitively diagnose or rule out streptococcal pharyngitis[47]. It is our opinion that perhaps approaches that focus on the development of cheaper rapid antigen tests would form a better strategy for our environment were up to 70% of all childhood cardiac morbidity is attributable to rheumatic heart disease.

The isolates were stored for further analysis of the emm type as this could not be done in this initial analysis due to limited funding. If these are found to be significantly different from those reported from temperate climates, the data will

provide justification for a larger study to more clearly investigate the epidemiology of GABHS in the general population. Since childhood pharyngitis acts as a reservoir for strains with invasive potential this study will also provide information on what proportion of the serotypes are included in the potential vaccines that are currently under development. Other areas for further study include a similar cross sectional study at primary care centres with a much larger sample size to try and elucidate the sensitivity of the aforementioned criteria.

CHAPTER SIX

6.0 CONCLUSION

We conclude that the prevalence of GABHS in children presenting with pharyngitis to the University Teaching Hospital is 15.2% and that the gold standard for diagnosis remains throat culture . However in view of the general poor sensitivity of the assessed clinical predictive rules, strategies to make cheaper rapid antigen tests for GABHS should begin to be pursued particularly for high rheumatic disease endemic areas such as our own. The assessed clinical scoring systems have too low a sensitivity to be used safely in a high endemic rheumatic disease area to decide which children should receive antibiotic treatment. This study illustrates that while GABHS remains sensitive to penicillin the use of erythromycin is of questionable value for treatment of pharyngitis in a substantial proportion of the children because of the noted reduced susceptibility. Of note also is the presence of a high isolation rate of other non-GAS streptococci- almost 50%- which pose a risk of invasive disease. Therefore antibiotic use in most children presenting with pharyngitis in our setting, given this high prevalence of bacterial pharyngitis remains a sound clinical decision. An urgent need remains for better and bigger studies to assess and possibly even formulate highly sensitive clinical scoring systems for use in a high endemic rheumatic disease area such as ours.

6.1. STUDY LIMITATIONS

As this was a hospital based study with selection bias the data may have limited applicability to the general population. Additionally as this is only a cross sectional study it is difficult to know for certain whether the GABHS isolates represent true GABHS pharyngitis as opposed to simple bacterial carriage in those who had inter current viral pharyngitis. In addition Kappa statistics for inter observer agreement on the various signs and symptoms could not be carried out due to shortage of staff. This may have introduced some bias into the study.

6.2. RECOMMENDATIONS

- Penicillin should remain the drug of choice for the treatment of suspected bacterial pharyngitis.
- Locally generated predictive rules designed for use at primary care level should be formulated
- Strategies to intensify development of cheaper rapid antigen tests for the detection of *Streptococcus pyogenes* should be embarked on by the government.

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Appendix I: INFORMATION SHEET

Prevalence of group A- beta haemolytic streptococcal isolates in children presenting with acute pharyngitis in health institutions in Lusaka.

Why are we giving you this form?

We are giving you this form, so as to give you information about the named study and also to give you a chance to ask questions about this study. Then you can decide if you would like to take part in this study that is trying to find out how often the germ causes that sore throat is found in children with sore throat at the University Teaching Hospital (UTH).

2. Who is carrying out this study?

Dr. Mwaba Chisambo as part of specialist training at the University Of Zambia School Of Medicine.

3. Background Information

You are being asked to take part in the above mentioned study, were we would like to find out how often Group A beta haemolytic streptococcus (a germ) are found in the throat of children complaining of sore throat at UTH's out patient department . By participating in this study we will be able to get the information that may help in order to make relevant policies and interventions for this problem of sore throat in children. We believe this is very vital information to all of us and you would help by participating in this study.

4. What Happens In This Research Study?

You will be interviewed now and then your child will have a throat swab taken for tests. The information collected will be kept confidential.

5. Possible Problems

We believe that the processes being used will not be harmful to you and the **child** participating in this study although **collection of the throat swab will be uncomfortable and may make the child want to vomit**. However if we notice

anything peculiar to you or your child during or after information is collected, we will let you know and facilitate your (you and your child) seeking appropriate medical help **at the UTH paediatrics emergency room.**

6. Benefits

It is hoped that the study will help reduce over and under diagnosis as well as over or under treatment of sore throat in children that is caused by this germ.

7. Confidentiality

Your name will never be made public by the investigators. The medical record will be treated the same as all medical records at the health centres. A code number that makes it very difficult for anyone to identify you will identify the research information gathered during this study from you. All information will be stored in a secure place. Information from this study maybe used for research purposes and may be published; however, your name will not be made public by the investigators. It is possible that, after the study is over, we may want to look again at the laboratory and interview record data collected during this study to help us answer another question. If this happens, still your name will not be made public by the investigators.

8. Research Related Injury

In the event that a problem results from a study-related procedure, Dr Mwaba in LUSAKA should be notified (0n +260 977 369695) or contact the ethics committee of the university of Zambia (see contact details section), and you or your child will be facilitated to seek and receive appropriate medical care at the health facility.

9. Contact Details

Should you want further information about this study or your rights as a participant please use the details provided below.

Dr. Mwaba **Principle Investigator.** **University Teaching Hospital,** **Department of Paediatrics and Child Health.** **Lamya: 260-977 369 695** **Email:chisambomwaba@yahoo.co.uk**	**Dr. Nkandu** **Chair, Research Ethics Committee** **Ridgeway Campus,** **Post Box 50110,** **Lusaka,10101. Zambia.** **Lamya: +260-211-256067.** **Fax:+260-211-250753.** **Email:unzarec@zamtel.zm**

Appendix II: CONSENT FORM

Prevalence of group A- beta haemolytic streptococcal isolates in children presenting with acute pharyngitis in health institutions in Lusaka.

Participant

I_____ (participant's parent or guardian's name, signature or thumb-print) have been informed about the study .I volunteer to have my child and I participate in the study. A copy of this form signed by me and one of the study investigators is being given to me.

Signature/Thumb_____

Date (D/M/Y) _____

Interviewer

I have explained this research study to the subject. I am available to answer any questions now or in the future regarding the study and the subject's rights.

Signature of Investigators & Printed Names Date of signature

Signature_____

Date (D/M/Y) _____

APPENDIX III: ASSENT INFORMATION SHEET

Prevalence of group A- beta haemolytic streptococcal isolates in children presenting with acute pharyngitis in health institutions in Lusaka.

1. Why are we giving you this form?

We are giving you this form, so as to give you information about the named study and also to give you a chance to ask questions about this study. Then you can decide if you would like to take part in this study that is trying to find out how often the germ that causes sore throat is found in children with sore throat at the University Teaching Hospital (UTH).

2. Who is carrying out this study?

Dr. Mwaba Chisambo who is training to become a children's doctor

3. Background Information

Sore throat is a very common illness in children your age. This is a study were we are trying to see the commonest germ that causes sore throat. The doctor will talk to you and your guardian then she will examine you. The doctor will then collect a swab from your throat. This will be a little uncomfortable and may make you feel like vomiting. In addition the doctor will collect 3 ml of blood. This will be a little painful.

The importance of you taking part in the study is that you will assist the doctor to try and come up with information that may be useful in helping to decide better ways of treating children with sore throat.

APPENDIX IV: ASSENT FORM

Prevalence of group A- beta haemolytic streptococcal isolates in children presenting with acute pharyngitis in health institutions in Lusaka.

Participant

I_____ (participant's name, signature or thumb-print) have been informed about the study .I volunteer participate in the study. A copy of this form signed by me and one of the study investigators is being given to me.

Signature/Thumb_____

Date (D/M/Y) _____

Interviewer

I have explained this research study to the subject. I am available to answer any questions now or in the future regarding the study and the subject's rights.

Signature of Investigators & Printed Names Date of signature

Signature_____

Date (D/M/Y) _____

APPENDIX IV: HISTORY DATA SHEET

Identification :

Name of participant :

Initials of participant :

Subject study number:

Part I: Demographics

Age (completed year)

Sex 1) Male 2) Female

Number of people in the home

Number of rooms in the house

Part II: Symptoms of sore throat

2.1 Painful throat 1) Yes 2) No

2.2 Headache 1) Yes 2) No

2.3 Abdominal pains 1) Yes 2) no

2.3 Antibiotics last 2 weeks 1) Yes 2) no

Part III: Past medical history

3.1 cardiac disease 1) Yes 2) No

APPENDIX V: PHYSICAL EXAMINATION DATA SHEET

Date :

Subject full name :

Subject initials :

Subject study number:

General appearance: 1) Well 2) Ill

Vitals

Pulse:

Respiratory rate:

Temp:

Anthropometry

Weight

Height

HEENT

Enlarged tonsils 1) Yes 2) No

Inflamed tonsils 1) Yes 2) No

Inflamed pharynx 1) Yes 2) No

Tonsillopharyngeal exudates 1) Yes 2) No

Runny nose 1) Yes 2) No

Injected conjunctiva 1) Yes 2) No

Lymphoglandular system

Cervical lymphadenopathy 1) Yes 2) No

If enlarged, tender? 1) Yes 2) No

Submandibular nodes? 1) Yes 2) No

Other lymph node groups

Chest

Abnormal sounds 1) Yes 2) no

CVS

Cardiac murmur 1) yes 2) no

If murmur, characterise . . . . . .

Abdomen

Hepatomegaly 1) yes 2) No

Slenomegaly 1) Yes 2) No

Musculoskeletal system

Joint tenderness/swelling 1) Yes 2) No

If yes, characterise . . . . . .

Integumentary system

Skin rash 1) yes 2) No

If yes, characterise . . . . . .

Central nervous system

Abnormality 1) yes 2) No

Treatment given, if any . . . . . .

Referrals . . . . . .

Clinician name signature

APPENDIX VI: LABORATORY DATA SHEET

Subject study number

Subject initials

Site

Date specimen collected

1.0 Growth of beta haemolytic colonies 1) Yes 2) No

Gram reaction	Positive		Negative	
Shape	cocci		Bacilli	
Arrangement	chain		Cluster	
Catalase	positive		Negative	
Bacitracin	sensitive		Resistant	
Group A				
Group B				
Group C				
Group G				

2.0 emm- sequence . . . . . .

3.0 emm-type of the isolate . . . . . .

4.0 Antibiotic sensitivity . . . .

Signature