PREVALENCE AND RISK FACTORS OF STREPTOCOCCUS PYOGENES INFECTION AMONG CHILDREN AGED 0-5 YEARS IN SOME HOSPITALS IN ZARIA, KADUNA STATE, NIGERIA.

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BY

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DEPARTMENT OF MICROBIOLOGY,

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AHMADU BELLO UNIVERSITY

ZARIA, NIGERIA.

DECEMBER, 2016.

DECLARATION

I hereby declare that the work in this dissertation entitled "PREVALENCE AND RISK FACTORS OF STREPTOCOCCUS PYOGENES INFECTION AMONG CHILDREN AGED 0-5 YEARS IN SOME HOSPITALS IN ZARIA, KADUNA STATE, NIGERIA" was carried out by me in the Department of Microbiology, Ahmadu Bello University, Kaduna State, Nigeria. Under the supervision of Prof. H.I. INABO and Prof. E.D. JATAU. The information derived from literature has been duly acknowledged in the text and a list of references provided. No part of this work has been presented for another degree or diploma at this or any other Institution.

Grace Bunmi OLAFEMI

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Name of Student Signature Date

CERTIFICATION

This project dissertation entitled "PREVALENCE AND RISK FACTORS OF STREPTOCOCC US PYOGENES INFECTION AMONG CHILDREN AGED 0-5 YEARS IN SOME HOSPITALS IN ZARIA, KADUNA STATE, NIGERIA" by GRACE BUNMI OLAFEMI meets the regulations governing the award of the degree of Master of Science, of Ahmadu Bello University, and is approved for its contribution to knowledge and literacy presentation.

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DEDICATION

This work is dedicated to God Almighty who has been the pillar of my life; my helper, sufficiency and source of strength.

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ABSTRACT

Group A beta-haemolytic streptococcal infections have a worldwide distribution among children and it poses an important health problem globally. The study therefore, was aimed at determining the prevalence and risk factors of *Streptococcus pyogenes* infection among children aged 0-5 years in the study area. A total of three hundred samples were collected from children aged 0-5 years who attended three different hospitals namely: Gambo Sawaba, Sickbay and Health Care Center for Children and Women. One hundred and fifty throat swab samples and one hundred and fifty blood samples were collected for *Streptococcus pyogenes* isolation, characterization using cultural method and classified using the Lancefield identification kit as well as the use of immunological assay such as Anti-streptolysin O (ASO) for further diagnosis and confirmation. The findings showed that the prevalence of group A *Streptococcus pyogenes* was 6.7% using the Lancefield identification kit. Other groups isolated were groups C, D and F with prevalence of 1.3%, 9.3% and 2.7% respectively. The highest prevalence of *Streptococcus pyogenes* infection was observed in the age group 0-1 years (11.5%) while the lowest was in the age group 2-3 years (1.9%).There was no significant difference in the prevalence of the infection and age groups (chi square =3.7439, p= 0.1538).The prevalence of *Streptococcus pyogenes* infection was higher in males (8.9%) than females (4.2%).There was no significant difference in the level of infection in both sexes (chi square = 1.2198, p= 0.2694). Erythromycin was found to be the most effective antibiotics against *Streptococcus pyogenes* from this study. A prevalence of 14.7% was recorded for ASO in sera samples. The level of ASO in patients that tested positive for the presence of ASO ranged from 200IU/ml to 16,000IU/ml. GS21 and GS7 showed the highest concentration of 16,000IU/ml respectively with a mean of 1772IU/ml. Two individuals were recorded to be both positive for *Streptococcus pyogenes* from throat swab samples as well as positive for Anti-streptolysin O antibody (ASO) from sera samples. The findings showed that children and parents who practiced good hygiene, cultured less *Streptococcus pyogenes* as well as those who had good ventilation in school, at home and those who were previously exposed to the infection and completed antibiotics treatment. The findings showed the effectiveness and sensitivity of the use of immunological assay (ASO) for diagnosis of *Streptococcus pyogenes* infection alongside the use of cultural method.

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LIST OF ABBREVIATIONS AND SYMBOLS

ASO- Antistreptolysin O antibody

SLO- Streptolysin O

SLS- Streptolysin S

CLSI- Clinical Laboratory Standard Institute

CDC- Centers for Disease Control

NPC- National Population Commission

FMH- Federal Ministry of Health

GAS- Group A *Streptococcus*

NS- Negative *Streptococcus pyogenes*

NA- Negative Antistreptolysin O antibody

S- *Streptococcus pyogenes* infection

GS- Gambo Sawaba Hospital

SC- Sickbay Clinic

HCC- Health Care Center for Children and Women

$\sum$ - Total number of samples collected

df- Degree of freedom

α- Alpha

γ - Gamma

β- Beta

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Streptococcus pyogenes is a spherical, Gram-positive, non-motile, non-sporing bacterium that occurs as long chains of cocci and occasionally in pairs. It is less than 2µm in length and forms colonies greater than 0.5mm in size (Murray *et al.*, 2007). It is catalase-negative and has been classified in group A, using Lancefield serotyping because it displays antigen A on its cell wall. Therefore, this bacterium is commonly called the group A (beta-haemolytic) *Streptococcus* (GABHS or GAS) (Todar, 2002). This pathogen is classified as group A *Streptococcus*, typically having a capsule composed of hyaluronic acid and are beta-haemolytic which is true for *Streptococcus pyogenes* (Todar, 2005).

Beta-haemolytic streptococci produce a toxin that forms a clear zone of haemolysis on blood agar, demonstrating its ability to destroy red blood cells. This haemolysis is attributed to toxins formed by group A streptococci called "Streptolysins" which can destroy not only the red blood cells but also the white blood cells responsible for destroying pathogens (Tortora *et al.*, 2007). This bacterium is the causative agent for bacterial pharyngitis, tonsillitis and a wide range of both invasive and non-invasive infections. The name is derived from the Greek word "streptos", meaning 'twisted chain' due to the fact that the bacterium resembles a string of small pearls when viewed under the microscope.

Infection of group A *Streptococcus* may spread through direct contact with mucus or sores on the skin (CDC, 2012). A sore throat infection is often a symptom of a bacterial or viral infection.

This bacterium causes over 50,000 deaths per year (Cohen-Paradosu and Kasper 2007). Despite the emergence of antibiotics as treatment for group A beta-haemolytic *Streptococcus* infection, this bacterium has become an increasing problem, particularly in the continent of Africa (Carapetis *et al.*, 2005). Usually persons with suppressed or compromised immune system may be susceptible to certain diseases caused by group A *Streptococcus* than other persons who are immuno-competent. Group A *Streptococcus* may be present on the skin or in the throat of people and show no symptoms, known as carriers. Non-invasive infections occur when the bacteria colonise the throat area, where they colonise epithelial cells (CDC, 2012).

The two most important infections of group A streptococci are invasive and non-invasive infections. Non-invasive infection includes: pharyngitis where it causes 15-30% of childhood cases and 10% of adult cases including scarlet fever (Cohen-Paradosu and Kasper 2007). The invasive infections caused by group A beta-haemolytic *Streptococcus* tend to be more severe. This occurs when the bacterium is able to infect areas where bacteria are not usually found, such as blood and organs, as a result of haematogenous spread of the organism.

Group A streptococci may lead to further complications and health conditions such as: rheumatic fever, streptococcal toxic shock syndrome (STSS), necrotizing fasciitis (NF), Post-streptococcal glomerulonephritis (Cohen-Paradosu and Kasper 2007). All severe group A streptococci infections may lead to shock, multi-system organ failures and death. Early recognition and treatment is critical (Dwyer, 2012).

There are currently 74 species under the genus *Streptococcus* while *Streptococcus pyogenes* is one of the most virulent species causing human infections (Euzeby, 2012). *Streptococcus pyogenes* is one of the most common pathogens found worldwide and also the most pathogenic

species in the genus *Streptococcus*. This bacterium can rapidly colonise and multiply within a host, causing acute infections such as "Strep throat" and impetigo to the severe necrotizing fasciitis "flesh eating" and Streptococcal toxic shock syndrome (Todar, 2002).

The pathogenesis of *Streptococcus pyogenes* infection usually begins in the throat and on the surface of the skin. From there, the bacterium begins to spread into deeper areas of the skin which can potentially lead to life-threatening diseases (Facklam, 2002). This bacterium can be found as a commensal in the upper respiratory tract, particularly in children (Cheesbrough, 2010). The main site of colonization is the oro-pharyngeal mucosa but other locations such as gastrointestinal tract and lower female genital tract can also be colonized. Person-to-person transmission involves respiratory droplets and direct contact.

Acute respiratory infections cause four and a half million deaths among children per annum, especially those in the developing countries (Berman, 1991). Acute respiratory tract infection remains an important cause of childhood morbidity and mortality in developing countries though this infection is potentially treatable and preventable (Gbadegesin *et al.*, 1997).

Transmission of streptococcal infection is through the respiratory tract by inhalation of droplets, hand contact with nasal discharge and direct contact with lesions. In the last century, infections by *Streptococcus pyogenes* claimed many lives especially, since the organism was the most important cause of puerperal fever (Sepsis after child birth) (Todar, 2005).

The type of haemolytic reaction displayed on blood agar has long been used to classify the streptococci. Beta-haemolysis is associated with 'complete lysis' of red cells surrounding the colony, while alpha-haemolysis represents 'partial' or 'green' haemolysis associated with

reduction of blood haemoglobin. Non-haemolytic colonies have been termed gamma-haemolytic (Todar, 2005).

1.2 Statement of the Research Problem

There are at least 517,000 deaths reported globally each year (Carapetis *et al*., 2005). These deaths have been attributed to severe *Streptococcus pyogenes* infection and its prevalence increasing to 30-80%, with at least 18.1 million cases each year and 1.78 million new cases each year. Rheumatic fever alone causes 233,000 deaths (Carapetis *et al*., 2005). There are about 1,800 invasive *Streptococcus pyogenes* related deaths in the USA yearly with necrotizing fever killing 30% of patients and Streptococcal toxic shock syndrome with a mortality rate of 30%-70% (Stevens, 1995; Murray *et al.*, 2007; Torralba and Quismorio, 2009).

It was reported that 15-20% of school aged children has *Streptococcus pyogenes* in its carrier form in their throat and are more at risk of having the disease (Vincent *et al.*, 2004; Bessen, 2009). If untreated, patients with streptococcal pharyngitis are infective during the acute phase of illness usually 7-10 days (Vincent *et al.*, 2004). As of 2007, there were at least 18.1 million cases of invasive infections which are predominant in older population (Murray *et al.*, 2007).

Streptococcus pyogenes can remain in the body of its carrier (humans) without causing illness in the host, for weeks or months and remains transmissible in this state (Bessen, 2009).

1.3 Justification for the Study

Streptococcus pyogenes is responsible for a wide range of both invasive and non-invasive infections (CDC, 2012). Despite the availability of effective chemotherapy, this pathogen is still a major health problem in most countries of the world (Bernaldo *et al.*, 1997).

There are 616 million cases of pharyngitis attributed to *Streptococcus pyogenes* world wide each year (Bessen, 2009; Carapetis *et al.*, 2005). Group A *Streptococcus* (GAS) infection remains a major public health problem in developing countries, constituting an important cause of morbidity and mortality.

Streptococcus pyogenes infection is very contagious which can be transmitted by breathing in air with droplet containing streptococci when an infected person coughs, sneezes, touches the nose and throat secretions of an infected person. The incidence of the infection was reported to be higher in children due to poor hygiene, overcrowding and improper development of innate immune system (Cunningham, 2008). Antibiotic resistance can develop as a result of therapeutic exposure to antibiotics. Despite the emergence of antibiotics as a treatment for *Streptococcus* infection, it has become an increasing problem particularly in the continent of Africa (Carapetis *et al.*, 2005).

In a research work carried out by Mawak *et al*.(2005) on bacterial aetiologic agents associated with upper respiratory tract infections in children attending some selected hospitals in Jos, Nigeria, *Streptococcus pyogenes* was identified from the upper respiratory tract of children with a prevalence rate of 10.45%.

Acute respiratory tract infection remains the important cause of childhood morbidity and mortality in developing countries. Despite this, the infection is potentially treatable and preventable (Gbadegesin *et al.*, 1997). The infection is attributed to abuse of antibiotics prescribed even after the disappearance of symptoms to prevent further complications. The infection can also be contracted during breastfeeding of a child because *Streptococcus pyogenes* can be found on the skin (mammary gland). Hence, the need to study the prevalence rate of this infection in Zaria, cannot be overemphasized.

1.4 Aim of the Study

The aim of this study was to determine the prevalence and risk factors of *Streptococcus pyogenes* infection among children aged 0-5 years in some hospitals in Zaria, Kaduna State, Nigeria.

1.5 Objectives were to:

1. Determine the socio-demographic and risk factors associated with *Streptococcus pyogenes* infection in the study population using structured questionnaire.
2. Isolate, characterize and determine the prevalence of *Streptococcus pyogenes* from throat swab samples of children aged 0-5 years.
3. Confirm the presence of *Streptococcus pyogenes* using Lancefield grouping test kit.
4. Determine the antibiogram of the *Streptococcus pyogenes* isolates.
5. Screen for the presence of antibody to *Streptococcus pyogenes* in the blood samples collected using Anti-Streptolysin O (ASO) titer test kit.

1.6 Research Hypothesis

H_O: *Streptococcus* pyogenes infection is not prevalent among children aged 0-5 years attending some hospitals in Zaria, Kaduna State.

H_A: *Streptococcus pyogenes* infection is prevalent among children aged 0-5 years attending some hospitals in Zaria, Kaduna State.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 General description of *Streptococcus pyogenes*

Streptococcus pyogenes or Group A *Streptococcus* (GAS) is a Gram positive coccus, which grows in chains and occasionally in pairs. Its causes numerous infections in humans including: pharyngitis, tonsillitis, scarlet-fever, cellulitis, rheumatic-fever, post-streptococcal glomerulonephritis, necrotizing fasciitis, lymphangitis (Prescott *et al.,* 2011). .

The only known reservoirs for GAS in nature are the skin and mucus membranes of the human host. Group A *Streptococcus* requires complex media containing blood products, grow best in an environment of 10% carbondioxide and produce pin point colonies on blood agar plates which are surrounded by a zone of complete beta-haemolysis. The exhaustive work of Rebecca Lancefield established the classification of streptococci into types A through O based upon acid extractable carbohydrate antigens of cell wall material (Lancefield, 1938).

Group A *Streptococcus* can be subdivided into over 100 serotypes by the M-protein antigen that is located on the cell surface and by fimbriae (hair like fuzz) that project from the outer edge of the cell (Beall *et al.,* 1996). A molecular approach has been adopted, using the polymerase chain reaction and DNA sequencing of the emm gene encoding the M-protein. More than 130 distinct M-genotypes have been identified using emm typing (Steer *et al.,* 2009). Group A *Streptococcus* produce and release into the surrounding medium a large number of biologically active extracellular products. Some of these are toxic to humans and other mammalian cells. *Streptococcus pyogenes* has several virulence factors that enable it to attach to host tissues

successfully, evade immune response and spread by penetrating host tissue layers (Patterson, 1996).

In 2005, there was a research report that showed that group A *Streptococcus* actually have pili which are important virulent structural factors which they use to attach to the target cells (Mora *et al.,* 2005). Adherence to target cells prevents the organism from being easily removed by mucus or salivary fluid (Cunningham, 2000). A carbohydrate-based bacterial capsule composed of hyaluronic acid surrounds the bacterium, protecting it from phagocytosis by neutrophils (Ryan and Ray, 2004). In addition, the capsule and several factors embedded in the cell wall, including M-protein, Lipoteichoic acid and protein F facilitates attachment to various host cells (Binso *et al.,* 2003). M-proteins inhibits opsonization by binding to fibrinogen (Ryan and Ray, 2004). M-proteins are unique to each strain and identification can be used clinically to confirm the strain causing an infection.

Streptococcus pyogenes uses lipoteichoic acid for adherence, hyaluronic acid capsule as an immunological disguise and to inhibit phagocytosis, invasins such as streptokinase, Streptodornase (DNase B), hyaluronidase and streptolysins as virulence factors. They also produce exotoxins such as pyrogenic-toxin which causes the rash of scarlet fever and Streptococcal toxic shock syndrome (Todar, 2002). Some of the extracellular enzymes and toxins produced by strains of *Streptococcus pyogenes* include:

1. Streptokinase: a protease that lyzes fibrin.

2. Hyaluronidase: facilitates spread in the tissue by destroying hyaluronic acid.

3. Erythrogenic toxin: responsible for rash in scarlet fever and Streptococcal toxic shock syndrome.

4. Leukocidin: destroys leucocytes.

5. Streptolysin: toxins that haemolyze red cells.

6. NADase (Nicotinamide adenine dinucleotidase): kills leucoytes.

7. DNases (Deoxyribonuclease): breaks down DNA and stimulate an antibody response particularly against DNase B (Cheesbrough, 2010).

Streptococcus pyogenes is known to be an opportunistic organism that causes an infection when the person's natural defenses to the disease is compromised. *Streptococcus pyogenes* is somewhat unique in that it does not cause just one disease, but it is capable of causing many different diseases (Duckworth, 2006). *Streptococcus pyogenes* is the most bacterial cause of sore throat, a painful red throat with white patches on tonsil which is characteristics of pharyngitis otherwise known as Strep throat. Pharyngitis is the inflammation of the part of the throat behind the pharynx. It is an infection of the Pharynx by group A *Streptococcus pyogenes* which produces sore throat which may be associated with tonsillitis. Tonsillitis is the inflammation of the tonsils due to bacterial or viral infection which stimulates an inflammatory response and the lysis of white and red blood cells, causing discomfort, fever and headache (Prescott *et al.,* 2011).

Streptococcus pyogenes when left untreated can lead to scarlet fever, or rheumatic fever. Scarlet fever is characterized by body temperatures above 38^{o}C with a red or pinkish rash on the skin of the patient that can spread to other parts of the body. The rash is caused by exotoxins produced

by *Streptococcus pyogenes* which has a sand paper like structure and appears as tiny red pin points (NIAID, 2005).

The Center for Disease Control recommends that persons with streptococcal infections stay home from work, school, day-care until they have been taking antibiotics for at least 24 hours to reduce the risk of transmitting the infection (CDC, 2005).

Streptolysin S (SLS) is a small oxygen-stable toxin responsible for beta-heamolysis of group A *Streptococcus* on blood agar while Streptolysin O (SLO) is an oxygen-labile cholesterol dependent toxin. Both SLS and SLO injure cell membranes and not only lysing red blood cells.

Streptolysin O is antigenic while Streptolysin S is not. Group A streptococci produce bacteriocin, low molecular weight proteins that can kill a varieties of other Gram positive bacteria and may play a role in promoting infection or even persistent colonization (Tagg *et al.*, 1976).

Streptococcus pyogenes usually colonizes and initiates an infection on the surface of the skin or in the throat, from there the bacterium begins to spread into deeper areas of the skin, which can potentially lead to life threatening diseases (Facklam, 2002).

Streptococcus pyogenes initiates infection by adhesion of bacterial organism to human epithelial cells, including those in the oral, nasal cavities and skin. Bacterial pathogens express various molecules that are anchored in the cell wall as fimbrial- like structures. Several surface fimbrial–like protein also bind to human extra cellular matrix proteins, which includes, fibronectin (Sela *et al.,* 2003), Laminin and collagen (Podbielski *et al.,* 1999). The fibronectin proteins of *Streptococcus pyogenes* were identified and characterized between 1993 and 2002.

The fibronectin proteins have been reported to be adhesion factors and invasion factors (Talay *et al.,* 1994).

The genome of an M-1 strain of *Streptococcus pyogenes* has been sequenced and was found to contain 1,852,442, base pairs and about 1,752 predicted protein encoding genes (Ferretti, 2001). Also, more than 40 putative virulence associated genes have been identified, and this could explain why than any other bacterial species (Ferretti, 2001).

2.2 Epidemiology

Group A beta-heamolytic *Streptococcus* is a common cause of a wide variety of infections in infants**,** children and adults. This infection has long been associated with serious morbidity and mortality, but towards the middle of the 20th century, a marked decline in the incidence and severity of such infections occurred. However, over the past 15 years, there has been a resurgence in the incidence of severe invasive group A streptococcal infections (Stevens, 1994). These include: necrotizing fasciitis, myositis, Streptococcal toxic shock syndrome and Streptococcal bacteraemia.

This resurgence has been partly attributed to a change in the virulence of the organism and a change in the epidemiology of group A *Streptococcus* (Stevens, 1999). Some have suggested that changes in the susceptibility of group A streptococci to commonly used antibiotics may have contributed as well (Martin *et al.,* 2002).

An estimated 700 million infections occur worldwide each year, while the overall mortality rate for this infection is 0.1%, over 650,000 of the cases are severe and invasive and have a mortality rate of 25% (Aziz *et al.,* 2010). In 1928, Rebecca Lancefield published a method for serotyping

Streptococcus pyogenes based on its M-protein, a virulence factor displayed on its surface (Lancefield, 1928). Later in 1946, Lancefield describe the serologic classification of *Streptococcus pyogenes* isolates based on their surface T-antigens (Lancefield and Dole 1946). Four of the 20 T-antigens have been revealed to be pili, which are used by bacteria to attach to host cells (Mora *et al.*, 2005).

Group A streptococci are highly communicable and can cause disease in individuals of all ages (Wannamaker, 1970). Severe invasive group A streptococci infections had become uncommon in the U.S during the second half of the 20^{th} century, however, since the late 1980s, there has been a worldwide increase in severe invasive group A streptococci infection (Stevens, 1996).

It has been estimated globally that at least 663,000 cases of invasive group A Streptococci diseases occur each year resulting in 163,000 deaths (Carapetis *et al.*, 2005). Group A streptococci can gain access to sterile site via direct inoculation following an injury that breaches the mucous membrane or skin (Olsen *et al.*, 2009). Epidemiologic data suggests that the oropharynx is the primary site of origin for systemic group A streptoccoci isolates, thus invasive disease is likely to occur as a result of transient blood stream infection originating from the oropharynx, possibly as a result of direct tissue penetration by group A streptococci (Johansson *et al.*, 2010).

Important risk factors of severe invasive group A streptococci includes: Varicella, Diabetes mellitus, Human immunodeficiency virus infection, Intra venous drug use, and chronic pulmonary or cardiac disease. Acute rheumatic fever still remains a major public health burden in most of the developing world. There are currently at least 15.6 million people worldwide with it. There are about 282,000 new cases and 233,000 deaths annually directly attributed to acute

rheumatic fever (Carapetis *et al.*, 2005). The incidence of acute rheumatic fever in some developing countries exceeds 50 per 100,000 children.

In developing countries, pharyngo- tonsillitis caused by beta-heamolytic *Streptococcus pyogenes* remains an endemic disease with annual incidence ranging from 100-200 per 10,000 school children and it is a major cause of cardiovascular mortality. Group A streptococci are the most common bacterial cause of acute pharyngitis accounting for approximately 15-30 % of cases in children and 5-10% of cases in adults (Binso, 2001). Certain M-protein serotypes, such as M-types 1,3,5,6,14,18,19 and 24 of group A streptococci are found associated with throat and rheumatic fever (Stollerman, 1997; Mandor *et al.*, 2013).

Several epidemiological studies have been carried out on group A *Streptococcus pyogenes* infection of such studies are: A research carried out on invasive infections due to *Streptocooccus pyogenes*, seasonal variation of severity and clinical characteristics in Island, 1975-2012, gave a total of 288 cases of positive culture including children and adult with certain risk factors for invasive infections such as: Immunosuppression, diabetes mellitus, loss of skin intergrity (Aebi *et al.*, 1996). Likewise, in a research, carried out in Zaria, Nigeria by Ella and Okafor,(2015) on: "Anti-streptolysin O" titer in comparison to positive blood culture in determining the prevalence of group A streptococcal infections in selected patients (12-20) years in Zaria, Nigeria", out of the 100 samples observed, 16% were found to be positive (male and female) to group A *Streptococcus pyogenes*.

In a research reported in India by Nirmal *et al.*(2014), prevalence of group A streptococci infections among school children of urban community was 30.7% and attributed to group A beta-

haemolytic *Streptococcus pyogenes* amongst the 300 samples collected, which was found majorly in children aged 5-10 years belonging to nuclear families.

Mawak *et al.*(2005), observed that *Streptococcus* species had the highest frequency among bacterial agents associated with upper respiratory tract infections among hospitalized children in Jos.

Acute respiratory tract infection remains an important cause of childhood morbidity and mortality in developing countries, despite it is potentially preventable and treatable.

Acute respiratory tract infection causes four and half million deaths among children, especially those in the developing countries. Infections of the upper respiratory tract are the most common cause of illness in childhood, accounting for about 50% of all illness in children younger than 5 years of age.

2.2.1 Classification of *Streptococcus pyogenes*

Rebecca Lancefield in 1928, published a method for serotyping *Streptococcus pyogenes* based on its M-protein, a virulence factor displayed on its surface. Later, in 1946, Lancefield described the serologic classification of *Streptococcus pyogenes* isolated, based on their surface T antigen (Lancefield and Dole 1946). Lancefield grouping is a method of grouping beta-haemolytic bacteria based on the carbohydrate composition on bacterial antigens found on their cell wall. It is the only member of Lancefield group A Streptococci. It is the most pathogenic species and it is present as a commensal in the throat of less than 10% of people mostly children. It produces a large number of enzymes and toxins. Lancefield grouping test is an agglutination immunological

assay that detects carbohydrate in the cell wall of Streptococci. Lancefield grouping of beta-haemolytic *Streptococcus* includes:

Group A:	*Streptococcus pyogenes*
Group B:	*Streptococcus agalactia*
Group C:	*Streptococcus equisimilis*
	Streptococcais equi
	Streptococcus zooepidemicus
	Streptococcus dysgalactiae
Group D:	*Streptococcus bovis,*
	Enterococci
Group E:	*Streptococcus milleri*
	Streptococcus mutans
Group F:	*Streptococcus anginosus*
Group G:	*Streptococcus canis*
	Streptococcus dysgalactiae
Group H:	*Streptococcus sanguinis*
Group L:	*Streptococcus dysgalactiae*

Group N: *Lactococcus lactis*

Group R and S: *Streptococcus suis*

Other *Streptococcus* species are classified as non-Lancefield Streptococci (Lancefield, 1933). *Streptococcus pyogenes* has an incubation period of about 1-3days (Vincent *et al.,* 2004). If untreated, patients with Streptococcal pharyngitis are infective during the acute phase of the illness, usually 7-10 days. Antibiotics usage reduces the infective period of the bacteria to 24 hours (Vincent *et al.,* 2004). The bacterium can remain in the body of its carrier state without causing illness in the host for weeks or months and it is transmissible in this state (Bessen, 2009). Humans are primary reservoir for this bacterium (Bessen, 2009).

2.3 Clinical Manifestations

Symptoms of *Streptococcus pyogenes* throat infection typically appear several days after exposure to the bacteria. The most common symptoms of *Streptococcus pyogenes* infection are: sore throat, difficulty in swallowing food/water, swollen tonsils, headache, fever, stomach-ache, fatique, loss of appetite, vomiting, white rash may develop on the tonsils or the throat may have stringy pus (Duckworth, 2006).

2.3.1 Scarlet fever

It is characterized by a red rash on the chest that may spread to the rest of the body. The rash has a sandpaper-like texture and appears as tiny red pinpoints (NIAID, 2005). It is caused by exotoxins produced by *Streptococcus pyogenes.*

2.3.2 Impetigo

It starts as a red sore and form crusty sore after a few days. The sore most commonly occur on the face, but can also be found on the extremities. They usually itch, but are extremely contagious through direct contact, so scratching the sores could spread the infection throughout the body (NIAID, 2005).

2.3.3 Cellulitus

It is inflammation of the skin and deep tissues. Cellulitis begins when *Streptococcus pyogenes* infects the site of a minor injury such as a bruise, burn, or wound. It causes the skin to turn red, widespread pain, swelling and tenderness of the infected area. Other symptoms includes: fever, chills, swollen glands, blistering of the skin and malaise (Sharma, 2006).

2.3.4 Necrotizing fasciitis

It is a very serious infection caused by *Streptococcus pyogenes* that is popularly termed "flesh eating bacteria". The bacteria typically enters the body through a minor trauma or surgical wound in persons of compromised immune systems and causes infection just below the skin that spreads to deeper tissues. Necrotizing fasciitis is an extremely quick moving infection that is characterized by the rapid destruction of tissue. It is fatal in 30-40% of cases (Sharma, 2006).

2.3.5 Streptococcal toxic shock syndrome

It is a serious disease, following an initial infection of *Streptococcus pyogenes*. Symptoms includes: significant pain, swelling and redness of infected area, dizziness, difficulty in breathing, low blood pressure (Sharma, 2006).

2.3.6 Glomerulonephritis

It is also called Bright's disease. It is an inflammatory disease of the renal glomeruli (membranous structures within the kidney where blood is filtered). Clinically, the affected person exhibits edema, fever, hypertension and haematuria (blood in the urine) (Prescott *et al.*, 2011).

2.3.7 Rheumatic fever

It is characterized by inflammatory lesions involving the heart valves, joints, subcutaneous tissues and central nervous system. Clinically, the affected person exhibits difficulty in breathing, joint pains, fever, loss of appetite (Prescott *et al.*, 2011).

2.4 Mode of Transmission

Transmission is via respiratory droplets, hand contact with nasal discharge, skin contact with nasal discharge and skin contact with impetigo lesions are the most important modes of transmission (Vincent, 2004). The pathogen can be found in its carrier state in the anus, vagina, skin and pharynx and contact with these surfaces can spread the infection (Bessen, 2009).

2.5 Pathogenesis

The first step in group A streptococcal disease involves successful colonization of the upper respiratory mucosa or skin of human host. A large number of adherence for epithelial cells have been described including: lipoteichoic acid, M-protein, pili and fibronectin-binding proteins (Binso *et al.*, 2003).

Group A *Streptococcus* biofilm formation facilitates persistence within the human host (Doern *et al.,* 2009). *Streptococcus pyogenes* has several virulence factors that enable it to attach to host tissues, evade immune response and spread by penetrating the host (Patterson, 1996). It has a protein called protein F, which is a fibronectin binding protein that allows it to adhere to respiratory epithelial cells (Hanski *et al.,* 1992). This protein is an important virulence factor because by binding to the epithelial cells, the organism is able to stick to the cells of the host tightly and not leave.

Both M-protein and fibronectin- binding proteins are important for subsequent endocytotic uptake of group A *Streptococcus* into respiratory epithelial cells (Binso *et al.,* 2003). The capsule and several factors embedded in the cell wall, including M-protein, lipoteichoic acid and protein F, facilitates attachment to various host cells (Binso *et al.,* 2003). The M protein found on some serotypes is able to prevent opsonization by binding to fibrinogen (Ryan and Ray 2004). M-proteins are unique to each strain and identification can be used clinically to confirm the strain causing an infection. Hyaluronic acid capsule is vital in order for it to survive in its host and also acts as an important adherence factor in the pharynx, since it binds CD44 on epithelial cells (Schrageri *et al.,* 1998).

Infections due to certain strains of *Streptococcus pyogenes* can be associated with the release of bacterial toxins. Throat infections associated with the release of certain toxins lead to scarlet fever, while other toxigenic *Streptococcus pyogenes* infections may lead to streptococcal toxic shock syndrome, which can be life-threatening (Ryan and Ray 2004).

Streptococcus pyogenes uses lipoteichoic acid for adherence, hyaluronic acid capsule as an immunological disguise and to inhibit phagocytosis. They also produce invasins such as streptokinase, streptodornase, hyaluronidase and streptolysins as virulence factors (Todar, 2002).

A carbohydrate-based bacterial capsule composed of hyaluronic acid surrounds the bacterium, protecting it from phagocytosis by neutrophils (Ryan and Ray 2004). It produces bacteriocins, low molecular weight proteins that can kill a variety of other Gram-positive bacteria, which play a role in promoting infection and persistence of colonization (Tagg *et al.*, 1976). This process of intracellular invasion allows group A *Streptococcus* access to a priviledged intracelleular niche and aids in the pathogenesis of systemic infection (Lapenta *et al.*, 1994). The propensity of group A *Streptococcus* to produce serious infection depends on the capacity of the pathogen to resist host innate clearance mechanism that normally functions to prevent microbial dissemination (Kwinn and Nizet 2007).

Group A *Streptococcus* gains access to deeper tissues through cellular invasion or a break in epithelial intergrity. It develops specific peptidases that cleave and inactivate the neutrophil chemo-attractants, interleukin-8 and complement factor 5a. Streptococcal pyrogenic exotoxin (Spe) B, can degrade host immunoglobulins and cationic antimicrobial peptidase (Kwinn and Nizet 2007).

M-protein collaborates with the group A *Streptococcus* virulence factor streptokinase to bind to host plasminogen to the group A *Streptococcus* surface, where upon plasmin activity is generated, effectively coating the bacterial surface with a powerful protease to facilitate tissue spread (Sun *et al.*, 2004).

The pore-forming toxins streptolysin S and streptolysin O are toxic to multiple host cell types including macrophages and neutrophils, thus promoting group A *Streptococcus* tissue damage and resistance to phagocytic clearance (Nizet, 2002).

Streptococcal pyrogenic exotoxin C are responsible for the rash of scarlet fever, stimulating formation of specific antitoxin antibodies that provide immunity against future scarlatiniform rashes, but not against group A *Streptococcus* infections (Alouf and Mueller, 2003).

The group A streptococcal strain producing scarlet fever does so because it carries the genes for one or more of the streptococcal pyrogenic exotoxins (Cunningham, 2000). The type specific antibody against M-protein is not usually detectable until 4-8 weeks after infection (Denny *et al.,* 1957), therefore its primary role is not in the termination of active infection, but rather in the prevention of reinfection by the same serologic type (Daikos and Weinstein, 1951).

2.6 Laboratory Diagnosis

The diagnosis of group A streptococci infection is usually conducted using the following methods:

2.6.1 Culture

Throat swabs are usually cultured on blood agar plates, to check for distinct colonies related to *Streptococcus pyogenes,* which still remains the gold standard. Pharyngeal exudates, blood, tissue or other body fluids can also be examined carefully in the laboratory for diagnosis. Pharyngeal culture is followed by a Gram-staining test to show Gram- positive cocci in chains (Kellogg *et al.,* 2001).

2.6.2 Rapid antigen detecting test (RADT)

This test have proven useful for the rapid diagnosis of streptococcal pharyngitis (Shulman *et al.,* 2000).

2.6.3 Antistreptolysin O (ASO) test

It is an immunological reaction test which helps to detect if antibody in a serum sample was de veloped against streptolysin O. Likewise, DNase B test is conducted for diagnosis for diagnosis of group A Streptococci (Martin, 2000). Routine use of back-up throat swab helps to prevent future re-occurrence of infection.

2.7 Treatment

Group A streptococci infection is usually treated, using specific antimicrobial therapy to eradicate the organism. The drug of choice is penicillin and the duration of treatment is well established as being 10days minimum (Falagas *et al.,* 2008). In individuals with a penicillin allergy, erythromycin, other macrolides and cephalosporins have been shown to be effective treatments (Khan, 2014). The use of immunoglobulin intravenous (IGIV) therapy is used for patients with severe invasive group A Streptococci (Kaul *et al.,* 1999). Surgery is often needed to remove damaged tissue and stop the spread of infection in the case of necrotizing fasciitis (CDC, 2014).

2.8 Prevention /Control

Group A streptococci are highly contagious and epidemics of pharyngitis, scarlet fever, rheumatic fever, glomerulonephritis, bacteremia, streptococcal shock syndrome, necrotizing

fasciitis have been described (Stevens, 2000). The acquisition of group A streptococci in the family environment poses problems for individuals in the environment.

In the hospital environment, group A streptococci can spread rapidly to patients with surgical wounds, burns or chicken pox, therefore, strict adherence to infection control measures is crucial (Stevens, 2000). Strict isolation procedures should be employed in patients who are admitted to hospitals with group A Streptococcal infections (Stevens, 2000).

Routine throat culture/culture of skin lesions of all individuals with persistence or recurrence of streptococcal infection and found to be positive to culture, should all be treated immediately (Robinson *et al.,* 2003).

The Centers for Disease Control and Prevention, recommend that health care providers inform household contact of persons with invasive group A streptococci about the clinical manifestation of pharyngeal and invasive group A streptococcal infections and emphasize the importance of seeking immediate medical attention if contacts develop such symptoms (CDC, 2002). Tonsillectomy (surgical removal of tonsils) may help to reduce the number of acute infections in children with recurrent group A streptococci pharyngitis (Binso, 1996). Patients who develop acute rheumatic fever require continuous prophylaxis to prevent recurrent streptococcal infections and recurrent episodes of acute rheumatic fever (Congeni, 1992).

All prescribed drugs should be taken even after symptoms have gone away to prevent infection from coming back (Adult Health Advisor, 2004). Patients should avoid close contact with other people until they have been treated with antibiotics for 24-48 hours, so they do not spread the strep bacteria to those uninfected (Adult Health Advisor, 2004). Do not share food and eating utensils with others, use tissues when you cough and dispose them carefully, do not share

drinking cups and hand towels, wash your hands after cough (Adult Health Advisor, 2004). Hand washing is the best method of prevention of diseases. Hands should be washed before eating, before touching dishes, before touching napkins (Adult Health Advisor, 2004). Hand hygiene still remains the first line of defense against many infectious diseases and against respiratory and gastrointestinal disorders (Conway, 2003). Hand hygiene encompasses all practices of disease prevention which are usually the result of hands being vehicles for transmission of infectious agents. Such procedures includes: washing of hands, drying of hands, use of hand gloves, cutting of nails (CDC, 2012).

2.9 Vaccines

Development of an effective group A streptococcal vaccine continues to be of interest. Researchers are currently carrying out researches on effective vaccines against group A streptococcal infections. Currently, none are commercially available. Researchers have looked at the conserved region of the M-protein since the region is shared by all serotypes of group A streptococci, to provide immunity against about 150 known M-types of group A streptococci and would need to be polyvalent (Fischetti, 2000).

2.10 Anti-streptolysin O (ASO)

Anti-streptolysin O test is an immunological titer test used to demonstrate the reaction of the body to an infection caused by group A beta-haemolytic Streptococci. Group A Streptococci, produce the enzyme streptolysin O which can destroy (lyse) red blood cells, because streptolysin O is antigenic (contains a protein foreign to the body). The body reacts by producing anti-streptolysin O (ASO), which is a neutralizing antibody (Pagana, 1998). Many researches have been conducted, using ASO to diagnose group A streptococcal infection in the study population.

A research carried out in Zaria, Nigeria by Ella and Okafor.(2015), documented prevalence rate of ASO in the studied population to be 68.75%, which was quite significant in the study. However, a research carried out in India in 2003, documented the prevalence of ASO titer in the study population to be 52% which was also significant. The most commonly available and easiest assay to perform is the anti-streptolysin O titer test (ASO), which is particularly effective at detecting upper respiratory tract *Streptococcus pyogenes* infection (Ferrieri, 1986).

Anti-streptolysin O, is the antibody response most often examined in serological tests to confirm recent streptococcal infection and also helps in the diagnosis of rheumatic fever (Cunningham, 2000). Anti-streptolysin O remains useful in the diagnosis of streptococcal infections and their complications, follow-up, as well as in evaluating the effectiveness of treatments (Periwal *et al.,* 2006). A rise in ASO titer occurs in the second week after infection and reaches its maximum value at 4-6 weeks, an increase in ASO titer is usually accepted as serologic confirmation of recent infections (Ferrieri, 1986). Anti-streptolysin O titers can vary, depending on the geographic location, age, climatic conditions (Ozturk *et al.,* 2004). Anti-streptolysin O is also helpful when the throat culture technique is ineffective or when the patient has already taken antibiotics. Significant findings have shown that an ASO positive measurement might be used in conjunction with throat culture to identify group A *Streptococcus* carriers (Manandhar *et al.,* 2013).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was carried out on children attending some hospitals in Zaria, Kaduna State. Zaria is one of the major cities in Kaduna State in Northern Nigeria, covering a total land of 300km^3. It is made up of two Local Government Areas – Zaria and Sabon Gari Local Governments with 12 districts and a population of 408,198 people with annual growth rate of 3.3% (NPC, 2006). The selected hospitals include: Hajiya Gambo Sawaba Memorial Hospital located in Zaria City Area, Sick Bay Clinic, Ahmadu Bello University, Samaru-Zaria, ABUTH Women and Children Health Care Centre, Cemetery Road, Sabon Gari, Zaria, Nigeria.

3.2 Study Design

The study was based on cross-sectional and experimental research. It involved the use of structured questionnaires and the analysis of throat swab samples and serum samples from consented parents of children aged 0-5 years.

3.3 Sample Size Determination

For the purpose of this study, a prevalence rate of 10.45% as reported from a study conducted by Mawak *et al.*(2005) in Jos, Nigeria was used to determine the sample size. The sample size was calculated using the equation below, as derived by Sarmukaddam and Gerad (2006) at 95% confidence interval.

$$n = \frac{Z^2pq}{L^2}$$

Where,

n = sample size

Z = Standard normal distribution at 95% confidence interval = 1.96.

P = known prevalence of *Streptococcus pyogenes* infection among Children in Jos = 10.45% (Mawak *et al.*, 2005)

q = (1-P) converting 10.45% = 10.45 = 0.1045

q = 1 – 0.1045 = 0.8955

L = allowable error, which is taken as 5% = 0.05

Substituting

$$n = \frac{(1.96)^2 \times 0.1045 \times 0.8955}{0.05^2}$$

$$n = \frac{3.8416 \times 1.092025}{0.0025} = 144 \text{ samples} \sim 150 \text{ samples}$$

For the purpose of this study, 150 samples were collected each for blood sample and throat swab samples, giving a total of 300 samples.

3.4 Study Population

The target population for this study comprised children aged (0-5) years, who showed some clinical signs and symptoms related to *Streptococcus pyogenes* infection who attended the selected hospitals.

3.5 Inclusion and Exclusion Criteria

The inclusion criteria for the study population included all children (male and female) in the age group 0-5 years who presented clinical signs and symptoms (pharyngitis, tonsillitis, cough, fever,

red-rash on the skin) related to *Streptococcus pyogenes* infection who attended the selected hospitals and whose consent was sought. The exclusion criteria included all children above 5 years with and without clinical signs and symptoms (pharyngitis, tonsillitis, cough, fever, red rash on the skin) related to *Streptococcus* pyogenes infection, as well as children aged 0-5 years, who showed clinical signs and symptoms related to *Streptococcus pyogenes* infection whose parents did not give their consent and who attended the selected hospitals.

3.6 Ethical Consideration

Ethical approval (Appendix I) was obtained from the Kaduna State Ministry of Health (MOH/Adm/744/Vol.1/319), before the commencement of the study. All consented parents who enrolled in the study were issued a consent form (Appendix II) which they signed and/or thumb printed prior to data and sample collection.

3.7 Data Collection

All consented parents were administered structured questionnaire (Appendix III), which was aimed at obtaining socio-demographic data and clinical information related to *Streptococcus pyogenes* infection.

3.8 Sample Collection

Blood and throat swab samples were collected from each child, whose parent consented to the study (Appendix IV). A total of 150 samples of blood and 150 samples of throat swab were collected. All samples collected were labeled appropriately with the name of patient, sex, age, hospital name, serial number, date of collection, and sample type collected.

3.9 Collection and Processing of Throat Swab Samples

Throat swabs were collected using a sterile cotton swab to obtain a sample from the pharynx gently and a disposable tongue depressor was used to depress the tongue so that the pharynx was visible to see with the assistance of a laboratory scientist. The swab collected was returned into the swab holder and was immediately placed into a tube of Mueller-Hinton broth which served as the transport medium to maintain the viability of the organism.

The throat swabs were transported in a cold ice pack to the laboratory for examination immediately. The swabs were then sub-cultured on blood agar containing 5% defibrinated sheep blood and incubated at 37^{o}C for 20 hours under atmosphere containing 10% carbondioxide. A control plate was prepared without any organism inoculated on it and was incubated along-side the plates. After 20 hours, the plates were observed and the colonial morphology of colonies were recorded. The phenotypically distinct colonies were sub-cultured on fresh blood agar to obtain pure bacteria culture. The freshly inoculated plates were incubated at 37°C for 19 hours. Tiny white to grey colonies with a clear zone of beta-haemolysis on blood agar were observed and identified.

3.10 Identification, Biochemical Test Principles and Procedures.

Biochemical characterization and identification of isolates (Appendix V) was carried out, using the following test:

3.10.1 Haemolysis test

Principle: Haemolysis test is the breakdown of red blood cells. The ability of bacterial isolates to induce haemolysis when grown on blood agar is used to classify certain micro-organisms. This is

useful in classifying streptococcal species. A substance that causes haemolysis is a haemolysin. Beta-haemolysis is the complete lysis of red cells in the media around and under the colonies, the area appears lightened and transparent. Streptolysin, an exotoxin is the enzyme produced by the bacteria which causes the complete lysis of red blood cells.

Procedure: A sterile nutrient agar was prepared according to the manufacturer's instruction and was allowed to cool to about 45°C and 5% sterile defibrinated blood was added to the already prepared nutrient agar, swirled gently in a conical flask near a flame to mix thoroughly in order to avoid bubble formation. It was then dispensed gently near a flame into sterile petrish dish plates. The plates were inoculated on and incubated at 37°C for 20 hours, after which the plates were observed for haemolytic reaction.

Beta-haemolysis was checked out for, which had a clear zone of haemolysis, indicating complete lysis of red blood cells which was suggestive of *Streptococcus pyogenes* (Cheesbrough, 2006).

3.10.2 Gram staining

Principle: Gram staining is a microscopic technique used to enhance the clarity of a microscopic image. It helps to differentiate between Gram positive and Gram negative bacteria based on the biochemical and structural differences of their cell walls. Stains and dyes are widely used in the scientific field to highlight the structure of the biological specimens, cells and tissues. The differential staining technique is based on the ability of micro-organisms to retain colour of stains used during the Gram stain reaction (typical for Gram positive organisms) or to be decolourised by alcohol, losing the primary stain (typical for Gram negative organisms).

Procedure: A colony was selected among the grey translucent beta-haemolytic colonies for Gram staining. A smear of the colony was made on a clean and dry glass-slide and heat fixed. The heat fixed smear was stained with the primary dye-crystal violent for one minute and then rinsed gently with distilled water. A mordant, Gram's iodine, was added for another one minute and rinsed with distilled water. A decolourizer (acetone) was added for 10 seconds and rinsed off immediately. A secondary safranin (counter stain) was added for one minute and rinsed with distilled water. The slide was then carefully air-dried and viewed under the microscope, using and ×100 magnification with the aid of an oil immersion (Cheesbrough, 2010).

3.10.3 Catalase test

Principle: Catalase test is conducted to check for the presence of the enzyme catalase on bacteria that hydrolyses hydrogen peroxide into water and oxygen. If the bacteria possess catalase enzyme, it is evident by formation of bubbles in the test due to liberation of oxygen. Lack of catalase is indicated by absence of bubble formation. Catalase test is used to differentiate the genus Staphylococcus which is catalase positive and genus Streptococcus which is catalase negative.

Procedure: Two drops of hydrogen peroxide was placed on a clean dry glass slide, to which a colony of the isolated organism added and emulsified using a sterile wire loop. The reaction was observed for 30 seconds. A positive test was indicated by bubble formation while a negative test showed no bubble formation (Cheesbrough, 2010).

3.10.4 Oxidase test

Principle: Oxidase test is used to identify bacteria that produce the enzyme cytochrome oxidase. An organism which is oxidase producing, will oxidase the phenylenediamine in the oxidase reagent, producing a deep purple colour.

Procedure: A piece of filter paper was placed in a clean petri-dish and 2 drops of freshly prepared oxidase reagent was added. A sterilized wire loop was used to remove a colony of the test organism and smeared on the filter paper. Development of a blue-purple colour within 10 seconds showed a positive oxidase test and no blue-purple colour within 10seconds showed a negative test (Cheesbrough, 2010).

3.10.5 Motility test

Principle: Motility test is a biochemical test performed to check the motility (movement) of an organism. The motility medium contains a low concentration of agar (0.2 – 0.5%) and motile organisms are able to move away from the line of inoculation through the sloppy area.

Procedure: Isolates were inoculated into the motility medium by making a fine stab with a sterile inoculating needle to a depth of about 2cm bottom of the tube in a slant wise manner. The tube was incubated at 35°C for 24 hours. At the end of the incubation period the tube was examined. A positive result showed a well dispersed growth of organism along the area of stab and the line of inoculation was not sharply defined (turbid), while a negative result showed restricted movement to the line of inoculation which was sharply defined and the rest of the medium was clear, which was indicative of non-motile organism (Cheesbrough, 2010).

3.10.6 Bacitracin test

Principle: Bacitracin test is an antibiotic interfering with the synthesis of peptidoglycan, a major component of bacterial cell walls. Different types of bacteria have different degrees of susceptibility to bacitracin. This test determines whether the bacterium is sensitive or resistant to bacitracin.

Procedure: A sterile wire loop was used to pick 3 colonies of isolates from a freshly prepared cultured plate and emulsified into a tube containing 2ml of normal saline. It was gently shaken for uniform distribution and its turbidity was matched to 0.5 Mcfarland Standard. A sterile cotton swab stick was inserted into the solution and pressed gently around the neck of the tube to reduce excess fluid. The sterile cotton swab stick was then spread evenly and gently on an already prepared Mueller-Hinton agar plate, supplemented with 5% defibrinated sheep blood. The petri-dish plate was allowed to dry for 5 minutes before bacitracin disc (0.04U) was laid gently on the surface, using a sterile forcep. The plates were incubated at 37°C for 23 hours. After the incubation period, the plates were observed if the bacteria were susceptible or resistant to bacitracin. A positive test showed a visible zone of inhibition forming around the disc, representing the area where the antibiotics concentration has prevented bacterial growth, indicating it's susceptibility to the disc, while a negative result showed a lawn of cells, which formed visible growth up to the margin of the disc, indicating resistance to the disc. *Streptococcus pyogenes* is inhibited by small amount of bacitracin while other beta-haemolytic Streptococci usually are not (Cheesbrough, 2010).

3.10.7 Preservative medium

All samples that showed beta-haemolysis on blood agar, Gram positive cocci shaped morphology in pairs and in chains under microscopy and positive to all the biochemical tests conducted were stored on blood agar base slant, supplemented with 5% sheep blood and kept in the refrigerator prior to Lancefield grouping test.

3.10.8 Lancefield grouping of Beta-haemolytic *Streptococcus*

All samples positive for *Streptococcus pyogenes* after isolation and identification were grouped into serotypes, using the Lancefield grouping Latex Kit.

Principle: Lancefield grouping is a method of grouping beta-haemolytic bacteria based on the carbohydrate composition of bacterial antigens found on their cell wall. Lancefield grouping test is an agglutination immunological assay that detects carbohydrate in the cell wall of Streptococci.

Procedure: The Microgen Strep reagents were brought out from the refrigerator and then allowed to cool to reach temperature prior to use. The bottle of enzyme was reconstituted prior to use by adding 10ml of distilled water. It was mixed gently to ensure complete reconstitution. 0.4ml of the extraction enzyme was dispensed into a sterile test tube, through the aid of a Pasteur pipette.

Streptococcal colonies (3) were picked from freshly prepared cultured plates, using a sterile bacteriological wire loop and emulsified thoroughly in the extraction enzyme. The tube was capped using sterile cotton wool and incubated for 15 minutes in a 37°C water bath. The

tubes were shaken after the first 5minutes of incubation to obtain even suspension of the antigen and the water bath was closed back.

One drop of each latex reagents (A, B, C, D, F and G) were dispensed separately into six circles on a reaction card. One drop of well mixed extract (positive control) was transferred into the six separate circles next to the drop of the latex reagent. The content of each circle were mixed gently using a separate mixing stick for each circle, in order to spread the liquid to cover the area of the circle.

The reaction card was slowly and gently rocked and rotated for one minute, for proper mixing of the reagents. The card was inspected for agglutination during the one minute reaction time under a good light source. Agglutination reaction on each card circle (A, B, C, D, F, and G) was indicative of a positive reaction to the Group. A negative result was indicated when the latex particles retained their original milky appearance, without any significant aggregation while a positive result showed the aggregation of the latex particles into visible clumps during the one minute reaction time.

3.10.9 Antibacterial susceptibility test

Principle: Sensitivity test is a laboratory test used to determine the sensitivity pattern of a given microorganism for a range of antibiotics. It helps in the determination of effective antibiotic therapy that can be prescribed by a physician to cure certain diseases. Antibiotics kill or inhibit the growth of microorganisms.

3.10.10 Preparation of 0.5 Mcfarland standards

A 1% v/v solution of sulphuric acid was prepared by adding 1ml of concentrated sulphuric acid to 99mls of distilled water and mixed gently in a conical flask. Barium chloride solution was prepared by dissolving 1g of dehydrated barium chloride solution into 99mls of distilled water in another conical flask. Aliquots of 0.05ml of barium chloride solution was added to 9.95ml of the sulphuric acid solution and properly mixed in another conical flask. The turbid suspension was transferred into a sterile test tube (Cheesbrough, 2003).

3.10.11 Standardization of test organism

Isolates of about 3 colonies were picked, using a sterile wire loop from a freshly prepared cultured plate and emulsified into 2ml of normal physiological saline contained in a labeled sterile tube. The contents were gently mixed for even distribution and the turbidity of the suspension was then matched to that of the standard (0.5 Mcfarland Standard) in a good light against a white sheet of paper (Cheesbrough, 2003).

3.10.12 Inoculation on Mueller-Hinton agar

Mueller-Hinton Agar plate supplemented with 5% defibrinated sheep blood was prepared. The surface of the plates were made to dry with each plate, containing 15ml each of the prepared agar. A sterile cotton swab stick was dipped into the turbid suspension, excess fluid was removed by pressing and rotating the swab stick against the side of the tube above the level of the suspension.

Inoculation was done on the surface of the Mueller-Hinton Agar plate and one swab stick was used for each plate. The swabs were streaked evenly over the surface of the medium in three

dimensions by rotating the plates at an angle of 60° to ensure even distribution. The petri-dish plates were allowed to dry for 5minutes before antibiotic discs were laid on the surface.

3.10.13 Antibiotic discs

Kirby- Bauer disc diffusion technique was used. The appropriate discs with known concentration were aseptically impregnated and placed at about 15mm from the edge of the plates, using a pair of sterile forceps. The discs were lightly pressed down to ensure contact with the agar. The discs were left to diffuse on the plate and incubated at 37°C for 23 hours. After 23 hours, the plates were observed for susceptibility and resistance to the antibiotic discs.

3.10.14 Measurement of the inhibition zone diameter

After incubation, the plates were examined to ensure confluent growth. The diameter of each zone of microbial growth inhibition was measured, using a transparent ruler in millimeters (mm) on the outside of the plate.

The inhibition zone diameter (IZD) was evaluated according to the Clinical and Laboratoty Standard Institute (CLSI, 2015) and Cowan and Steel (2003). Guidelines were interpreted as sensitive or resistant depending on their respective MIC (Minimum Inhibition Concentration) break point. The first line action drugs were used as choice of antibiotics discs, which included: Ampicillin, Erythromycin, Vancomycin and Rifampacin (Cohen and Kasper, 2007*;* Bessen, 2009).

3.10.15 Collection and processing of blood samples

Approximately, 2mls of venous blood was collected into a sterile plain bottle with the assistance of a medical personnel. Each of the blood sample collected was vortex-mixed 5 times and allowed to stand for 10 minutes so that it can clot. The blood samples were transported in a cold ice pack to the laboratory for examination.

The sample was centrifuged at 1000rpm for 20 minutes. The serum was harvested and aseptically transferred into another labeled plain sterile screw capped container, using a 2ml sterile syringe and refrigerated prior to examination.

3.10.16 Anti-Streptolysin 'O' titer test

Principle: Anti-streptolysin O (ASO) titer is a blood test to measure antibodies against streptolysin "O", a substance produced by group A *Streptococcus* bacteria. It is used to demonstrate the body's reaction to an infection caused by group A beta-haemolytic streptococci.

Group A beta-haemolytic streptococci produces the enzyme streptolysin "O", which can destroy red blood cells because streptolysin O is antigenic, the body reacts by producing the anti-streptolysin O (ASO) which is a neutralizing antibody.

3.10.17 Procedure for Anti- Streptolysin O (ASO titer)

Anti-streptolysin O test is a stabilized buffered suspension of polystyrene latex particles that have been coated with Streptolysin O. When the latex reagent is mixed with serum containing ASO, agglutination occurs.

All blood samples collected were examined by using the ASO titer test kit to check for the presence of Anti-Streptolysin O (ASO) in the serum of the patient which helped to confirm if *Streptococcus pyogenes* infection was responsible for the clinical signs and symptoms (pharyngitis, tonsillitis, cough, fever, red rash on skin) and to check if antibody was developed to the antigen responsible for the infection, observed in the study population.

The sera samples collected were analysed using two methods, namely: Qualitative and Quantitative.

3.10.18 Qualitative analysis

The qualitative test is a screening test to determine the presence of the ASO antibody in the serum (Appendix VI). The ASO latex vial was shaken gently to disperse and suspend the latex particles. One drop of ASO (40µl) positive control was placed on circle of field (1) of a reaction slide, using a disposable serological Pasteur pipette. Also, one drop of ASO (40µl) negative control was also placed on another circle of field (2) of a reaction slide, they were both tested with each series of test that was conducted. A disposable serological Pasteur pipette was used to place (40µl) 0f undiluted test serum sample to field (3), field (4), field (5) and field (6) respectively, using different disposable Pasteur pipette tips for different samples.

The ASO latex reagent was gently re-suspended and one drop was added to each test field on the reaction slide. The contents on the reaction slide was well mixed, using a stirring stick and was gently tilted and rotated for three (3) minutes and read immediately to check for the presence of agglutination reaction under a good light source. A positive result showed agglutination reaction which was indicative for the presence of anti-streptolysin “O” in the serum while a negative result showed no agglutination but rather a milky suspension, indicating the absence of

anti-streptolysin “O” in the serum. Sera that tested positive in the qualitative test were re-tested in the titration test to provide verification for borderline interpretations.

3.10.19 Quantitative analysis

For each test sample to be titrated, five different Wells were set up, using a micro-titer plate labeled 1:1, 1:2, 1:4, 1:8, 1:16. The first Well contained 0.05ml undiluted test serum, and to the other Wells, 0.025ml physiological normal saline was added with the aid of a micro dispenser and a pipette filler.

To Well number one 0.05ml undiluted test serum was added and two fold dilutions were serially made by mixing contents of well number one gently with a micro diluter and 0.025ml was transferred to well number two, using a micro pipette, pipette filler, which was mixed gently, using another micro diluter. Each serial transfers were repeated for each of the remaining Wells, using a different micro diluter and micro dispenser for each of the test serum. Thereafter, one drop each of the positive and negative controls were placed on different slide rings and one drop, each of the five (5) dilutions made were placed on successive field of the reaction slides. The ASO Latex reagent was gently re-suspended and one drop was added to each slide ring containing the positive and negative controls as well as the five (5) different dilutions made. The content on each ring was properly mixed, using a stirring stick and was gently tilted and rotated for three (3) minutes and read immediately under good light source for agglutination reaction. A uniform milky suspension with no agglutination was observed with the ASO negative control while agglutination with large aggregates were observed with the ASO positive control. The specimen reaction (containing the dilutions) were compared to the ASO positive and negative control and read within three (3) minutes. A positive reaction was indicated by observable

agglutination in the reaction mixture. The last dilution showing a positive reaction was recorded and used to calculate the concentration of ASO in the sera samples.

The concentration (titer level) of ASO was determined by multiplying the last positive dilution factor of the serum sample with the concentration of the positive control (200IU/ml).

1UL/ml of sample = Conc. of positive control (200) × specimen titer.

Dilution	**1U/ml**
1:1	200
1:2	400
1:4	800
1:8	16,000
1:16	32,000

Note: The upper limit of anti-streptolysin O titer, for this test was 200IU/ml for adults and 100IU/ml for children. Titer above the upper limit was indicative of a streptococcal infection.

3.10.20 Analysis of results.

Results obtained from the laboratory and information from the questionnaires were reduced to percentages and presented in tables, charts and analyzed statistically using the Statistical Analysis System (SAS) software version 9.1(2011).

Pearson chi-square analysis was used to determine association between factors and infection at 95% confidence interval and 0.05 significant leve

CHAPTER FOUR

4.0 RESULTS

Seventy eight (78) males and seventy two (72) females were examined during the course of the research.

The risk and socio-demographic factors associated with *Streptococcus pyogenes* infection was revealed on Table 4.1. In this study, there was no statistical significance between sex (p=0.2694), age (p=0.1538), occupational status of parents (p=0.6667) and educational status of parents (p=0.6443).

There was no statistical significance between ventilation in school (p=0.3934), ventilation at home (p=0.09), level of hygiene of children (p=0.4476), level of hygiene of nursing parents (p=0.6745), previous history of disease (p=0.8933), previous administration of drugs (p=0.5562), record of outbreak in locality (p=0.2422) as depicted on Table 4.2

Likewise, as depicted on Table 4.3 there was no statistical significance between *Streptococcus pyogenes* infection and difficulty in swallowing food/or water (p=0.7175), swollen lymphnodes (p=0.7554), throat having sorethroat (p=0.424), fever (p=0.0722), cough (p=0.0704) and red rash on the skin (p=0.4382).

Table 4.1: The relationship between *Streptococcus pyogenes* infection and socio-demographic factors.

Socio-demographic Factors	Number examined	Number positive (%)	p-value	Chi square value	Odds ratio	Lower limit - Upper limit	df
SEX							
Female	72	3(4.2)		1.2198		0.811-3.485	1
Male	78	7(8.9)	0.2694				
					2.15		
Age (Years)							
0−1	61	7(11.5)	0.1538			0.101-2.280	
2−3	53	1(1.9)		3.7439			2
4−5	36	2(5.6)			1.19		
Occupational status of parents							
Civil servant	27	5(18.5)		0.8107			
Business women/men	61	11(18.0)			1.27		2
Unemployed	62	16(25.8)	0.6667			0.279 - 2.254	
Educational status of parents							
Primary School	45	11(24.4)		1.6672			
Secondary School	24	3(12.5)				0.405 - 2.886	
Tertiary Education	26	4(15.4)			1.65		3
None	55	14(25.3)	0.6443				

p-value significant at ≤ 0.05 p-value insignificant at > 0.05 Confidence Interval (CI) at 95% OR>1

Table 4.2: The relationship between *Streptococcus pyogenes* infection and risk factors.

Risk Factors	Number examined	Number positive (%)	p-value	Chi square value	Odds ratio	Lower limit - Upper limit	Df
Ventilation in school							
Yes	33	5(15.2)		1.8657		0.109 - 2.611	
No	67	14(20.9)					
Those not in school	50	5(10.0)	0.3934		1.36		2
Ventilation at home							
Yes	90	13(14.4)		2.837		0.189 - 3.754	
No	60	17(28.3)	0.09		1.97		1
Level of hygiene of children							
Good	17	2(11.8)		0.5767		0.108 - 3.560	
Poor	133	28(21.10)	0.4476		1.83		1
Level of hygiene of nursing parents							
Good	12	1(8.3)		0.1764		0.270 - 2.901	
Poor	45	6(13.3)					
Non-nursing parents	93	0(0.0)	0.6745		1.59		2
Previous history of disease							
Yes	83	17(20.5)		0.018		0.185 - 2.210	
No	67	13(19.4)	0.8933		1.19		1
Previous administration of drugs							
Yes				1.1731		0.202 - 2.481	
With completion	36	5(13.9)					
Without completion	47	12(25.5)					
No	67	13(19.4)	0.5562		1.34		2
Record of outbreak in locality							
Yes	53	17(32.1)		0.395		0.200 - 0.647	
No	97	13(13.4)	0.2422		0.42		1

p-value significant at ≤ 0.05 p-value insignificant at > 0.05 Confidence Interval (CI) at 95% OR>1

Table 4.3: The relationship between *Streptococcus pyogenes* infection and clinical signs with symptoms.

Clinical signs and symptoms	Number examined	Number positive (%)	p-value	Chi square value	Odds ratio	Lower limit - Upper limit	df
Difficulty swallowing food/water							
Yes	105	20(19.0)		0.1309		0.250 - 2.105	
No	45	10(22.2)	0.7175		1.17		1
Swollen lymphnodes							
Yes	18	3(16.7)		0.097		0.209 - 2.245	
No	132	27(20.5)	0.7554		1.23		1
Throat with sorethroat							
Yes	73	17(23.3)		0.64		0.208 - 2.136	
No	77	13(16.9)	0.424		1.2		1
Fever							
Yes	130	30(23.1)		3.2326		0.978 - 3.374	
No	20	2(10.0)	0.0722		2.17		1
Cough							
Yes	140	25(17.9)		3.2727		0.88 - 4.808	
No	10	5(50.0)	0.0704		2.8		1
Red rash on the skin							
Yes	26	7(26.9)		0.6011		0.409 - 1.807	
No	124	23(18.5)	0.4382		1.1		1

p-value significant at ≤ 0.05 p-value insignificant at > 0.05 Confidence Interval (CI) at 95% OR>1

The prevalence of *Streptococcus pyogenes* in this study was 6.7% (10/150), using Lancefield kit from Microgen biproducts Ltd. Surrey, UK, as presented in Figure 4.1.

The haemolytic pattern of the isolates on cultured plates, showed one hundred and one (101) isolates for beta-haemolysis, four (4) isolates for alpha-haemolysis, eleven (11) isolates for gamma-haemolysis while thirty four (34) plates had no growth on them as revealed in Figure 4.2. Those that showed beta-haemolysis were further subjected to analysis, using the Lancefield grouping kit.

The occurrence of *Streptococcus pyogenes* infection and other Lancefield groups in relation to sex is presented in Figure 4.3. The males recorded higher frequency of *Streptococcus pyogenes* isolates, with frequency of 7 for group A, 2 for group C, 9 for group D and 2 for group F, while the females had frequency of 3 for group A, 5 for group D and 2 for group F. The males were seen to be at higher risk of acquiring *Streptococcus pyogenes* infection than the females. There was for no group identified for B and G in both males and females.

The occurrence of *Streptococcus pyogenes* infection and other Lancefield groups among age groups is depicted in Figure 4.4. Children aged 0-1, had the highest frequency of *Streptococcus pyogenes* isolates, with frequency of 7 for group A, 1 for group D and 2 for group F compared to age group 2-3 with frequency of 1 for group A, 3 for group D and 2 for group F. Ages 4-5 had frequency of 2 for group A, 1 for group C, 7 for group D and 1 for group F. In all the age groups, there was no group identified for groups B and G.

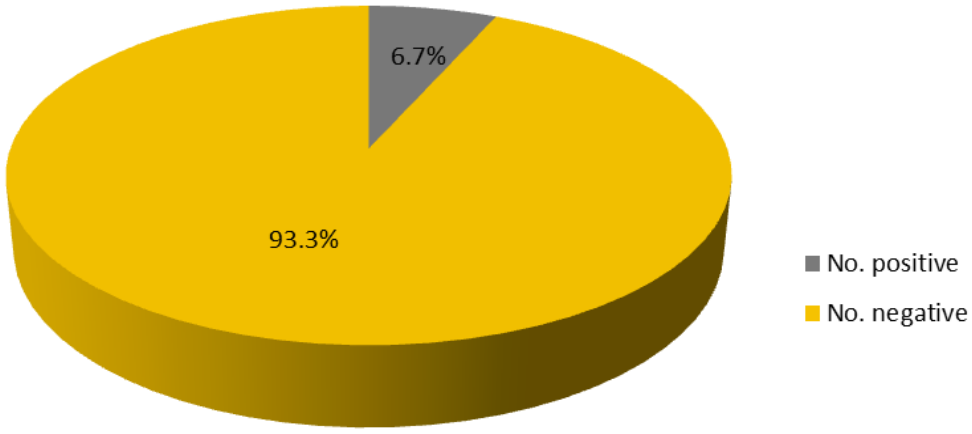


Figure 4.1: Prevalence of *Streptococcus pyogenes* in the study population.

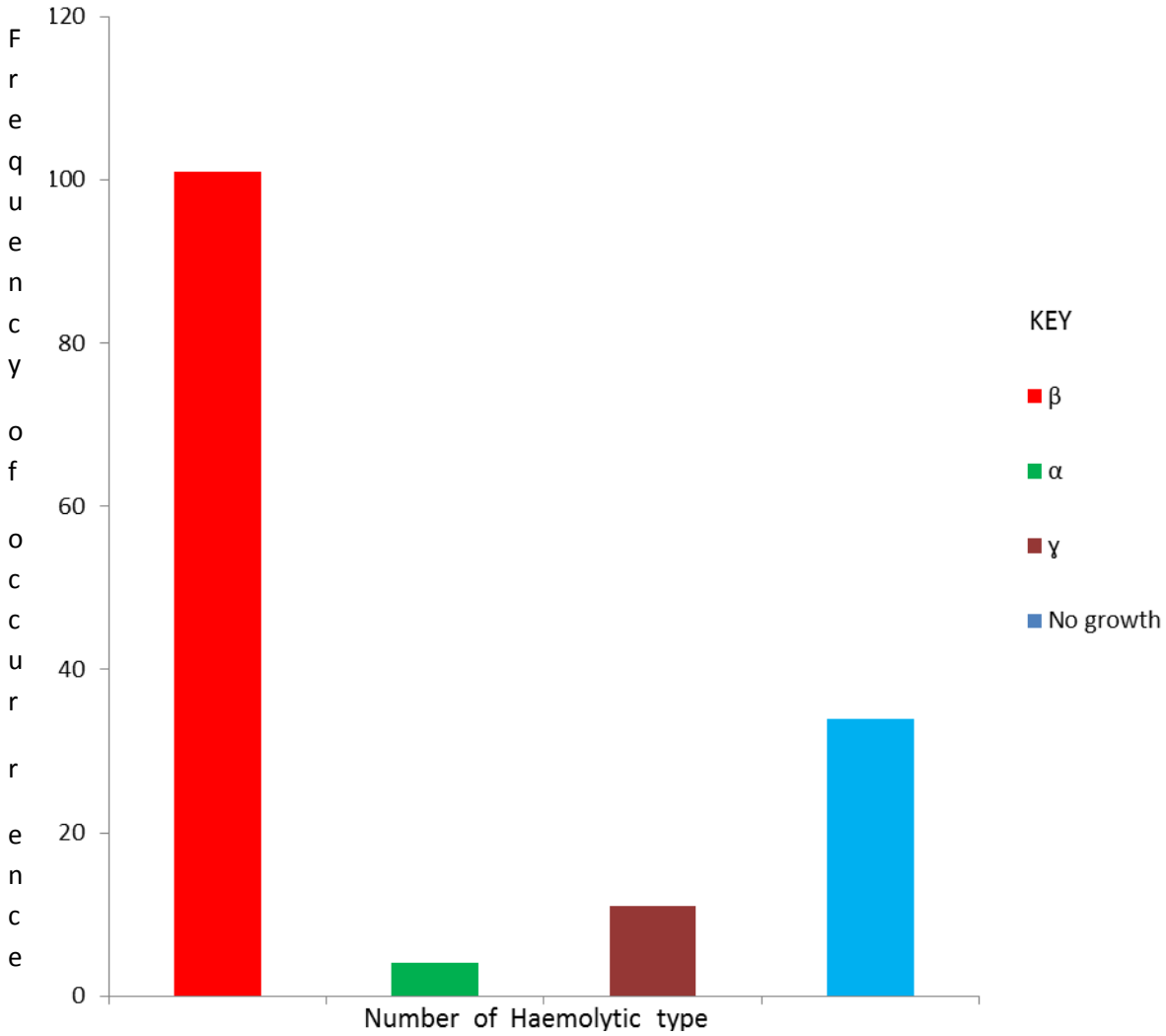


Figure 4.2: Haemolytic pattern of isolates on cultured plates.

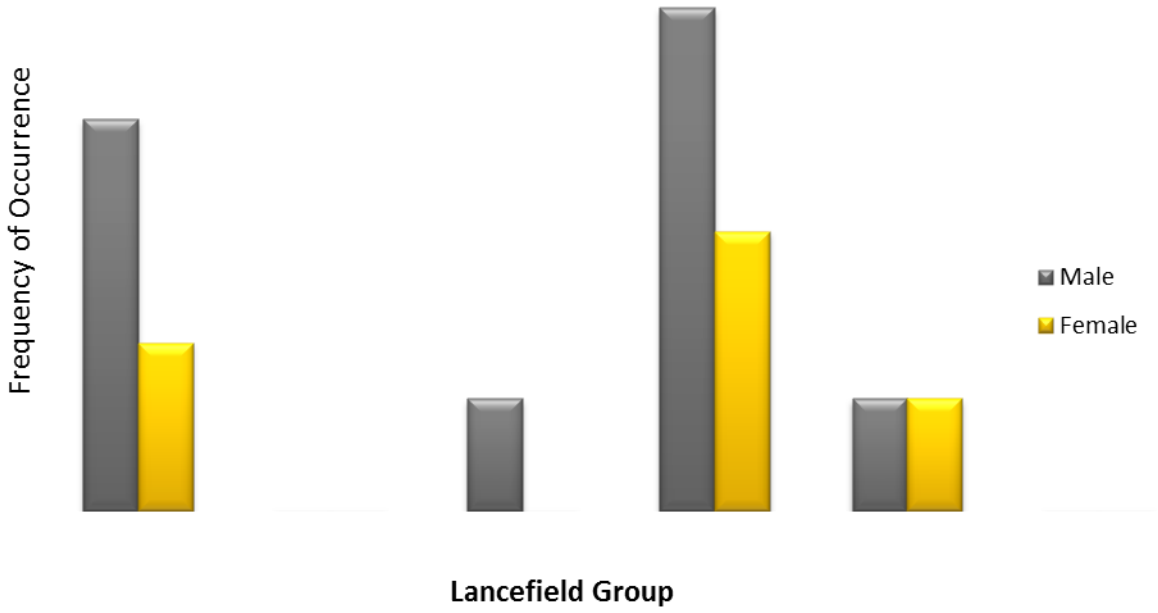


Figure 4.3: Occurrence of group A *Streptococcus pyogenes* infection and other Lancefield groups among children in the study population in relation to sex using Lancefield grouping kit.

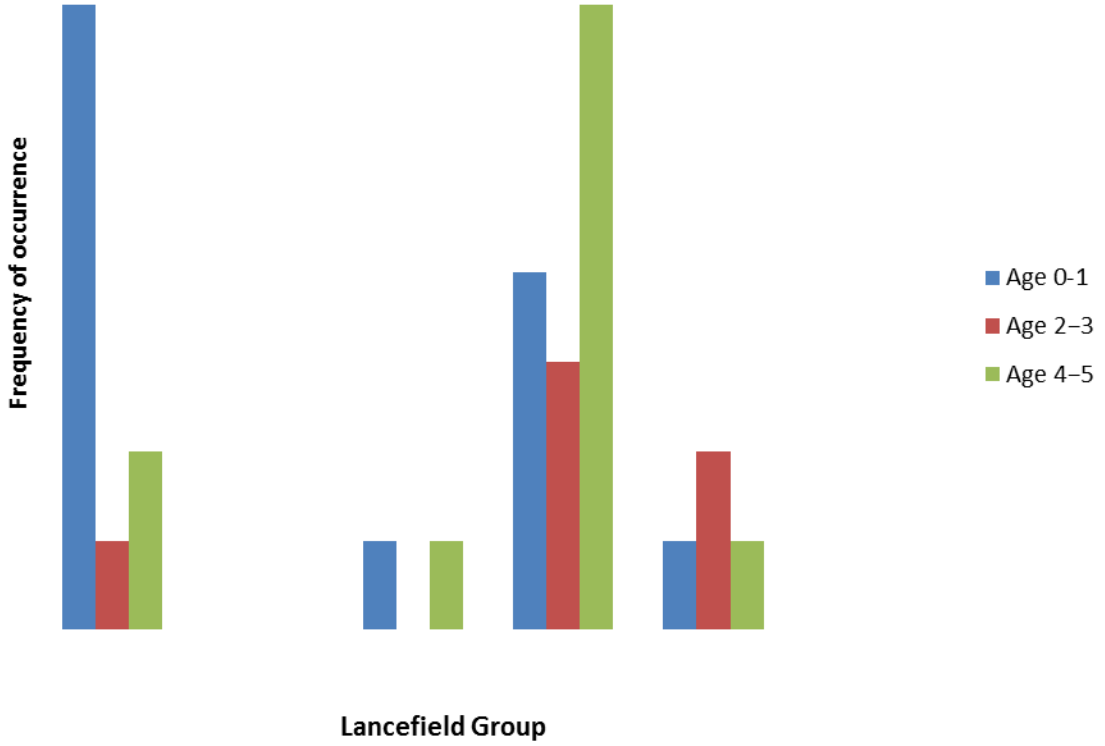


Figure 4.4: Occurrence of group A *Streptococcus pyogenes* infection and other Lancefield groups among children in the study population in relation to age using Lancefield grouping kit.

The prevalence of *Streptococcus pyogenes* isolated from each hospital is depicted on Table 4.4. Gambo Sawaba hospital had the highest prevalence 8.8% (6) positive isolates, compared to Sickbay Clinic with 8.0% (2) isolates and Health Care Centre with 3.5% (2) isolates. However, not statistically significant with p=0.603.

The susceptibility pattern of the ten (10) positive isolates to four (4) antibiotics namely: Erythromycin, Vancomycin, Ampicillin and Rifampacin is shown on Table 4.5. Erythromycin was found to have the highest activity against *Streptococcus pyogenes* while Ampicillin was found to have the least activity against *Streptococcus pyogenes.*

The prevalence for Anti-streptolysin O (ASO), was found to be 14.7% (22), as revealed in Figure 4.5.

The qualitative and quantitative analysis conducted using ASO kit is shown on Table 4.6 Out of the one hundred and fifty (150) sera samples tested, twenty two (22) were found to be positive to qualitative analysis. The twenty two (22) samples were further subjected to analysis quantitatively and were all found to be positive.

The titer level of the twenty-two (22) positive sera samples gotten from quantitative analysis were found to be significant, which were all higher than the cut off titer for children (100IU/ml). The titer ranged from 200IU/ml to 16,000IU/ml with a mean of 1772IU/ml as shown on Table 4.7.

Table 4.4: Prevalence of *Streptococcus pyogenes* infection in the study population in relation to hospital using Lancefield grouping kit.

Hospital	Number examined	Number positive for *Streptococcus pyogenes* (%)	
GS	68	6(8.82)	
SB	25	2(8.00)	P-Value 0.603
HCC	57	2(3.51)	
Total	**150**	**10(6.67)**	

df=2 p-value significant at $\leq$ 0.05 p-value insignificant at > 0.05

KEY:

GS- Gambo Sawaba Hospital (Zaria-City).

SB- Sickbay Clinic (Samaru).

HCC- Health Care Centre for Children and Women (Sabon-Gari).

Table 4.5: Antimicrobial Susceptibility profile of *Streptococcus pyogenes* isolates using Clinical Laboratory Standard Institute (CLSI, 2015).

Antibiotics (Disc potency)	Susceptibility n = 10		
	Sensitive (%)	Intermediate (%)	Resistance (%)
Erythromycin (15µg)	5(50.0)	2(20.0)	3(30.0)
Vancomycin (30µg)	4(40.0)	0(0.00)	6(60.0)
Ampicillin (10µg)	1(10.0)	0(0.00)	9(90.0)
Rifampacin (15µg)	3(30.0)	0(0.00)	7(70.0)
Total	**13**	**2**	**25**

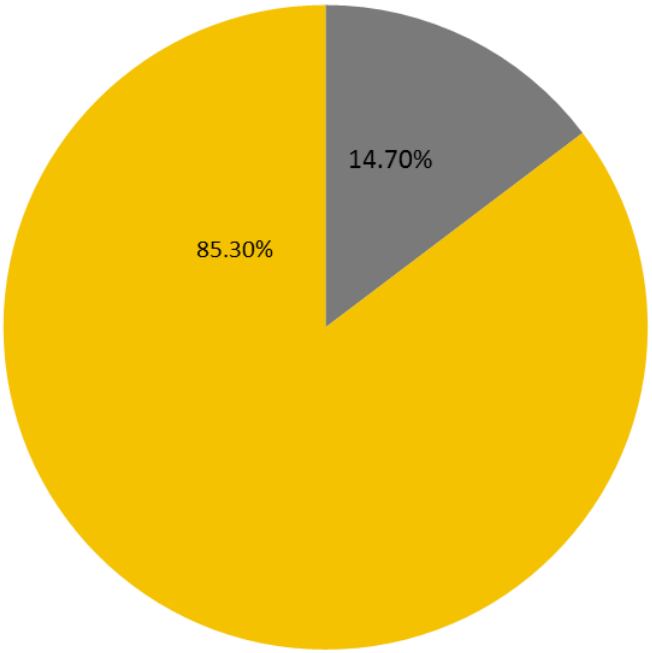


Figure 4.5: Prevalence of Anti-streptolysin “O” titer in the study population**.**

Table 4.6: Detection of Anti-streptolysin "O" titer in the study population.

	Number examined for ASO	Number positive (%)
Qualitative analysis	150	22(14.7)
Quantitative analysis	22	22(100.0)

Table 4.7: Titer levels for positive sera samples.

Specimen	Dilution	Concentration (IU/ml)	Result
GS21	1:8	16,000	Positive
GS33	1:1	200	Positive
GS35	1:1	200	Positive
GS24	1:1	200	Positive
GS30	1:1	200	Positive
GS29	1:2	400	Positive
GS18	1:2	400	Positive
GS19	1:2	400	Positive
GS16	1:1	200	Positive
GS7	1:8	16,000	Positive
GS14	1:1	200	Positive
GS9	1:4	800	Positive
GS4	1:1	200	Positive
GS2	1:4	800	Positive
SB77	1:2	400	Positive
SB91	1:4	800	Positive
HCC93	1:1	200	Positive
HCC105	1:2	400	Positive
HCC106	1:1	200	Positive
HCC113	1:2	400	Positive
HCC126	1:1	200	Positive
HCC136	1:1	200	Positive

KEY: Cut off titers
200IU/ml for adults
100IU/ml for children

GS – Gambo Sawaba Hospital
SB – Sickbay Clinic
HCC – Health Care Centre Hospital

As explained in Figure 4.6, ten (10) individuals were found to be positive to *Streptococcus pyogenes* infection from throat swab samples and twenty two (22) individuals were found to be positive to ASO analysis from sera samples. From this study, two (2) individuals were found to be positive to both *Streptococcus pyogenes* infection in the throat and Anti-streptolysin O antibody in the serum.

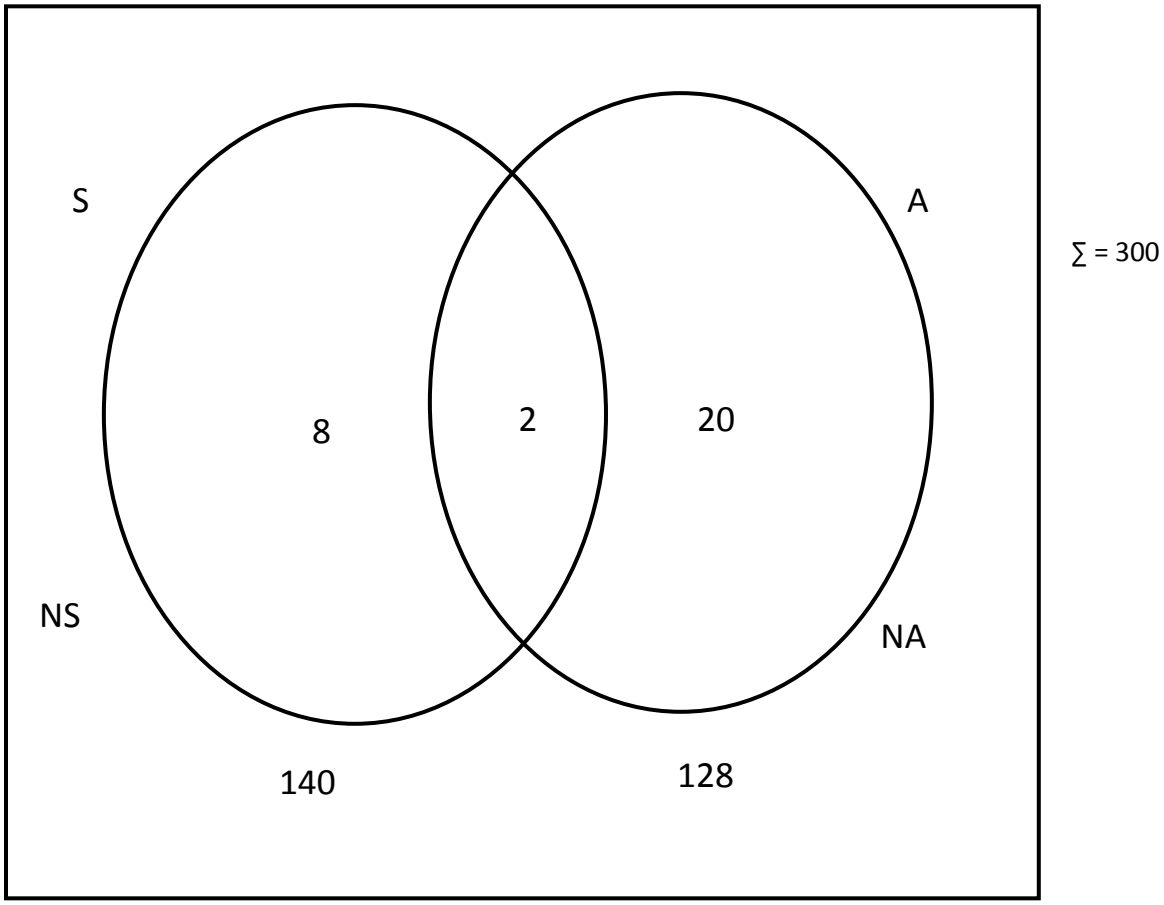


Figure 4.6: Venn diagram showing *Streptococcus pyogenes* infection and Anti-streptolysin "O" titer in the study population.

KEY:

S- *Streptococcus pyogenes* infection.

A- Anti-streptolysin O test.
c Total number of samples collected.

NS- Negative *Streptococcus pyogenes* infection.

NA- Negative Anti-streptolysin O antibody.

The microscopic appearance of *Streptococcus pyogenes* isolated from one of the throat swab samples is shown in Plate I.

Plate II shows the screening of presumptive isolates for the presence of *Streptococcus pyogenes* using Lancefield grouping test kit.

Plate III depicts the qualitative screening of sera samples for the presence of Anti-streptolysin O.

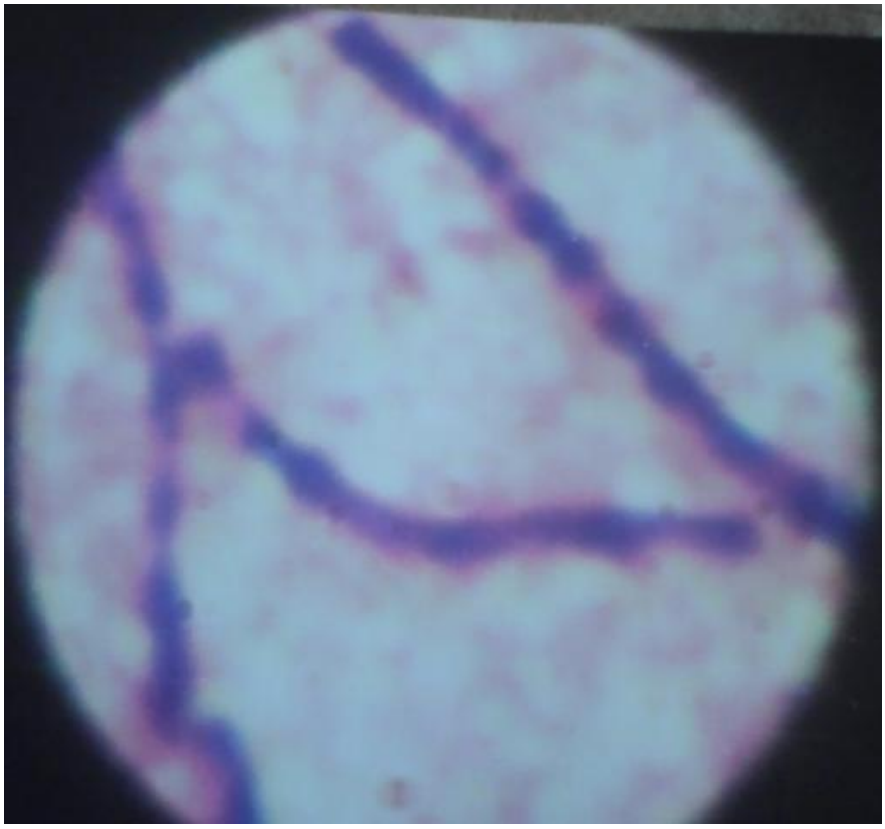


Plate I: The microscopic appearance of *Streptococcus pyogenes* under x100 objective.

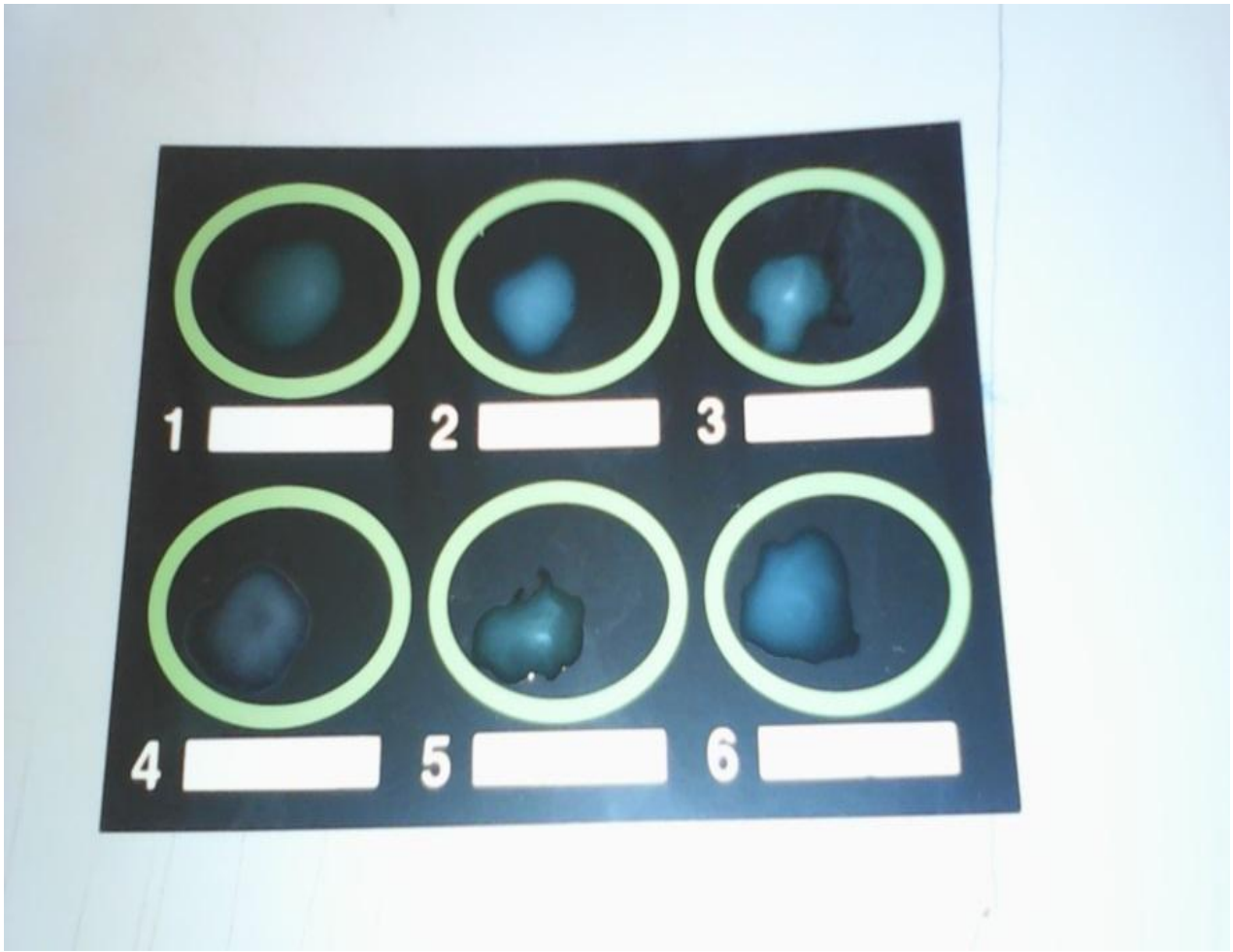


Plate II: Screening of presumptive isolates for the presence of *Streptococcus pyogenes* using Lancefield grouping test kit.



Plate III: Qualitative screening of sera samples to check for the presence of Anti-streptolysin O.

CHAPTER FIVE

5.0 DISCUSSION

Acute respiratory tract infection remains an important cause of childhood morbidity and mortality in developing countries despite the fact that it is potentially treatable and preventable (Gbadegesin *et al.,* 1997).

Various kinds of micro-organisms that infect the respiratory tract are transmitted through nasal and throat secretions of infected people, which are expelled as aerosols when they cough or sneeze (Constantainescu *et al.,* 2002). The organisms responsible for upper respiratory tract infections ranges from bacteria, fungi, viruses and other micro-organisms that are pathogenic and are present in dust and air. It is therefore, important to diagnose if a respiratory tract infection is a bacteria source or not, so as to control the indiscriminate use of antibiotics.

The relationship between *Streptococcus pyogenes* infection and sex, shows that the prevalence of 8.9% in males was higher than that recorded in females (4.2%). This report shows that male children were found to be at higher risk of acquiring the infection than the females. This could probably be because the males are often involved in outdoor activities than the females; they have higher chances of interacting more with other children, exchange more playing materials. However, it was not statistically significant (p= 0.2694), with Odds ratio 2.15 This is in agreement with the findings of Mawak *et al*.(2005) who reported a prevalence of 42.0% and 32.5% for males and females respectively. However, a study reported by Jitendra *et al*.(2014) was in contrast to this study with a prevalence of 31.4% in females and 29.8% in males.

In relation to the age group, the prevalence of *Streptococcus pyogenes* was shown to be highest in the age group 0-1 years (11.5%). However, it was not statistically significant (p= 0.1538), with Odds ratio 1.19. This could probably be due to maternal waning immunity seen in a child at this stage, thereby presenting the child with low immunity. Likewise, it could also be that some breast feeding mothers were found not to clean the surface of their breast before breast feeding and some were found to be putting money around their breast which could serve as source of infection for these children. This study agrees with a work reported in Jos Nigeria by Lar *et al*.(2005) who reported highest prevalence of 16.5% in children between age group 0-5 months.

In relation to occupation, the highest prevalence of 25.8% was reported in children whose parents are low income earners and are of low socio-economic status. Though, it was not statistically significant (p= 0.6667), with Odds ratio 1.27. The high prevalence observed in this group could be due to lack of adequate finance to take care of infected children, by taking them to the hospital for proper treatment. These findings, is in contrast to a work done by Nirmal *et al.*(2014) who reported a higher prevalence of 47.6% among children whose parents were of higher socio-economic group than those from low socio-economic groups. This could be due to negligence from parents towards children because of their busy schedules. Owobu *et al.*(2013) reported a prevalence of 40% among children whose parents were from middle socio-economic group.

Prevalence of *Streptococcus pyogenes* infection in relation to the educational status of parents was highest in children whose parents had no form of formal education, with prevalence of 25.3%. However, it was not statistically significant (p= 0.6443), with Odds ratio 1.65. This could probably be that parents are not enlightened and therefore lack knowledge on good hygiene

practices and preventive measures of the spread of the disease. This is in agreement with a work reported by Idika *et al.*(1999) in Abia State Nigeria.

In this study, higher prevalence of *Streptococcus pyogenes* infection was recorded in children without adequate ventilation in school (20.9%) and at home (28.3%) respectively. They were at higher risk of acquiring the infection than those with good ventilation, with Odds ratio 1.36 and 1.97 respectively. Though this was not statistically significant (p= 0.3934) and (p= 0.09) respectively. These reasons could be that the infection is more widely spread in areas where there is poor ventilation and over- crowding. This is in agreement with a work done by Madhumsti *et al.* (2014) who reported a prevalence of 62.2% for *Streptococcus pyogenes* infection among children who had poor ventilation at home.

Children with poor level of hygiene had higher prevalence (21.10%); likewise, parents with poor level of hygiene had higher prevalence (13.3%) as compared to those who practice good hygiene. However, it was not statistically significant (p= 0.4476) and (p= 0.6745), with Odds ratio 1.83 and 1.59 respectively. The reasons could probably be because poor hygiene practices are known to be predisposing factors to this infection.

In this study, there was no statistical significance in the previous history of exposure to *Streptococcus pyogenes* infection (p= 0.8933). Those who had an earlier exposure to the infection had a higher prevalence (20.5%) as compared to those who had no exposure with prevalence (19.4%), with Odds ratio 1.10. The reasons could be due to mis-diagnosis of infection or ineffective treatment during the first encounter with the infection.

Previous administration of drugs (antibiotics) was not statistically significant with the prevalence of *Streptococcus pyogenes* infection (p=0.5562), with Odds ratio 1.34. However, highest

prevalence (25.5%) was recorded for those who had incomplete medication compared to those who completed their medication with prevalence (13.9%) and (19.4%) for those who were not given any form of antibiotics. This result could be because those who had incomplete drug administration were prone to drug resistance and re-infection.

Record of outbreak in locality in this study, was statistically insignificant (p= 0.2422). Though, higher prevalence of 32.10% was reported in areas of outbreak compared to prevalence of 13.4% reported in areas of no record of outbreak. This result could probably account for *Streptococcus pyogenes* infection being an air borne infection which can easily spread through air droplets, fluids and contacts with infected skin from an infected person to another. This result agrees with other studies.

The result presented on Table 4.3 shows the relationship between *Streptococcus pyogenes* infection and clinical symptoms. A prevalence of 22.2% was recorded for patients who had no difficulty in swallowing food/water, compared to 19.0% who had difficulty in swallowing food and water, which was not statistically significant (p= 0.7175), with Odds ratio 1.17. A prevalence of 20.5% was recorded for those who do not have swollen lymphnodes and (16.7%) for those who had swollen lymphnodes. However, statistically insignificant (p= 0.7554), with Odds ratio 1.23.

A higher prevalence (23.3%) was recorded for those with sore throat compared with those without sore throat with (16.9%) which was statistically insignificant (p= 0.424), with Odds ratio 1.20.

Patients with fever had prevalence (23.08%) while those without fever had prevalence (50.0%). However, it was statistically insignificant (p= 0.0722), with Odds ratio 2.17.

Patients without cough had higher prevalence of 50.0% compared to those with cough with (17.9%) Though, it was statistically insignificant (p= 0.0704), with Odds ratio 2.8.

Patients with red rash on the skin had higher prevalence of 26.9% compared to those without rash on the skin with (18.5%). However, it was statistically insignificant (p= 0.4382), with Odds ratio 1.10.

In this study, those with sore throat and red rash on the skin had higher prevalence of 23.3% and 26.9% respectively. This could be because *Streptococcus pyogenes* infection is known to be attributed to sore throat and red rash on the skin. Higher prevalence recorded in those without difficulty in breathing (22.2%), those without swollen lymphnodes (20.5%), those without fever (50.0%) and those without cough (50.0%), could probably be because from the results obtained, lower number of positives were gotten compared to the large numbers examined. This findings is in agreement with a research carried out by Gray *et al*.(1991) who reported that the diagnosis of *Streptococcus pyogenes* infection has a condition confounded by a lack of consistently reliable diagnostic symptoms or clinical signs.

The prevalence of *Streptococcus pyogenes* infection in this study was 6.7%. However, this prevalence is lower than the prevalence of 30.7% reported in India by Nirmal *et al*.(2014), 38% reported in Niger by Sani *et al.* (2012). The lower prevalence reported in this study, may be due to the fact that majority of the patients examined were found to be on antibiotics. However, the prevalence recorded in this research was higher than a prevalence of 4.9% reported in Kwara by Oluwagunke (2013).

The result depicted in Figure 4.2 reveals the haemolytic pattern of isolates on cultured plates. One hundred and one (101) isolates showed beta-haemolysis on cultured plate, four (4) showed

alpha-haemolysis, eleven (11) showed gamma-haemolysis while thirty four (34) showed no growth. Biochemical tests were carried out which included: haemolysis, catalase, oxidase, motility and bacitracin test. From the biochemical test conducted, thirty (30) presumptive isolates were gotten and kept on slant for further confirmation, using the Lancefield grouping test kit. Out of the thirty (30) isolates, ten (10) were confirmed to belong to Group A (6.7%), two (2) Group C (1.3%), fourteen (14) Group D (9.3%) and four (4) Group F (2.7%). This result is in agreement with a work done by Benjamin and Perriello.(1976) who reported that there is a growing recognition of other sero-groups in human infectious diseases, with a number of tonsillitis associated with beta-haemolytic Group C.

The result presented in Figure 4.3 shows the occurrence of *Streptococcus pyogenes* infection and other Lancefield groups in relation to sex, using Lancefield grouping kit. In this study, male children were at higher risk of acquiring the infection with frequency of 7 for group A, 2 for group C, 9 for group D and 2 for group F, while females had frequency of 3 for group A, 5 for group D and 2 for group F. Both sexes had no group identified for groups B and G. The higher occurrence seen in males may be due to the fact that male children are more involved in outdoor activities, exchange more play materials with other children than the females and were at higher risk of acquiring the infection. This result agrees with other researches carried out in Nigeria and other countries.

The result depicted in Figure 4.4 shows the occurrence of *Streptococcus pyogenes* infection and other Lancefield groups among age groups. Children between ages 0-1 years, had the highest risk of acquiring the infection with frequency of 7 for group A, 1 for group C, 4 for group D and 1 for group F compared with ages 2-3 years with frequency of 1 for group A, 3 for group D and 2 for group F, ages 4-5 years had frequency of 2 for group A, 1 for group C, 7 for group D and 1 for

group F. The reasons, could attributable to the low immunity seen in children at this age group due to the waning maternal immunity common to them, as well as poor hygiene practices by some parents who do not clean the surface of their breast before breast feeding, while some were found to put money around their breast which could serve as source of infection for these children.

The result presented on Table 4.4 reveals the prevalence of *Streptococcus pyogenes* infection in relation to hospitals. Children in Gambo Sawaba hospital had the highest prevalence of 8.82% compared to children in Sickbay with (8.0%) and Health Care Centre with (3.51%). However, it was not statistically significant (p= 0.603) indicating that the occurrence of infection is not hospital specific. The result obtained could probably be because the highest number of samples were gotten from Gambo Sawaba hospital.

The susceptibility pattern of some isolates to some antibiotics discs namely: Erythromycin, Vancomycin, Ampicillin and Rifampacin is depicted on Table 4.5. These antibiotics were used because they are known to be the first-line drug for treatment of *Streptococcus pyogenes* infection. The highest susceptibility pattern was recorded for Erythromycin with prevalence of 50.0% for sensitivity, (20%) for intermediate susceptibility and (30.0%) for resistance. Lowest activity was recorded for Ampicillin with prevalence of 10.0% for sensitivity and (90.0%) for resistance. The high susceptibility pattern recorded for Erythromycin could be that the use of the antibiotics is not abused while resistance to Ampicillin could be as a result of inappropriate administration of drugs, use of expired antibiotics, self-medication and use of counterfeit drugs. This study is in agreement with the work of Okonko *et al.*(2009), whose work reported resistance of *Streptococcus pyogenes* to Ampicillin. Likewise, reports from other researches confirm

Erythromycin to have high activity against *Streptococcus pyogenes* infection. However, this is in contrast to a research carried out in Nigeria by Nkang *et al.* (2009).

The result depicted in Figure 4.5 shows the prevalence of 14.7% for Anti-streptolysin O (ASO). This prevalence was lower than the prevalence of 68.75% reported in Zaria, Kaduna State by Ella and Okafor (2015). However, the prevalence was higher than the prevalence of 3.5% in a research carried out by Gray *et al*.(1993).The differences in prevalence could be as a result of the different methods used as well as the population used in each study.

The result presented on Table 4.6 reveals the prevalence of 14.7% for patients who were positive to qualitative analysis and prevalence of 100.0% was recorded for patients who were positive to quantitative analysis, having all titers above the cut-off titer for children.

The titer level for all the positive sera samples is revealed on Table 4.7. The twenty two (22) positive sera samples gotten from qualitative analysis were subjected to further analysis quantitatively, in order to check for significance in titer level. Out of the twenty two (22) positive sera samples examined, all were found to be significant with titers above the cut-off titer for children (100IU/ml), indicating the possibility of a recent infection caused by *Streptococcus pyogenes.* However, the titer level obtained in this study was lower than the titer reported by Sethi *et al.*(2003). The differences could be due to the different population used in each study. The level of ASO in patients who tested positive for ASO varied from 200IU/ml to 16,000IU/ml. GS21 and GS7 showed the highest concentration of 16,000IU/ml. The mean ASO was 1772.7IU/ml.

The result presented in Figure 4.6 depicts the venn diagram showing infection of *Streptococcus pyogenes* infection in the throat and Anti-streptolysin O antibody in sera samples. Two (2)

individuals were found to be positive for *Streptococcus pyogenes* infection in the throat and ASO in the sera of the same patients. Out of the one hundred and fifty (150) throat swabs examined, ten (10) patients were found to be positive to *Streptococcus pyogenes* infection and out of the one hundred and fifty sera samples examined; twenty-two (22) were found to be positive to ASO antibody in the sera samples. The two (2) individuals who were found to be positive to culture and ASO could probably have been previously exposed to the infection without proper treatment, mis-diagnosis, use of counterfeit drugs thereby leading to re-infection. The result gotten in this research was lower than the eleven (11) reported in a work carried out in Kaduna State Nigeria, by Ella and Okafor (2015). The reasons could be due to different methods and study population used.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Some risk factors were found to be predisposing factors to *Streptococcus pyogenes* infection in this study, though they were not statistically significant. Such risk factors included: poor ventilation in school, poor ventilation at home, poor level of hygiene of children, poor level of hygiene of nursing mothers, previous exposure to infection and incomplete drug administration.

In this study, the prevalence of *Streptococcus pyogenes* infection from throat swabs of children aged 0-5 years was 6.7% and Anti-streptolysin O antibody had prevalence of 14.7%. ASO was found to be more sensitive than culture, probably because as with many infections, pathogen recovery may be impeded by culture due to empirically initiated antibiotics administration.

In this study, two (2) patients were found to be positive both to *Streptococcus pyogenes* infection in the throat swab (culture) and ASO antibody in the sera.

Erythromycin was found to be the most effective drug for treatment of *Streptococcus pyogenes* infection in this study.

Anti-streptolysin O remains useful, in the diagnosis of streptococcal infections and their complications, follow- up as well as in evaluating the effectiveness of treatment. ASO is a much easier method than cultural method and could provide baseline information use for diagnosis.

6.2 Recommendations

i. Proper and adequate ventilation should be encouraged in schools, day-cares and houses.

ii. Early and accurate diagnosis of streptococcal infection is important in order to avoid dissemination of the bacteria.

iii. Effective hand hygiene should be encouraged in our localities.

iv. Strict isolation procedures should be employed in patients who are admitted to hospitals with Group A streptococcal infection.

v. Health care givers should educate and encourage patients on the importance of drug regimen compliance.

vi. Government should engage in enlightenment campaign programs to enlighten localities on the clinical manifestations, mode of transmission, complications and the importance to seek immediate medical attention if contacts develop symptoms related to *Streptococcus pyogenes* infection.

vii. Culture and ASO assay should be deployed routinely in laboratories which help in effective diagnosis and in the prescription of appropriate antibiotics.

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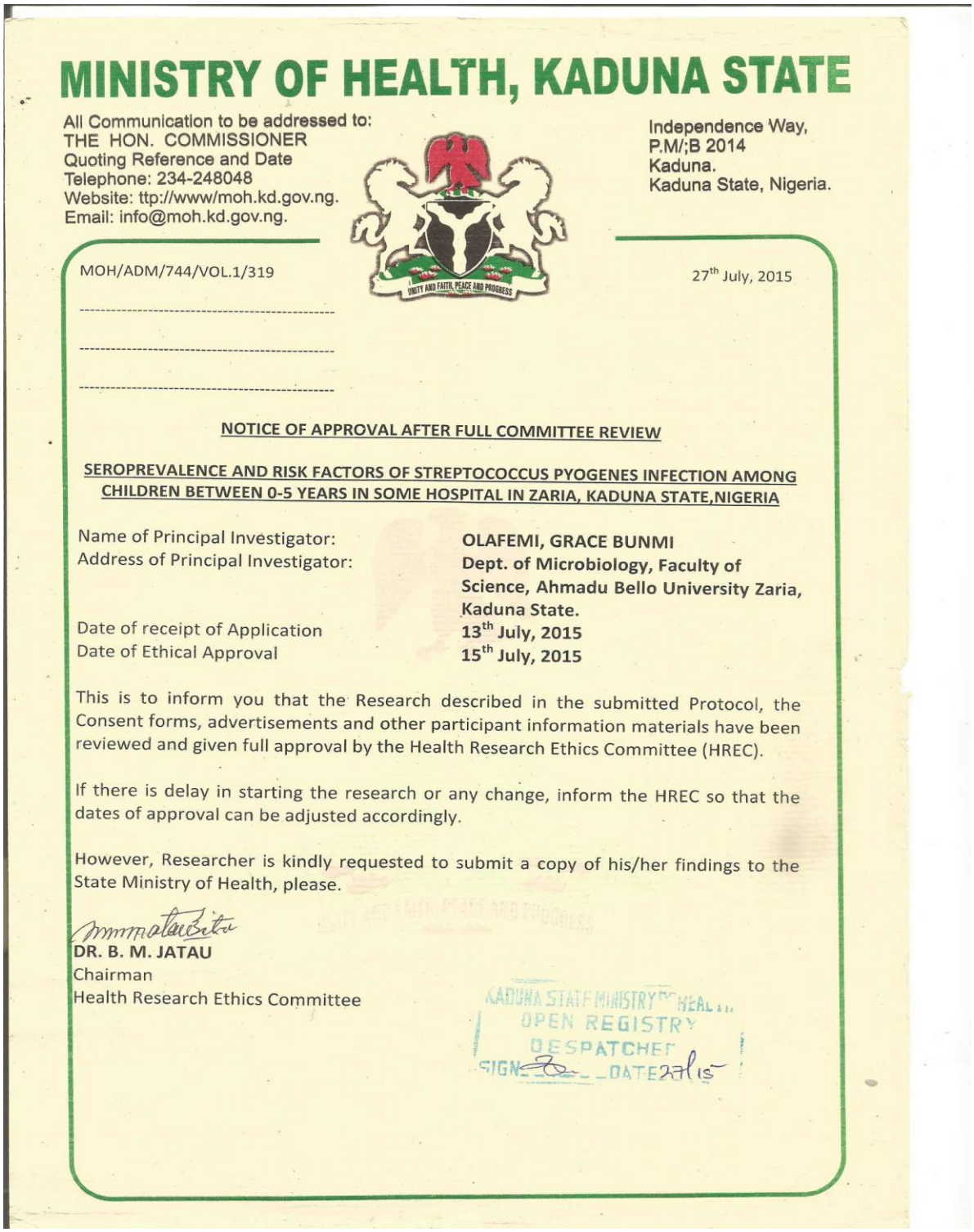
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APPENDICES

Appendix I: Ethical Approval Letter from Kaduna State Ministry of Health



MINISTRY OF HEALTH, KADUNA STATE

All Communication to be addressed to:
THE HON. COMMISSIONER
Quoting Reference and Date
Telephone: 234-248048
Website: ttp://www/moh.kd.gov.ng.
Email: info@moh.kd.gov.ng.

Independence Way,
P.M/;B 2014
Kaduna.
Kaduna State, Nigeria.

MOH/ADM/744/VOL.1/319

27th July, 2015

NOTICE OF APPROVAL AFTER FULL COMMITTEE REVIEW

SEROPREVALENCE AND RISK FACTORS OF STREPTOCOCCUS PYOGENES INFECTION AMONG CHILDREN BETWEEN 0-5 YEARS IN SOME HOSPITAL IN ZARIA, KADUNA STATE,NIGERIA

Name of Principal Investigator:	**OLAFEMI, GRACE BUNMI**
Address of Principal Investigator:	**Dept. of Microbiology, Faculty of Science, Ahmadu Bello University Zaria, Kaduna State.**
Date of receipt of Application	**13th July, 2015**
Date of Ethical Approval	**15th July, 2015**

This is to inform you that the Research described in the submitted Protocol, the Consent forms, advertisements and other participant information materials have been reviewed and given full approval by the Health Research Ethics Committee (HREC).

If there is delay in starting the research or any change, inform the HREC so that the dates of approval can be adjusted accordingly.

However, Researcher is kindly requested to submit a copy of his/her findings to the State Ministry of Health, please.

DR. B. M. JATAU
Chairman
Health Research Ethics Committee

KADUNA STATE MINISTRY OF HEALTH
OPEN REGISTRY
DESPATCHER
SIGN ... DATE 27/7/15

Appendix II: Consent Form

DEPARTMENT OF MICROBIOLOGY
SCHOOL OF POSTGRADUATE STUDIES
AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA.

INFORMED CONSENT FORM (ICF)

Serial No.: ..... *Hospital No.:* ..... *Age:* ..... *Phone No.:* .....

This Informed Consent Form is basically for collection of throat swab samples and blood samples. We are inviting you to participate in this research work titled "Seroprevalence and risk factors associated with *Streptococcus pyogenes* infection in children between ages [0-5years], in some selected hospitals in Zaria, Kaduna State". The research will involve the collection of throat swab samples and blood samples. The results obtained thereby may be used in any way to improve the understanding and management of the disease in our community.

RISK AND BENEFITS

The participants would not be exposed to any risk during the course of the research. Rather, the research would ensure that all cases that proof positive would be recommended to the right authorities for treatment. There would also be enlightment on the socio-demographic factors of the disease to all participants in order to avoid future occurrence of the disease both in positive and negative cases.

CERTIFICATE OF CONSENT

I .....of .....
hereby consent to participate in this study. The full procedures of the test/study have been explained to me by the investigator. I understand that throat swab samples and blood samples will be collected. I therefore give this consent voluntarily without being subjected to any pressure.

Name of Participant .....

Signature of Participant/ thumb print..... Date .....

Statement by Witness:

I have witnessed the accurate reading of the consent form and detail explanation of the study to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Name of witness.....

Signature of witness/thumb print..... Date .....

Statement by the Researcher/Person Taking Consent

I confirm that sufficient information, including about risks and benefits, to make an informed decision have been fully explained to the participant. The participant was given an opportunity to ask questions about the study, and all the questions asked by participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

Name of Researcher .....

Signature..... Date. .....

Appendix III: Research Questionnaire

TOPIC: Prevalence and risk factors of *Streptococcus pyogenes* infection among children between 0-5 years in some hospitals in Zaria, Kaduna State, Nigeria.

INSTRUCTION: Please kindly tick (√) as appropriate and provide answers where necessary, which will be treated and kept confidential.

SECTION 'A' BIODATA

1. Serial No. …… Hospital No:……
2. Name of Child: …….
3. Age (years): 0-1 [ ] 2-3 [ ] 4-5 [ ]
4. Sex: Male [ ] Female [ ]
5. Place of residence ……
6. Name of Hospital……
7. Occupation of parent:
   Civil servant [ ] Unemployed [ ]
   Others (specify) …….
8. Educational Status of Parents:
   Primary [ ] Secondary [ ] Tertiary [ ] None [ ]

SECTION 'B'

9. Has your child ever been down with this type of infection before?
   Yes [ ] No [ ]
10. Does your child attend any day care?
    Yes [ ] No [ ]
11. Is the daycare well ventilated/with windows?

Yes [ ] No [

12. What class is your child? ……..
13. Is he/her class well ventilated?
    Yes [ ] No [ ]
14. Is your house well ventilated?
    Yes [ ] No [ ]
15. Do you often clean the surface of your breast before breastfeeding?
    Yes [ ] No [ ]
16. Any history of antibiotics given to the child?
    Yes [ ] No [ ]
17. Any outbreak of sore throat infection in your locality?
    Yes [ ] No [ ]
18. If Yes, is there any complication during the outbreak in your locality?

Yes [ ] No [ ]

19. Do you often wash your child's hands before eating?

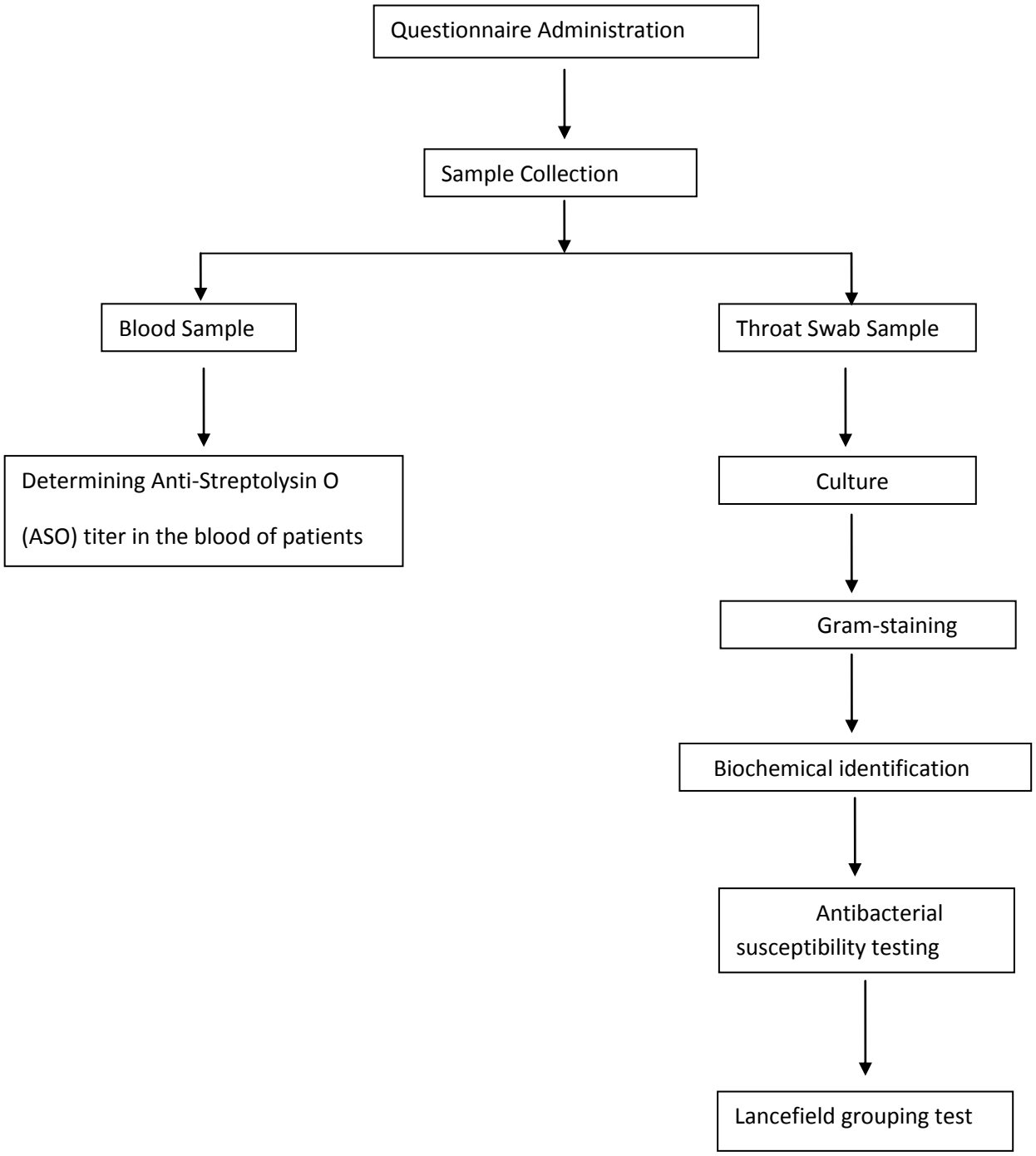
Yes [ ] No [ ]

20. If 'yes', with what?
    Water only [ ] Water and soap/detergent [ ]

SECTION 'C' CLINICAL INFORMATION

21. Difficulty swallowing food or water ? Yes [ ] No [ ]
22. Swollen lymph nodes? Yes [ ] No [ ]
23. Throat with pus, white patches/sore throat? Yes [ ] No [ ]
24. Fever? Yes [ ] No [ ]
25. Cough? Yes [ ] No [ ]
26. Red rash on the skin? Yes [ ] No [ ]

Appendix IV: Summary of Methods Used.



Appendix V: Biochemical characterization and identification of isolates.

Number of patients	Haemolysis test	Gram staining test	Catalase test	Oxidase test	Motility test	Bacitracin test	Lancefield test
1	β	Gram + cocci in pairs and short chains	-	-	-	+	Group A
2	β	Gram + cocci in pairs and short and long chains	-	-	-	-	Group D
3	β	Gram + cocci appearing in clusters	+	nil	nil	nil	nil
4	β	Gram □ rods in long chains	+	nil	nil	nil	nil
5	β	Gram □ rods in short and long chains	+	nil	nil	nil	nil
6	β	Gram + cocci appearing in pairs and short chains	-	-	-	-	Group D
7	β	Gram + cocci appearing in short chains	-	-	-	-	Group D
8	β	Gram + cocci appearing singly and in clusters	+	nil	nil	nil	nil
9	β	Gram + short rods	+	nil	nil	nil	nil
10	β	Gram □ short and long rods	+	nil	nil	nil	nil
11	β	Gram + cocci in pairs	-	□	□	□	Group D
12	ɤ	Gram □ short rods	+	nil	nil	nil	nil
13	α	Gram □ short and long rods	+	nil	nil	nil	nil
14	ɤ	Gram □ long rods	+	nil	nil	nil	nil
15	β	Gram □ cocci in short chains and few yeast cells	+	nil	nil	nil	nil
16	β	Gram □ short and long rods	+	nil	nil	nil	nil
17	β	Gram + cocci in pairs and long chains	□	□	□	□	Group D
18	β	Gram + cocci in short chains	□	□	□	□	Group F

19	β	Gram + cocci in appearing in clusters	□	nil	nil	nil	nil
20	ɤ	Gram □ short rods	+	nil	nil	nil	nil
21	β	Gram + cocci in pairs	□	□	□	□	Group C
22	β	Gram + cocci in pairs and short chains	□	□	□	□	Group C
23	β	Gram + cocci appearing in pairs	□	□	□	□	Group D
24	β	Gram + cocci appearing in pairs and short chains	□	□	□	+	Group A
25	β	Gram □ cocci appearing in pairs and yeast cells	+	nil	nil	nil	nil
26	β	Gram + cocci appearing in short and long chains	□	□	□	+	Group A
27	β	Gram + cocci appearing in short and long chains	□	□	□	□	Group D
28	β	Gram + cocci appearing in pairs	□	+	+	nil	nil
29	β	Gram + cocci appearing in short and long chains	□	□	□	□	Group D
30	β	Gram + cocci appearing in pairs	□	□	□	□	Group D
31	β	Gram + cocci appearing in short chains	□	□	□	□	Group F
32	β	Many yeast cells and few Gram □ negative rods	□	nil	nil	nil	nil
33	β	Gram + cocci appearing in clusters	+	nil	nil	nil	nil
34	β	Few yeast cells and Gram + cocci appearing in clusters	+	nil	nil	nil	nil
35	β	Gram + cocci appearing singly and few in clusters	+	nil	nil	nil	nil
36	β	Gram □ cocci appearing in chains	□	nil	nil	nil	nil
37	β	Gram □ rods appearing in pairs and short chains	□	nil	nil	nil	nil
38	No growth	nil	nil	nil	nil	nil	nil
39	ɤ	Gram + rods appearing in short chains	+	nil	nil	nil	nil
40	ɤ	Gram + rods appearing in pairs and short chains	+	nil	nil	nil	nil

41	β	Gram □ long rods mixed with Gram + cocci in clusters	+	nil	nil	nil	nil
42	ɤ	Gram □ cocci in short chains	+	nil	nil	nil	nil
43	No growth	nil	nil	nil	nil	nil	nil
44	β	Gram + cocci appearing singly and in clusters	+	nil	nil	nil	nil
45	β	Gram □ cocci in pairs	+	nil	nil	nil	nil
46	β	Gram □ short rods appearing in chains	+	nil	nil	nil	nil
47	No growth	nil	nil	nil	nil	nil	nil
48	No growth	nil	nil	nil	nil	nil	nil
49	No growth	nil	nil	nil	nil	nil	nil
50	No growth	nil	nil	nil	nil	nil	nil
51	No growth	nil	nil	nil	nil	nil	nil
52	No growth	nil	nil	nil	nil	nil	nil
53	β	Gram + cocci appearing in clusters	+	nil	nil	nil	nil
54	ɤ	Gram □ rods in short chains	+	nil	nil	nil	nil
55	No growth	nil	nil	nil	nil	nil	nil
56	β	Gram + rods in short and long chains	+	nil	nil	nil	nil
57	β	Gram + rods in short chains	+	nil	nil	nil	nil
58	β	Gram + cocci appearing in clusters	+	nil	nil	nil	nil

59	No growth	nil	nil	nil	nil	nil	nil
60	β	Gram + cocci appearing in short and long chains	□	□	□	+	Group A
61	No growth	nil	nil	nil	nil	nil	nil
62	β	Gram + cocci appearing in short chains	□	□	□	+	Group A
63	No growth	nil	nil	nil	nil	nil	nil
64	No growth	nil	nil	nil	nil	nil	nil
65	No growth	nil	nil	nil	nil	nil	nil
66	No growth	nil	nil	nil	nil	nil	nil
67	β	Gram □ rods in long chains	+	nil	nil	nil	nil
68	β	Gram + cocci in pairs and short chains	□	□	□	+	Group A
69	β	Gram + cocci appearing in clusters	+	nil	nil	nil	nil
70	β	Gram + cocci appearing in short chains	□	□	□	□	Group D
71	β	Many yeast cells	+	nil	nil	nil	nil
72	β	Gram + cocci in pairs and short chains	□	□	□	□	Group D
73	β	Yeast cells with few Gram □ cocci	+	nil	nil	nil	nil
74	β	Gram + cocci in short chains	□	□	□	□	Group D
75	No growth	nil	nil	nil	nil	nil	nil
76	β	Gram + rods appearing in chains	□	nil	nil	nil	nil
77	β	Gram + cocci appearing in clusters	+	nil	nil	nil	nil
78	β	Gram + cocci appearing in pairs	□	□	□	+	Group A

79	β	Gram + cocci appearing in pairs and long in chains	□	□	□	+	Group A
80	No growth	nil	nil	nil	nil	nil	nil
81	β	Gram + cocci in clusters and many yeast cells	+	nil	nil	nil	nil
82	No growth	nil	nil	nil	nil	nil	nil
83	No growth	nil	nil	nil	nil	nil	nil
84	β	Many yeast cells.	+	nil	nil	nil	nil
85	No growth	nil	nil	nil	nil	nil	nil
86	α	Gram + short rods appearing in long chains	+	nil	nil	nil	nil
87	α	Gram + short and long rods appearing in pairs	+	nil	nil	nil	nil
88	α	Gram □ cocci mixed with few yeast cells	+	nil	nil	nil	nil
89	No growth	nil	nil	nil	nil	nil	nil
90	β	Gram + cocci appearing in clusters	+	nil	nil	nil	nil
91	No growth	nil	nil	nil	nil	nil	nil
92	No growth	nil	nil	nil	nil	nil	nil
93	No growth	nil	nil	nil	nil	nil	nil
94	No growth	nil	nil	nil	nil	nil	nil
95	β	Gram + cocci appearing in clusters	+	nil	nil	nil	nil
96	β	Gram + cocci in long chains	□	□	□	□	Group D
97	β	Gram □ cocci appearing in chains and few yeast cells	+	nil	nil	nil	nil
98	β	Yeast cells	+	nil	nil	nil	nil
99	β	Gram + cocci in clusters	+	nil	nil	nil	nil

100	β	Gram + cocci in pairs	□	□	□	□	Group D
101	β	Gram + cocci in clusters and Gram □ short rods in pairs	+	nil	nil	nil	nil
102	No growth	nil	nil	nil	nil	nil	nil
103	β	Gram + cocci appearing in short chains	□	□	□	□	Group F
104	β	Gram + cocci appearing in pairs and short chains	□	□	□	□	Group F
105	β	Gram □ cocci in short chains	+	nil	nil	nil	nil
106	β	Gram + cocci appearing in pairs	□	+	+	□	□
107	β	Gram + cocci appearing in clusters	+	nil	nil	nil	nil
108	β	Gram + cocci appearing in pairs	□	+	+	□	□
109	β	Many yeast cells	+	nil	nil	nil	nil
110	No growth	nil	nil	nil	nil	nil	nil
111	β	Gram + cocci appearing singly and in clusters	+	nil	nil	nil	nil
112	β	Gram + cocci appearing singly and in clusters	+	nil	nil	nil	nil
113	β	Gram + cocci appearing in clusters	+	nil	nil	nil	nil
114	No growth	nil	nil	nil	nil	nil	nil
115	β	Gram + cocci appearing in pairs	□	+	+	□	□
116	β	Gram + cocci appearing in pairs	□	+	+	□	□
117	No growth	nil	nil	nil	nil	nil	nil
118	β	Gram + cocci appearing in clusters and few yeast cells	+	nil	nil	nil	nil
119	β	Gram + cocci appearing singly and in clusters	+	nil	nil	nil	nil
120	β	Gram + cocci appearing in clusters and many yeast cells	+	nil	nil	nil	nil
121	β	Gram + cocci appearing in clusters	+	nil	nil	nil	nil
122	β	Gram + cocci appearing in clusters	+	nil	nil	nil	nil

123	β	Gram + cocci appearing in short and long chains	□	□	□	+	Group A
124	ɤ	Gram □ cocci appearing in pairs	+	nil	nil	nil	nil
125	β	Gram + cocci appearing in long chains	□	□□	□	+	Group A
126	β	Gram + cocci appearing in clusters	□	nil	nil	nil	nil
127	β	Gram + cocci appearing in pairs and in few yeast cells	□	+	+	nil	nil
128	β	Gram □ cocci appearing in short chains	+	nil	nil	nil	nil
129	β	Gram □ cocci appearing in short chains	+	nil	nil	nil	nil
130	No growth	nil	nil	nil	nil	nil	nil
131	No growth	nil	nil	nil	nil	nil	nil
132	β	Gram + cocci appearing in pairs	□	+	+	nil	nil
133	ɤ	Gram + short rods appearing in pairs	+	nil	nil	nil	nil
134	β	Gram + cocci appearing in clusters	+	nil	nil	nil	nil
135	β	Gram □ cocci appearing in pairs and Gram □ rods in pairs	+	nil	nil	nil	nil
136	β	Gram + cocci in pairs	□	+	+	nil	nil
137	β	Gram + rods in pairs	+	nil	nil	nil	nil
138	ɤ	Many yeast cells	+	nil	nil	nil+	nil
139	No growth	nil	nil	nil	nil	nil	nil
140	β	Gram + cocci appearing in pairs and short chains	□	+	+	□	□
141	β	yeast cells	+	nil	nil	nil	nil
142	No growth	nil	nil	NIL	nil	nil	nil
143	ɤ	Gram + rods appearing in pairs	+	nil	nil	nil	nil
1444	β	Gram + cocci appearing in clusters	+	nil	nil	nil	nil
145	β	Gram + cocci appearing in pairs and short chains	+	+	+	nil	nil
146	β	Gram + cocci appearing in short chains	□	+	+	nil	nil
147	β	Gram + cocci appearing in pairs	□	+	+	nil	nil
148	β	Gram □ cocci appearing in pairs	+	nil	nil	nil	nil
149	No growth	nil	nil	nil	nil	nil	nil
150	β	Yeast cells	+	nil	nil	nil	nil

Appendix VI: Qualitative analysis result for Anti-streptolysin 'O' titer (ASO) in 150 sera samples.

samples	agglutination reaction	result interpretation
1	no agglutination	negative
2	Agglutination	positive
3	no agglutination	negative
4	Agglutination	positive
5	no agglutination	negative
6	no agglutination	negative
7	Agglutination	positive
8	Agglutination	positive
9	no agglutination	negative
10	no agglutination	negative
11	no agglutination	negative
12	no agglutination	negative
13	no agglutination	negative
14	Agglutination	positive
15	no agglutination	negative
16	Agglutination	positive
17	no agglutination	negative
18	Agglutination	positive
19	Agglutination	positive
20	no agglutination	negative
21	Agglutination	positive
22	no agglutination	negative
23	no agglutination	negative
24	Agglutination	positive
25	no agglutination	negative
26	no agglutination	negative
27	no agglutination	negative
28	no agglutination	negative
29	Agglutination	positive
30	Agglutination	positive

31	no agglutination	negative
32	no agglutination	negative
33	agglutination	positive
34	no agglutination	negative
35	agglutination	positive
36	no agglutination	negative
37	no agglutination	negative
38	no agglutination	negative
39	no agglutination	negative
40	no agglutination	negative
41	no agglutination	negative
42	no agglutination	negative
43	no agglutination	negative
44	no agglutination	negative
45	no agglutination	negative
46	no agglutination	negative
47	no agglutination	negative
48	no agglutination	negative
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50	no agglutination	negative
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53	no agglutination	negative
54	no agglutination	negative
55	no agglutination	negative
56	no agglutination	negative
57	no agglutination	negative
58	no agglutination	negative
59	no agglutination	negative
60	no agglutination	negative

61	no agglutination	negative
62	no agglutination	negative
63	no agglutination	negative
64	no agglutination	negative
65	no agglutination	negative
66	no agglutination	negative
67	no agglutination	negative
68	no agglutination	negative
69	no agglutination	negative
70	no agglutination	negative
71	no agglutination	negative
72	no agglutination	negative
73	no agglutination	negative
74	no agglutination	negative
75	no agglutination	negative
76	no agglutination	negative
77	agglutination	positive
78	no agglutination	negative
79	no agglutination	negative
80	no agglutination	negative
81	no agglutination	negative
82	no agglutination	negative
83	no agglutination	negative
84	no agglutination	negative
85	no agglutination	negative
86	no agglutination	negative
87	no agglutination	negative
88	no agglutination	negative
89	no agglutination	negative
90	no agglutination	negative
91	agglutination	positive
92	no agglutination	negative
93	agglutination	positive
94	no agglutination	negative
95	no agglutination	negative
96	no agglutination	negative
97	no agglutination	negative
98	no agglutination	negative

99	no agglutination	negative
100	no agglutination	negative
101	no agglutination	negative
102	no agglutination	negative
103	no agglutination	negative
104	no agglutination	negative
105	agglutination	positive
106	agglutination	positive
107	no agglutination	negative
108	no agglutination	negative
109	no agglutination	negative
110	no agglutination	negative
111	no agglutination	negative
112	no agglutination	negative
113	agglutination	positive
114	no agglutination	negative
115	no agglutination	negative
116	no agglutination	negative
117	no agglutination	negative
118	no agglutination	negative
119	no agglutination	negative
120	no agglutination	negative
121	no agglutination	negative
122	no agglutination	negative
123	no agglutination	negative
124	no agglutination	negative
125	no agglutination	negative
126	agglutination	positive
127	no agglutination	negative
128	no agglutination	negative
129	no agglutination	negative
130	no agglutination	negative
131	no agglutination	negative
132	no agglutination	negative
133	no agglutination	negative
134	no agglutination	negative
135	no agglutination	negative
136	agglutination	positive

137	no agglutination	negative
138	no agglutination	negative
139	no agglutination	negative
140	no agglutination	negative
141	no agglutination	negative
142	no agglutination	negative
143	no agglutination	negative
144	no agglutination	negative
145	no agglutination	negative
146	no agglutination	negative
147	no agglutination	negative
148	no agglutination	negative
149	no agglutination	negative
150	no agglutination	negative

KEY: Agglutination- Positive result.

No agglutination- Negative result.