EPIDEMIOLOGY OF GROUP A STREPTOCOCCAL INFECTIONS IN AND AROUND COIMBATORE

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Certificate

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CERTIFICATE

This is to certify that the dissertation work entitled "**Epidemiology of Group A Streptococcal infections in and around Coimbatore"** submitted by **Dr.S.Shanmuga priya** and this work was done by her during the period of study in this department from January 2015 to July 2016. This work was done under direct guidance of **Dr.S.Parvathi** Professor, Department of Microbiology, PSGIMS&R.

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Introduction

Streptococcus pyogenes is one of the pathogens which is common and can cause a wide range of diseases with varied differences in the severity. The frequent manifestation is pharyngitis.[1] *S.pyogenes* is one the leading cause of acute pharyngitis caused by bacteria resulting in about 15-30% of cases in children and 5-10% in adults[2]. *S.pyogenes* is capable of a wide range of infections which include suppurative and non-suppurative infections. The suppurative infections being streptococcal sore throat, pharyngitis, impetigo, scarlet fever, erysipelas, postpartum (puerperal) fever, necrotizing fasciitis, septicemia and toxic shock syndrome.

The non-suppurative complications being acute rheumatic fever,gas gangrene and acute post-streptococcal glomerulonephritis. During winter and spring, up to 20% of school-aged children who are asymptomatic may be Group A Streptococcus carriers falling in the age group of 5-15 years. Cellulitis is caused by *Staphylococcos aureus* mostly apart from *staphylococcus, Streptococci* also play a role in cellulitis. Person to person transmission is mainly through respiratory droplets or by direct contact with the nasal secretion of infected person or throat or by contact with infected wound on the skin. A study conducted in USA showed the highest mortality of 3/100,000 population due to *S.pyogenes* infections this is relatively uncommon when compared to other infections[3].

It has been estimated that nearly seven sore throat episodes occur per child per year with 13.5% of these being caused mainly by the Group A

Streptococcus (GAS)[2]. The detailed information on the occurrence of invasive streptococcal infections in India is limited. There are at least 517,000 deaths globally every year due to severe *S.pyogenes* infections and rheumatic fever disease are the major causes of 233,000 deaths. 1,800 invasive *S.pyogenes* disease-related deaths are reported in the USA per year, death caused by necrotizing fasciitis is about 30% of patients and streptococcal toxic shock syndrome has a mortality rate of 30-70%. Worldwide, there are estimated to be more than 600 million cases of GAS pharyngitis and more than 100 million cases of GAS pyoderma annually. Different clinical manifestations of this bacterium are more common in different parts of the world.[4]

Even though *S.pyogenes* is susceptible to penicillin, the infections continues to be a major health problem in developing countries because the epidemiology of streptococci is being poorly investigated, however the available data indicates that these infections are of high public health importance. Diseases caused by the Group A Streptococcus is a major problem in India and in some developing countries. Therefore, a continued study about these infections is imperative to monitor the epidemiological trends.

THROAT CARRIAGE OF STREPTOCOCCI

About 5 to 15% of cases of pharyngitis in adults caused by *S.pyogenes* and in case children 20 to 30 percent between 5 to 15 years of age. *S.pyogenes* is the most common cause of pharyngitis occurs among children between 5 and 15 years of age.

Untreated patients can develop purulent complications like otitis media, sinusitis, peritonsillar, oropharyngeal abscesses & cervical lymphadenitis. The importance of respiratory tract infection is related to its acute morbidity and its non suppurative sequelae i.e acute rheumatic fever and acute glomerulonephritis.

One of the leading causes of acquired heart disease in countries like Africa, India is Rheumatic fever, but it is uncommon in developed countries. Transmission rates of group A streptococcal infection is nearly 35% within a family or school if a patient is untreated. The carrier rate is different in developed and developing countries. It is usually spread directly by person to person contact through respiratory droplets or saliva. Respiratory droplets are the common mechanism of spread because this organism which is primarily located in the throat.

The symptoms of pharyngitis caused by *Streptococcus pyogenes* are sudden in onset.

The symptoms are

1. Throat pain
2. Fever and body pain
3. Headache
4. Nausea, vomiting
5. Abdominal pain mainly in younger children.

Scarlet fever may be associated with Streptococcal phryngitis which produces reddish popular lesions in the skin which spare the face and desquamate while recovery.

The diagnosis of streptococcal pharyngitis based on clinical grounds is unreliable. Symptoms and signs are variable and the severity of illness may vary from mild throat discomfort to classic exudative pharyngitis with high fever & prostration. The diagnosis is further complicated because the infection caused by many other agents may mimic the clinical symptoms of streptococcal pharyngitis.

If timely intervention is done by the physician for diagnosis of streptococcal pharyngitis by conventional methods, then the treatment can be

possible with the proper use of appropriate antibiotics and can prevent the long term non suppurative complication includes glomerulonephritis & rheumatic fever which has a prevalence of 4 to 6 per 1000 children per annum in the developing countries.

This study is aimed to find the epidemiology of Group A streptococcal infections in and around Coimbatore and also to study the prevalence of throat carriage of *Streptococcus pyogenes* among school children.

Review of Literature

Human pathogenic Beta Hemolytic Streptococci

Streptococci come under the family Streptococcacae. They are Gram-positive aerobic and facultative anaerobic organisms that occur in chains or pairs. Theodor Billroth in 1874 proposed the name *Streptococcus* and was the first to identify these organisms in patients with skin infections In Greek, *streptos* means chain and *kokhos* means berry, which refers to the globular shaped structures, hence the name of the genus, Streptococcus following which the individual streptococcal species became named after the diseases they caused or sites of infection. The species *pyogenes* was coined by Friedrich Julius Rosenbach in 1884.[5]

At the beginning of the 20th Century, the classification of streptococci was based on their differential capacity to induce hemolysis on blood agar following which a serological classification based on group specific polysaccharides was proposed by Rebecca Lancefield during the 1930s. Further subdivision of group A streptococci by her, was based on the M protein, which is found on the surface of the bacterial cell wall. It is a virulence factor and prevents phagocytosis. During the 1920s and 1930s, further researches contributed to the identification of the toxins (streptococcal pyrogenic exotoxins) produced by streptococci and their role in the pathogenesis of scarlet fever.

Streptococcus species and their Lancefield grouping

- *S. pyogenes* comes under Lancefield group A and it causes human infections alone.
- *S. agalactae* belongs to group B and it causes both human and bovine infections
- *S. dysgalactiae* Subsp. *Equisimilis* belong to various groups like A,C,G,L and it causes both animal and human infections.
- *S. equi* Subsp. *Zooepidemicus* comes under group C and it causes both animal and human infections
- *S. canis* belongs to group G and it causes infections in dogs and human.
- *S. anginosus* (group) belongs to Lancefield group A,C,F,G and it causes human infections
- *S. constellatus* , Subsp. *Pharyngis* come under group C and it causes infections in human.[5]

A study from USA reported about the re emergence of GAS infections mainly rheumatic fever which is one of the non suppurative complication of S. pyogenes. M1 and M3 sero types plays major role in the invasive infections of S.pyogenes.

Streptococcal toxic shock-like syndrome, a newer disease caused by Group A Streptococci was identified in 1980 by USA. The reports of which were reviewed by a CDC working group and a case definition for Streptococcal toxic shock-like syndrome was established. Necrotizing fasciitis, pneumonia, puerperal sepsis, septic arthritis, STSS and non-focal bacteraemia are some of the invasive diseases caused by *Streptococcus pyogenes.*[6]

Group A streptococci (GAS), *Streptococcus pyogenes*:

Carriage:

Streptococcus pyogenes produces human infections it does not cause any infection in animals. It can be carried asymptomatically on the superficial layers of the skin and the mucous membranes like, the oropharyngeal mucosa, the nasal mucosa, the perianal area and the genital tract. A pharyngeal carrier of GAS is defined as an asymptomatic individual with a positive throat culture, with no active immune or inflammatory response, or an asymptomatic child who after completing the antimicrobial treatment of GAS pharyngitis tests positive for GAS.[7-9]

Children in the age group of 5-15 years are considered to be the major reservoir of GAS and 15-25% or more of them are found to be pharyngeal carriers. The pharyngeal carrier rate in adults is found to be <5%, which when compared to children is very low. But the carrier state can vary with age, season and the geographical location. Carriers can either be a transient carrier

or a persistent carrier, and a new type of GAS can replace a carried strain. Persistent throat carriage may be due to the internalization of GAS into the epithelial cells, which protects the pathogen from the antimicrobial agents.[7]

Transmission:

The transmission of bacteria from carriers, especially pharyngeal carriers to other persons and the surroundings occur by

- Direct contact with respiratory secretions
- Aerosol spread by droplets

Disease spectrum

The mortality of invasive S. pyogenes infection is high, ranging between 10% and 30%. 650,000 deaths per year have been estimated to be due to these infections. *Streptococcus pyogenes* causes a wide range of clinical infections including respiratory, cutaneous, skin, soft tissue and other systemic infections. Suppurative infections commonly associated with this organism are as follows:

Non-focal bacteremia is a suppurative infection produced by *S.pyogenes* Impetigo, carditis, meningitis, cellulitis, erysipelas are the skin and soft tissue infections of *S.pyogenes*

Epiglottitis, Pharyngitis, Empyema, Scarlet fever, Mastoiditis, Tonsillitis, Otitis and Pneumonia are the upper and lower respiratory tract

infections. Peritonitis, Appendicitis are abdominal infections caused by *S.pyogenes* Puerperal sepsis, Vaginitis are the pelvic infections

Acute tonsillitis and acute pharyngitis are the 2 common infections produced by *S.pyogenes.* Certain strains of *S.pyogenes* express one or more pyrogenic exotoxins (A, B or C). Scarlet fever is caused by one such strain and is thus associated with toxin-mediated symptoms like rash, strawberry tongue, and skin desquamation. Before the advent of antimicrobials, Scarlet fever used to be fatal infection and caused epidemics with a high mortality. But in this modern era, with the availability of appropriate antimicrobials, it has now become less severe, that present as pharyngitis with skin rash.

In addition to these infections, *S. pyogenes* can also cause various other localized infections, such as pneumonia, puerperal sepsis, meningitis, peritonitis and septic arthritis. Puerperal sepsis, a dreadful disease, also known as childbed fever, is an infection predominantly caused by GAS. In Europe it was most common cause of high mortality in young women during 18^{th} century.

Streptococcal toxic shock syndrome is the most fatal complication associated with S.pyogenes infection. It is also associated with highmortality[7]. STSS and scarlet fever comes under toxin-mediated diseases caused by *S. pyogenes.*

Non suppurative complications

Infection with *S.pyogenes* also causes two potentially serious non suppurative complications which includes acute rheumatic fever and acute glomerulonephritis

Acute rheumatic fever:

Acute rheumatic fever (ARF) commonly occurs after two to three weeks following group A streptococcal pharyngitis which occurs most commonly in children. It has cardiac, rheumatologic and neurologic manifestations. Since many physicians from most developed countries have little or no practical experience with the diagnosis and management of ARF, its incidence has declined in these areas. But outbreaks occur occasionally in the United States and pose a threat to public health.

Diagnosis is based on various clinical manifestations that develop as a result of group A streptococcal pharyngitis. The major clinical manifestations include subcutaneous nodules, migratory polyarthritis, erythema marginatum, carditis and chorea. If the pharyngeal infection is treated promptly, acute rheumatic fever can be completely prevented. Hence larger attention has been drawn towards preventive measures of this condition.

Acute glomerulonephritis:

Non-complement fixing antibodies to glomerular epithelial cell membrane antigens and those resembling membranous nephropathy are induced by glomerular epithelial cell membrane insertion of the C5b-9 membrane attack complex of complement. The mechanisms of these effects at the cellular level are however unclear but may involve the activation of glomerular epithelial cell and the release of local mediators like proteases or oxidants, or detachment of glomerular epithelial cell from underlying basement membrane.

The circulating inflammatory cells like neutrophils, macrophages, platelets and/or the resident glomerular cells (mesangial cells) mediate inflammatory types of glomerular lesions. Glomerular injury mediated by neutrophil involves the local release of glomerular basement membrane degrading proteases or glomerular basement membrane halogenations which is induced by the interaction of neutrophil-derived myeloperoxidase with hydrogen peroxide and a halide. Glomerular injury thus induced by neutrophil is augmented further by platelets.

Other non-suppurative complications:

One of the fatal complications of *S.pyogenes* is Streptococcal Toxic Shock Syndrome.(STSS) Infection in any part in the human body may lead to STSS, but it most commonly occurs in association skin infection. It is

characterized by toxic signs and a rapidly progressive clinical course and is associated with a very high mortality rate exceeding 50%.

Psoriasis is one of the most common skin lesions found in adults and is distributed worldwide. It is characterized by chronic, well defined, erythematous, dry, plaques of different sizes, covered by silvery white, imbricated and lamellar scales, with corresponding histological skin changes. It is associated with considerable impairment in the quality of life of the affected patients. The prevalence of psoriasis differs in different populations ranging from 0.1% -11.85% in accordance to the published reports[10]. The etiology and pathogenesis of the disease is still very unclear but the evidence from various studies suggests that it is caused by the interaction of one or more genetic components & environmental factors including β-hemolytic streptococci.

S. pyogenes can also exist in the female genital tract. Bacterial vaginosis is caused by various organisms, and S.pyogenes was not previously considered as one of the causes of this bacterial vaginal infection.[11] In last two decades the incidence of *S.pyogenes* vaginosis has increased.

Studies regarding this increased prevalence suggest that the persons who carry *S. pyogenes* in their respiratory tract either as normal commensal or as a pharyngeal infection introduce the pathogen into the female genital tract.

Owing to the increase in the incidence of infection, most of the laboratories are making changes in the protocols related to the detection and

isolation of *S. pyogenes*. Many laboratories have now included *S. pyogenes* in their protocols as a potentially dangerous vaginal pathogen that, must be identified, in addition to other bacterial pathogens, when present.[9]

The recognition of targeted dysfunction of the basal ganglia has led to the hypothesis that Sydenham's chorea and other central nervous system illnesses have a post-streptococcal autoimmune etiology. Pediatric autoimmune disorders associated with Obsessive compulsive disorder or Tic disorders also appear to occur in association with Group A streptococcal infections. In addition, the post infectious complications of streptococcal infection also include dystonia, dystonic choreoathetosis and chorea encephalopathy as documented in various studies. Various antistreptococcal-antineuronal antibodies and also genetic markers in patients who are susceptible to these illnesses have been described and isolated by many investigators.[12]

Virulence factors

The successful colonization of mucosa of the upper respiratory tract and skin forms the initial step in the pathogenesis of group A Streptococcal infection.

The adherence factors include:

1. Lipoteichoic acid,
2. M protein and pili,

3. Fibronectin-binding proteins like Sfb1 and Sfb2

These are some of the adherence factors for epithelial cells which plays major role in the virulence. The fibronectin binding protein will help in the endocytotic uptake of the organism into the respiratory epithelial cells. This process of invasion into the cell permits the organism to gain access to a intracellular niche, and this is the first step in the pathogenesis.

The potency of Group A Streptococci to resist the normally functioning innate clearance mechanism of the host for preventing microbial dissemination is defined by the ability of the organism to cause serious infections in otherwise healthy adults and children [13-15]. For instance, when the pathogen invades the deeper tissues through a break in epithelial integrity or through cellular invasion, specific peptidases are produced which inactivate the neutrophils. Similarly, it also secretes cysteine protease, which along with the streptococcal pyrogenic exotoxin (Spe) B, can lead to degradation of the antibodies produced by the host.[16]

Streptolysin S (SLS) and Streptolysin O (SLO) are the pore-forming toxins secreted by *S.pyogenes* are lethal to macrophages and neutrophils present in the host and thus help in promoting tissue damage and resistance to phagocytic clearance. The accelerated apoptosis of immune cells is induced by Streptolysin O.[16]

The hyaluronic acid capsule of GAS prevents the phagocytic recognition by simulating a common human matrix and thus blocking the opsonin targets. Under the influence of innate immunity inside our body the highly invasive form of M1T1 GAS strain, develop mutation in the covR/s gene which increase the capsular component and other virulence factors phage mediated virulence factor DNAse Sda1 protects the GAS from neutrophil mediated killing. Serum inhibitor of complement (SIC) mainly acts by inactivating the complements. There are more than 15 types of bacterial super antigens that are grouped under SPE family, among which SPE A and C are encoded by bacteriophage[17].

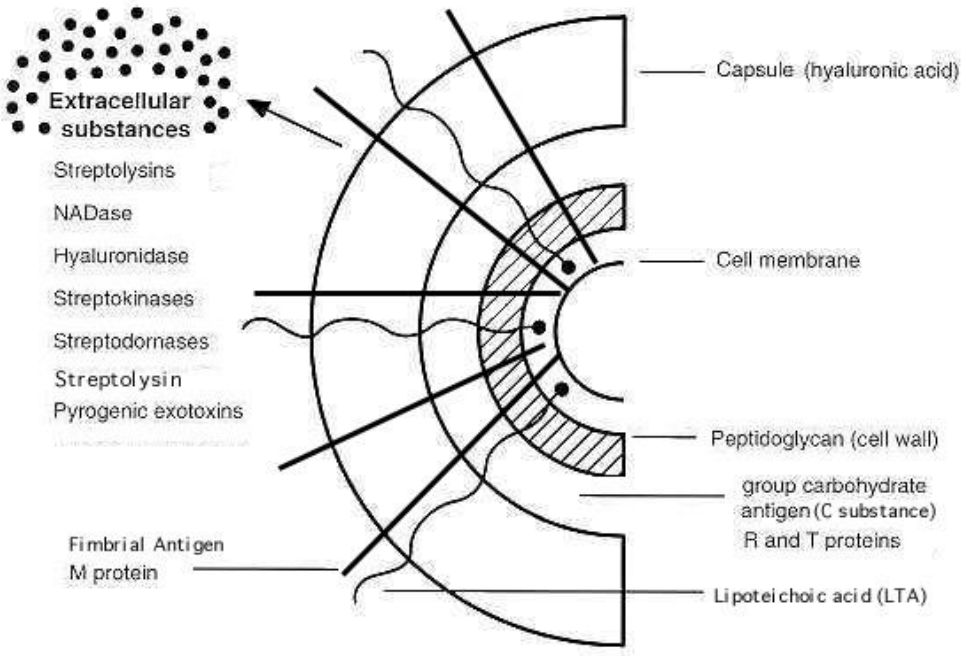
These super antigens causes enhanced activation of T lymphocytes, inhibit antibody synthesis, induce endotoxic shock, fever and also the release of pro inflammatory cytokines. It can lead to multi organ dysfunction, which is characteristic of STSS. SpeA producing strains have no antibodies to both M protein and super antigens but they have specific human leucocyte antigen. These strains are commonly associated with STSS in US. spe A and C in scarlet fever induce specific antitoxin antibodies which prevents the recurrence of rash in scarlet fever but doesn't prevent the recurrence of GAS infection.[18] *S. pyogenes* penetrate the host tissues by various virulence factors.

The hyaluronic acid capsule prevents the bacteria from the attack of neutrophils. M protein, lipoteichoic acid and protein F helps in the attachment of bacterium to the host cell. Among these the M protein helps the bacteria to escape from the alternate complement pathway and some serotypes capable of

binding to the fibrinogen and thus preventing opsonization. Our immune system mainly target against these M proteins which are unique to bacteria.[5]

M protein

The M proteins were discovered by Lancefield and constitute the structural basis for typing of GAS. They are fibrillar proteins projecting about 60 nm out from the streptococcal cell wall with a molecular weight of 35, 000 KDa. Two alpha-helical proteins form a so-called coil-coiled structure which was quite similar to the alpha-helical coiled-coil structure in host tissue proteins like tropomyosin and the keratin-desmin-vimentin and keratin-myosinepidermin-fibrinogen-families of molecule. Functionally, the M-protein inhibits phagocytosis, which is a primary virulence mechanism for survival in tissues.



The major function of M protein is tissue invasion & adhesion mainly in respiratory and skin tissues, this M protein plays role in tissue tropism based on serotypes. This M protein has variable n-terminal region which plays major role in the resistance of phagocytosis. In addition this M protein has property of binding to the human immune system by non overlapping site.

The complement inhibitor C4b-binding protein and IgA-Fc.[19] are the two main factors to which the M protein gets attached. emm gene codes the M protein as far now more than 150 emm types have been identified. Extensive study about this emm gene has major role in Group A Streptococcal vaccine development. PrtF1 and PrtF2 are the fibronectin proteins which also take part in the tissue adhesion and intracellular invasion by *S.pyogenes*[20-22]

The GAS genome

The size of the GAS gene is 1.9mb roughly, it has single chromosome which is circular in nature. 10 M types have been sequenced so far, the size of the genome varies between 1.815, 783-1.937. it has the base pair size of 112 bp and it varies with strains.

Pathogenesis of GAS infections:

GAS causes serious infections in humans by 3 main mechanisms like:

i. Suppuration, which is found in pyoderma and pharyngitis

ii. Elaboration of the toxin, which is the case in Staphylococcal toxic shock syndrome (STSS) and

iii. Immune mediated inflammation, which happens in PSAGN and Acute rheumatic fever [23]

Rheumatic fever (RF) is a condition where it affects arterioles around the connective tissues, and also untreated streptococcal throat infection can act as a predisposing factor. The mechanism of causing infection is supposed to be due to cross reactivity within antibodies. This basically is a reaction of class two hypersensitivity and hence it is known as molecular mimicry. Often, self reactive B cells can be anergic in the circulating periphery without a co-stimulation from the T cells. In case of infection, antigen-presenting cells (APC) which are mature like B cells can present antigen of the bacteria to CD4+ T cells which eventually differentiate into T_2 Helper cells. These cells further activate these B cells to transform into plasma cells and promote antibody production against the streptococcal cell wall. However, antibodies can react even against the joints and myocardium thereby, producing symptoms and signs of rheumatic fever.

The cell wall of *Streptococcus pyogenes* is made of polymers which are branched which can contain protein M which are very much antigenic. The immune system generates antibodies against the protein M which may cross react with the muscle cell protein of the heart called myosin, glycogen of the

smooth muscles of arteries and heart muscle, thereby inducing release of cytokine and destruction of tissues. The only cross reaction proven is that with the peri vascular connective tissue. This reaction occurs by directly attaching the complement and receptor Fc mediated recruitment of macrophages and neutrophils.

Aschoff bodies are characteristic, containing swollen eosinophilic collagen which is swollen and surrounded by macrophages and lymphocyte which is visible on light microscopy. Anitschkow or Aschoff giant cells are the transformed macrophages which are larger in size. Cell-mediated immunity is involved in rheumatic valvular lesions reaction as these contain macrophages and helper T cells.

The above said lesions can be found in any layer of the heart in rheumatic fever thereby causing carditis of different types. It can cause a pericardial exudate which is sero fibrinous known as 'bread and butter' pericarditis, and it usually resolves without any particular sequelae. Involving the endocardium results in the formation of a verrucae and fibrinoid necrosis along with the lines of closure of left sided heart valves. Projections which are warty can arise from the deposition, whereas lesions in the sub endocardium may induce thickening which are irregular, and known as MacCallum plaques.

Acute glomerulonephritis (AGN) consists of a particular set of renal diseases which are immunologic and inflammation is triggered and the

glomerular tissue gets proliferated which results in damaging the basement membrane, capillary endothelium or mesangium. Acute nephritic syndrome is the devastating form among the various renal syndromes.[23]

Antigenic sharing of Group A Streptococci

A study conducted in Japan about the antigenic sharing of *S.pyogenes* with *Streptococcus mutans* showed that there was a polysaccharide antigen shared by these two Streptococcus species. In this study they have used animals to investigate the antigenic property by using mono clonal antibody f-77 which reacts with the polysaccharide antigen. This antigen was isolated from patients with post glomerulonephritis.

Immunological response

Group A Streptococci has many antigenic components and that gives type specific protective immunity. The antibodies produced against this M protein will stay upto many years and give immunity against severe infections caused by GAS but not give any protection against pharyngeal carriers[24] the specific feature of these immunoglobulins are transplacental transmission.

The antibodies against M protein will appear one month after the initial infection, hence these antibodies will help in the prevention of re infection by the same serotype of S.*pyogenes*. Antistreptolysin O antibodies which are produced by host humoral immune system can be identified by neutralization test. This will be useful in the epidemiological study about the GAS infections.

Estimation of ASO titre is one of the useful test for identifying the streptococcal antibody but the sensitivity of the test is low since these ASO produced by both Group A and Group A Streptococci. The ASO titre will be high in children than in adult normally.

Characterization of strains and its classification

S.pyogenes has many surface proteins which are responsible for its virulence. There are three surface proteins namely protein T, protein M and serum opacity factor protein. Serogrouping of *S.pyogenes* is mainly based on these proteins.

As for now 25 serotypes have been identified based on agglutination in T protein. In M protein, based on hyper variability of N terminal it is classified into 80 serotypes. PCR method also plays a role in the serogrouping of M protein by using different probes.

Genotyping of the *S.pyogenes* based on emm gene (180bp) is the gold standard test currently. The original serologic M types were up to M 81, and the new types of *emm* from 82-124 are validated and accordingly added [7]. Currently over a hundred *emm* types have been sequenced and higher numbers are being identified and stored at CDC. Pulsed field gel electrophoresis where they target the whole bacterial genome and it is the typing method which is being used widely.

Epidemiology of *S.pyogenes* infection:

S. pyogenes is widely present all over the world. A study conducted by US showed that, over ten million cases of throat infections are documented annually. Of all the reported cases, only 5 to 10% was caused by streptococcal throat isolates, with Beta Hemolytic Streptococci being the most common agent.

It is estimated that around five to fifteen percent of normal individuals carry the bacteria which are known to cause sore throat called as colonisation. The mortality and morbidity rate of sore throat caused by *Streptococcus* is minimal. The mortality and morbidity rates arising from the complications of streptococcal throat infections are increasing. Data from CDC reports every year has reported an occurrence of 500 to 1000 cases of necrotizing fasciitis and 2000 to 3000 cases of STSS caused by GAS. Throat infections caused by *Streptococcus* may spread by direct or airborne contact with droplets like saliva or nasal discharge from any person infected with *Streptococcus pyogenes.* Some isolated reports of infection through contaminated food and indirect contact by handling objects of the person infected.

Streptococcus pyogenes will enter through the abraded skin open sores or through the oral cavity and reaches the blood for its nutrients. Children are prone to GAS infection than any other population as they are less caring at protecting themselves or the others from spreading of the infections. This is

more when children are in close contacts such as schools and daycares. The higher infection rates occur between the months of October to April. Since the easy availability of the antibiotics the prevalence rate of GAS infections mainly rheumatic fever has declined[7].

Age and sex specific rates of infection:

A study about the prevalence of GAS pharyngitis reported that approximately 616 million of Group A Streptococcal pharyngitis occurs per year globally. In developing countries like India the epidemiology of GAS infections are not documented properly.

Asymptomatic carrier of GAS pharyngitis which is common among children is distributed variedly from one country to other in the range of 9% to 34.1%. But in India the prevalence is around 4.2% to 13.7% which is as similar to the rates as reported from developed countries (USA)[25]. Similarly the prevalence in various parts of India varies from 11.2% to 34%. Around 0.3% in endemic and 3% in epidemic resulted in acute rheumatic fever and among these 60% developed RHD (rheumatic heart disease) by the damaged heart valves. Even though we could see a declining pattern of ARF/RHD in various parts of the world, it still continues to be a major cause of cardiovascular mortality and morbidity in India. The spread of GAS infection is common in crowded and closed areas. The gold standard diagnosis is throat culture. It takes around 2 to 5 days to enter and cause the first symptom in case of streptococcal pharyngitis.

In south India the research on Group A streptococcal infections are very cumbersome and it is mainly a hospital based study. As per these studies the prevalence of these streptococcal infections are common among low socio economic status children who are less than 15 years [26,27].

When compared with general population below 5 years Children and above 65 years have the higher incidence of GAS infections. A study conducted in Sweden showed the incidence rate of GAS infections in children was 2 or less per 100,000 populations. This is lower than the developed countries like USA, Canada. The incidence of GAS infections showed a slight male dominance in some countries.[28]

Mortality associated with invasive infections

Persons those who stay in long term care facilities are more prone to get GAS infection than others. These persons show high case fatality rate than general populations. Every year approximately 163,000 deaths reported due to GAS infections as per WHO estimation; this may be low because of the unavailability of data from many developing countries.

Estimation of case fatality rate is based on many factors like death within seven days of infection and other measures.[29]

Trends in emm type prevalence:

emm gene codes the M protein , which is responsible for most of the GAS infections. There are many types in emm gene but most prevalent types are emm type 1, 3, 12, 28 and 89. Approximately 50% of the isolates belong to any one of these five types [11]

Factors predisposing to invasive S.pyogenes infections:

There are many factors involving in the occurrence of GAS infections. Mainly age, sex, climate and socio-economic status of the person. Related to age, extremes of age is more prone to get infection than adults. Overcrowding and contact with the children who have the sore throat will increase the risk. Low socio economic status mainly poor hygienic practices may increases the chance of getting infections.[30]

Immunocompromised patients and persons with debilitating diseases like diabetes, cardiac illness and malignancy are the risk factors. The other risk factors include alcoholism, IV drug abusers and burns or trauma to the skin. Nosocomial spread also occur with GAS infections. Roughly about 17-20% of GAS infections occur without any risk factors[31-33]

Seasonal variation:

The main feature of *S.pyogenes* infections is seasonal occurrence. The frequency of invasive disease caused by *S.pyogenes* varies depending on

season; the frequency is higher between late winter and early spring. Late summer/autumn shows lower frequency but few exceptions have been reported in Europe. The invasive disease like scarlet fever and streptococcal pharyngitis show the similar seasonal pattern. The etiology of impetigo is shared between *Staphylococci & Streptococci* and it has the peak in summer and it may be related to insect bite. USA and Canada shows the similar seasonal pattern apart from Europe and it has been reported in a study from USA. A study about *Streptococcus suis* infection in pigs showed the same seasonal pattern and the similar seasonal pattern showed by invasive pneumococcal disease also[34]. The reasons behind the seasonal variations are change in environmental conditions, effect of climatic changes on our defense mechanism, effect of ultraviolet rays on our immune system and also seasonal viral infections add to this.[7]

Treatment and preventive measures

Control of *S. pyogenes* in community settings

There are only limited chances to prevent the occurrence of *S.pyogenes* infections Varicella infections are the important risk factors among children and so preventing such infections could lead to reduction in streptococcal infections. A study conducted in Canada showed that varicella vaccination if administered to children universally it would result in prevention 10% of the streptococcus infections. Only certain developed countries have decided to administer varicella vaccination in their immunization schedule.[35] Recurrence

of infections occur among drug users and so steps should be taken to detect signs of disease among them at the earliest. The management of critical situations depends on the timely decisions and the administration of appropriate antibiotics. Activities like military camps and sports result in increased chance for occurrence of soft tissue infections. Under these situations they should be advised not to share personal things and also about decontamination of shared items.

The limited data available about the risk among household contacts is the reason for limitations in secondary control measures to prevent the infections. Collecting such data will provide information of all the involved cases in a particular population in that period, risks involved among households. But these estimates are prone to have increased margin of error since the sensitivity of surveillance system is poor and also there are difficulties in obtaining information about household contacts. A study was conducted by USA and Canada to find out the household contacts in a particular time period in which they have identified only five household clusters and another five have been identified in UK. This sample determines that the risk to household contacts from less sample size is not significant. The strategies to control the household cases vary between different countries which depend on the evaluated risk[5, 6].

The antibiotic prophylaxis varies between countries , in USA they give antibiotic prophylaxis to household members those who are at risk where as in

Canada they recommend prophylaxis to all family members where a GAS infection arise in the community. Recommendation of antibiotic prophylaxis and community contact management guidelines was first introduced by UK[36]

S.pyogenes infections are still there in maternity care centre in many developing countries, though they provided with adequate hospital hygiene guidelines. This results in death of patient occasionally. In case of outbreaks it is important to break the transmission cycle by early isolation and identification and antimicrobial prophylaxis. In case of varicella outbreak along with immunization, antibiotic prophylaxis should be given to prevent secondary bacterial infections[36]

Control of *S. pyogenes* in hospital and institutional settings

Puerperal infections and post partum mortality will be markedly reduced if the hospital infection control strictly follows the effective hand hygiene measures and droplet precaution measures. In developed countries like USA, Canada, Sweden, they have made specific strategies to control the infection caused by *S.pyogenes* in maternity settings. They advised screening test for the staff nurses those who are dealing with affected patients and also to their household contacts. In case of outbreak of *S.pyogenes* infection in a institutional settings, it is advised to use antibiotic prophylaxis to break the transmission cycle.

Infection Control

Centre for disease control has published certain standards and guidelines to control the infections caused by *S.pyogenes.*

Patient-to-Personnel Transmission:

Strict adherence to the standard precaution will help in prevention of hospital acquired infection by *S.pyogenes*. The persons who deal with the cases should wear gown and glove when collecting secretions from the patients and while doing wound debridement to avoid contact with the secretions. Hand washing is mandatory to avoid the spread of infection from one patient to another patient. Once the results obtained the materials like pus, throat swabs, blood, wound swab and body fluids should be disposed in a proper manner. This will help in the secondary spread of infection to those who handle the secretions from the infected person.

Personnel-to-Patient Transmission

The carriers who harbor the Group A Streptococci are responsible for an outbreak of surgical wound infection in operation theatre. By the same way the carriers who work in the delivery room will cause outbreak of postpartum infections. Pharynx, female genital tract and skin are major reservoir of GAS carriers. The main mode of transmission of Group A Streptococci is by direct contact. The occurrence of wound infection is uncommon by S.*pyogenes*.

Isolation of > 2 cases of Group A Streptococci needs epidemiological investigation.

Restriction from patient care activities and food handling is indicated for personnel with Group A Streptococcal infections until 24 hours after they have received proper antibiotic therapy. However, work restrictions are not necessary for personnel who are colonized with Group A Streptococci unless they have been epidemiologically connected to transmission of infection within the facility. The most important means of controlling any Group A Streptococcal disease is correct and timing identification and treatment of infections. Children with GAS throat infections should not return to the preschool or child care centre until at least 24 hours after starting antimicrobial therapy and until they become afebrile. Contacts of recorded cases of streptococcal infection who have recent or present clinical evidence of GAS infection should have appropriate cultures taken and must be treated if the culture is positive.

Antimicrobial resistance

Due to the development of rapid resistance to various treatments of streptococcal infections deciding a appropriate for invasive streptococcal infection is a big challenge. In Nepal a study was conducted and found that there is high resistance to Co-trimaxazole about 71.0% which was the commonly used drug to treat children with various diseases. The risk of spread

of this resistance to other microorganisms is also high. There were no resistant strains to Ampicillin and penicillin. During the last seventy years[10]

Penicillin and its derivatives with stable minimum inhibitory concentration remained the treatment of choice for Streptococcal Pharyngitis. But nowadays there are reports showing decreased susceptibility to penicillin. In Nepal where cuture facilities were not available, like sub health centers Penicillin derivatives were commonly used. Though until now there is no review reported penicillin resistance to GAS,in developing countries like Nepal where they use Penicillin and its derivatives in peripheral health centers also, induces the development of resistance towards this drug also 5.2% of the isolates were found resistance to both ciprofloxacin and erythromycin,20 to 30% were resistant to macrolides. In addition to Penicillin and Ampicillin this study found that Azithromycin 100% susceptible drug against GAS.[36-38]

In recent days Azithromycin is the most common prescribed drug for respiratory tract infections because of its short duration of infection. But this can also development of resistance to azithromycin in future. so it is recommended to review the already exiting antimicrobials and their regimen before altering the therapeutic regimen.

Surgical management:

S.pyogenes will cause wound infections and soft tissue infections which needs surgical intervention. GAS will produce gangrene and necrosis of the affected tissue which should be treated immediately. For necrotized and gangrenous tissues, wound debridement along with hyperbaric oxygen should be given; this will support the oxygen supply to the necrotic tissues. After surgical intervention proper follow up should be advised.

Treatment

The main clinical goal in treating GAS infection is based on two factors

1. Accurate diagnosis at the correct time
2. Appropriate Antibiotics

American Academy of Family Physicians says that follow up culture should be done if rapid test for *S.pyogenes* antigen is negative

Clinician must know about the fatal complication and post infection sequlae of S.*pyogenes* impetigo is the minor infection which may produce glomerulonephritis as a post infection sequlae. Hence physician must be aware of minor infections caused by Group A Streptococci.

Procedures includes in the *S.pyogenes* infection management:

Endotracheal intubation, skin aspiration, surgical drainage, lumbar puncture and debridement of wound.

Immediate fluid management should be given in case of shock, central or peripheral venous can be used for fluid replacement. Tonsillectomy is proven beneficial in children with recurrent pharyngitis.

Children with varicella infection are more prone to get secondary bacterial infection with *S.pyogenes*, hence aggressive management is mandatory in such children.

Rehabilitation

In case of neuropsychiatric manifestations, inpatient care is advisable..[40, 41] physician and neurologist opinion should be obtained. supportive therapy is essential in case of neurological defects.

Standard treatment of Group A Streptococcal positive pharyngitis for patient's first or second case of GAS pharyngitis in a three month period[40]

ANTIBIOTIC	ROUTE	REGIMEN	DURATION
Penicillin V Give as first choice Given in empty stomach	Per oral	Children;20mg/kg/day in 2-3 divided doses, maximum 500mg 3 times daily Adult :500mg twice daily	10 DAYS
Erythromycin ethyl succinate Given if allergy to penicillin	Per oral	Children:40mg/kg/day in 2-4divided doses, maximum 1g/day Adult :400mg twice daily	10 DAYS
Benzathine penicillin G (BPG) Give if compliance with 10 dayregime likely to be a problem	Intramuscular	Children<20kg:600,000U once only Adult and children> 20kg:1,200,000 U once only	SINGLE DOSE
Amoxicillin Useful alternative as can be given with food improve compliance	Per oral	Weight<30kg:750mg once daily Weight>30kg:1500mg once daily	10 DAYS

Guidelines for treatment of recurrence (≥ 3 episodes of GAS pharyngitis in a period of three months)[40]

ANTIBIOTIC	REGIMEN	DURATION
Oral Clindamycin	Children: 20-30mg/kg/day in 3 divided doses Adult : 600mg twice daily	10 days
Oral Amoxicillin;clavulanic acid	Adult : 500mg twice daily	10days
Parenteral with or without oral Benzathine penicillin G	IM dose	1 dose
Parenteral with or without oral Benzathine penicillin G with Rifampicin	IM dose	4 days

The reason for treatment failure with penicillin in GAS tonsillo pharyngitis include any recent exposure, carrier state, lack of compliance, in vivo bacterial co aggregation, in vivo co pathogenicity of β-lactamase producing normal pharyngeal flora, , poor antibiotic penetration into tonsillo

pharyngeal tissue, early initiation of antibiotic therapy which eventually leads to suppression of host immune response which was adequate, intracellular localization of GAS, development of tolerance to penicillin or orthodontic appliances.[45]

There is very little evidence to support the above mentioned explanations for the treatment failure in GAS tonsillo pharyngitis, and the already reported studies are mostly observational or laboratory based without any clinical confirmation.[46]

In Korea, Taiwan Italy the increased resistance of macrolides has been documented. In United States the resistance of macrolides drug has been documented as 3% - 9%.

PUBLIC HEALTH MANAGEMENT

Treatment of cases

Group A Streptococcal infections have rapid progression and it has been studied that it has high mortality, hence the isolation and identification should be done at the earliest for better prognosis. *S.pyogenes* responds well to penicillin and it is more effective drug . The combination of Clindamycin with penicillin is most effective in case of most severe GAS infections and those who do not respond to penicillin alone.Certain wound infection caused by *S.pyogenes* needs wound debridement and exploration. In case of multi organ

failure the patient should be supported with intravenous fluids and appropriate antibiotics along with this, supportive measures should be given[2,3].

Contacts:

When compared with general population, the persons those who are in close contact with invasive GAS infections are more prone to develop GAS infections. The screening and antibiotic prophylaxis for contacts is not recommended unless they have underlying illness and culture positives. The contacts with culture positive should be treated. Persons those who have household patients with toxic shock syndrome are more risk of developing invasive GAS infections. But this risk is not sufficient to advice for screening test and antibiotic prophylaxis. Since the mortality rate among the older age group and immune compromised patients are high, these are the people targeted for chemoprophylaxis. In children the risk of getting these invasive infections are less hence they are not recommended for chemo prophylaxis.

Development of a group A streptococcal vaccine

Factors which are associated with mortality helps in prevention of diseases caused by *S.pyogenes* emm types 1,3 & 12 should be included in the development of vaccine to prevent the Group A Streptococcal infections. Group A Streptococcal vaccine should be targeted to the people those who are in long term care facilities. The mortality rate is high in case of elderly and certain invasive GAS infections, so these are the target group for vaccination.

Vaccines are licensed for use in elderly since it has been proved effective only in this age group[6,7]

There are wide number of strategies currently available for preventing morbidity and mortality due to invasive GAS infection. But they are restricted to promotion of routine infection control and healthy practices and prevention of secondary cases in post surgical patients and postpartum patients. A study done in US has proved that the proposed 26-valent GAS vaccine prevents 40% to 50% of cases associated with invasive GAS infections among children and 50-60%of deaths among elderly persons. The infection could be successfully prevented by vaccination because of the stability of the distribution of emm types for prolonged period.

Invasive Group A Streptococcal infection contributes to the significant mortality in the United states. But compare to the total burden of GAS infections the invasive infections are in less proportion only. Development of Group A streptococcal vaccine depends on the non-invasive cases like pharyngitis in children in industrialized countries. The 26-valent Group A Streptococcal vaccine gives protection against pharyngitis in children and may give to rheumatic fever which is one of the non suppurative complication of Group A Streptococci.[48]

The distribution of emm and clonal types for GAS infection vary from developing countries from USA[49, 50]

Multivalent conjugate vaccines are eliminated because of the newer technology in the development of protein based vaccine. On the other hand the changes in the emm distribution, disease burden in the community should be monitored and the population who are at risk should be identified for vaccination. This data will help in the vaccine cost-benefit analysis. Certain international standards are mandatory to monitor the disease burden in many countries. It will help to design the vaccines which will have global utility.

S.pyogenes has the surface protein which is variable in nature. The main aim of vaccine research is the variable surface M-protein.the vaccine against the variable surface M protein gives strong protective immune response. The variable nature of M protein at the amino acid terminus is a big challenge in vaccine development. A study conducted in Isrel about the prevalence of emm type showed 26 valent vaccine gives 60% protection against GAS infections cases. Others have developed hexavalent M-protein-based vaccines, and a vaccine using the more conserved C-terminus.[5,6] the surface M protein will produce strong immunity in rheumatic fever and the antigenecity also highly variable, these two are the major reason for the withdraw of the M protein based vaccines..

There is one more vaccine available for prevention of GAS infection which is based on C5a peptidase. This protein is shared between *S.pyogenes* and *S.agalactiae* , hence this vaccine gives protection against both of these organism.[7]

The Group A Streptococci gets colonized in the throat or skin by using surface pilus. Pilin is the subunit of pilus which is protein in nature. The T typing is based on the pilus and it plays major role in vaccine production. [6,7]

Aim and Objectives

Aim:

To find the epidemiology of Group A Streptococcal infections in and around Coimbatore and to study the prevalence of throat carriage of *S.pyogenes* among school children

Objectives:

Primary: To estimate the incidence of Group A Streptococcal infections in patients and study the prevalence of asymptomatic carriage among school children

Secondary:

- Isolation of Group A streptococcal infections from throat swabs of outpatients and inpatients attending PSG Hospitals
- Characterization of the isolates by conventional biochemical tests
- Confirmation of serogrouping by manual and commercial antigen extraction methods
- Comparative evaluation of the above two methods
- Detection of asymptomatic throat carriage of *Streptococcus pyogenes* among school children

- Detection of emm gene among a representative sample of isolates obtained.

- Sequencing and BLAST analysis for identification of subtypes.

Materials and Methods

TYPE OF STUDY:

Prospective observational study

STUDY POPULATION:

Outpatients and Inpatients attending PSG Hospitals and school children (in and around) for asymptomatic throat carriage of *S.pyogenes*

STUDY LOCALE:

PSG Hospitals

SAMPLE SIZE:

638 (495 school children and 143 isolates from patients)

SAMPLE SIZE ESTIMATION:

$n=4pq/d^2$

n = required sample size

p=expected prevalence

q=100-p

d= degree of prevalence

n=638

SAMPLING METHOD:

Convenience method for patient isolates and school children aged 5-15 years

INCLUSION CRITERIA:

Outpatients and Inpatients with suspected cases of acute pharyngitis and rheumatic fever, cellulitis, impetigo ,erysipelas, necrotizing fasciitis , mastoiditis, tonsillitis, otitis, pneumonia and bone and joint infections.

Healthy school children aged 5-15 years.

EXCLUSION CRITERIA:

Children who were on antibiotics for suspected throat infections.

CONFIDENTIALITY:

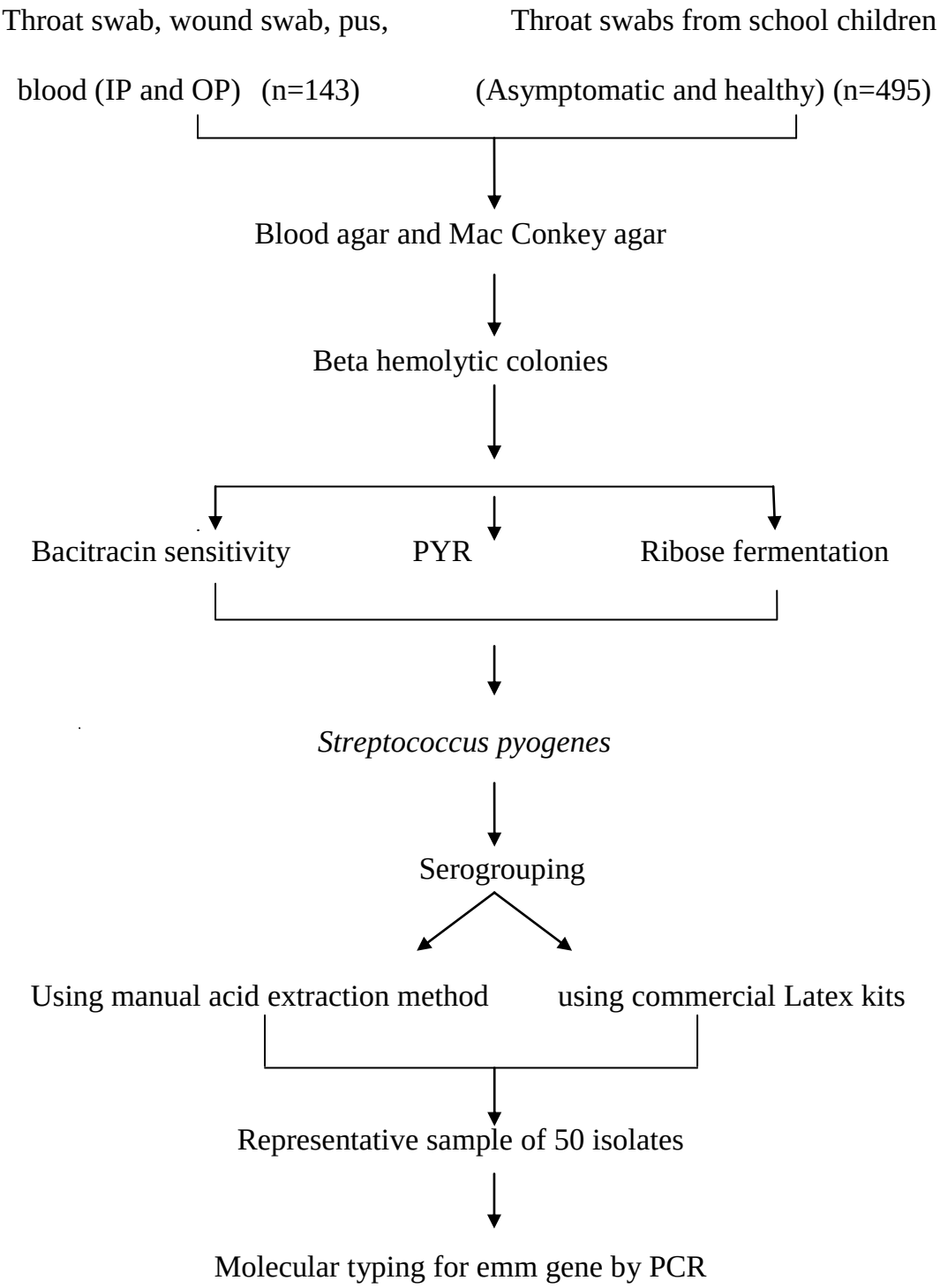
Confidentiality of the reports was maintained.

ETHICAL CLEARANCE:

The Institutional Human Ethical clearance was obtained before the commencement of the study. Informed consent was obtained before the collection of throat swabs from parents of the school children (Assent form)

A questionnaire was designed and the details obtained were filled.

ALGORITHM FOR IDENTIFICATION OF GROUP A STREPTOCOCCCI



THROAT SWABS:

Sterile swabs were taken and swabs were collected from the throat from school children. The same was streaked on sheep blood agar and were kept at 37^0C in candle jar for 24 hours.

1. **Growth on sheep blood agar:** Following overnight incubation of the sample, colonies of Streptococcus seen as grey colonies of about 0.5 - 1mm in diameter with beta (complete) hemolysis were taken as presumptive streptococcal colonies.
2. **Catalase test:** A drop of catalase reagent (3% hydrogen peroxide) was added to the slide. The growth was further applied to it with an applicator stick. Appearance of bubbles was taken as positive. Streptococcus colonies showed no bubbles which was taken as negative.
3. **Gram stain:** Smears made from colony showed Gram positive cocci in chains.

STORAGE:

If the isolate was catalase negative, gram stain showing the presence of gram positive cocci in chains and growth in blood agar showing beta hemolytic colonies, they were sub cultured and pure growth was stocked in Robertson's cooked meat medium in tubes. The tubes were sealed and stored at 4-8^0C and when further tests were to be done they were further sub cultured.

Storage by Lyophilization:

For long term storage the streptococcal isolates were stored by lyophilization method.

Two to three colonies of beta hemolytic streptococci was taken and lawn culture was made on blood agar and kept at 37^0C for 24 hours. The vials were autoclaved and kept. Brain heart infusion broth and milk broth were prepared for the storage of the isolates. The colonies from the lawn culture in blood agar was scrapped and mixed well in the vial which contain 0.5ml of milk broth and 0.5ml of brain heart infusion broth. The vials were kept at -20^0C for 48 hours and lyophilization done.

IDENTIFICATION TESTS:

1) **Bacitracin sensitivity**: A well isolated colony of Streptococcus from the culture was taken and lawn culture was made on blood agar plate. Then 0.04-0.05 international units of Bacitracin disc was kept over the lawn culture. Following overnight incubation at 37^0C the zone of clearance was measured. Zone of inhibition by greater than 14mm was taken as sensitive for *Streptococcus pyogenes.*
2) **PYR test**: The colonies were inoculated in PYR broth and incubated for a period of 4 hours. Later 3 drops of PYR reagent was added to it and change of colour to red was confirmed positive indicating *Streptococcus pyogenes* where as absence of colour change indicates negative test.

3) **Ribose fermentation:** Species identification was further done by sugar utilization by inoculating the organism in peptone with 1%sugar with Andrade indicator. Any change in colour from colorless to pink indicated the positive sugar fermentation test. Absence of colour change was taken as negative. The ribose was not fermented in *Streptococcus pyogenes.*

4) **Serogrouping:** Serogrouping was done by two methods.

a) Manual acid extraction method:

Micro nitrous acid extraction method:

1) 20 micro litres of 2% sodium nitrite solution was taken in a test tube.
2) 3 to 4 colonies of beta hemolytic streptococci were added to the test tube.
3) Glacial acetic acid (3microlitres) was added to the suspension and kept it at room temperature for 15 minutes.
4) 16 to 24 micrograms of sodium bicarbonate was added for neutralization.
5) Finally 60microlitres of distilled water was added.

Agglutination method:

1) One drop of micro nitrous acid extract was added to the circle on the slide by using Pasteur pipette.
2) To this one drop of latex reagent was added to each circle.
3) The slide was mixed by rotation for about 1 minute.
4) Evidence of agglutination against diffuse light was considered as positive.
5) The group reagent which gave the strongest agglutination denoted the group of the streptococcal isolate.

b) Commercial Latex kits method:

Principle:

Streptococci have group specific antigens in their cell walls which is carbohydrate in nature. After extraction by a specially prepared enzymatic method these antigens will react with latex particles coated with the corresponding antibody and produces agglutination. The latex remains in smooth suspension if there are no of group specific antigens.

Name of the kit: **STREP TEST KIT- PLASMATEC**

Kit presentation

1. Latex determination for the grouping of streptococci A, B, C, D, F, G.
2. Polyvalent positive control 2ml
3. Freeze dried extraction Enzyme. Two vials, reconstituted each with 10ml of distilled H2O.
4. Disposable test cards 50 in number.
5. Mixing sticks 300 and kit insert.

Method:

1) By using sterile bacteriological loop 2-6 colonies of streptococci was taken and emulsified in 0.4ml of extraction enzyme.
2) The tube which contains the bacterial colonies with extraction enzyme was kept for10 minutes at water bath 56^0C , the tube was shaken vigorously after 5 minutes of incubation.
3) The latex reagent was re-suspended by gentle agitation; one drop of latex reagent was added to each circle on the test slide.
4) One drop of the extract was added to each drop of latex reagent by using Pasteur pipette and the content was mixed well by using separate mixing stick.

5) The slide was rocked for not more than one minute, and the agglutination was observed

6) Visible agglutination of the latex particle was noted in the circle which marked as A which indicates positive result.

MOLECULAR STUDY OF ISOLATES:

The isolates of Group A streptococci were streaked on blood agar plate and incubated for 24 hours at 37^0C.The pure colonies were taken and inoculated into 10ml of Brain Heart infusion broth in sterile tarson tubes and then incubated for 24 hours at 37^0C.

After 24 hours those tubes were centrifuged for 5 minutes and the supernatant fluid was discarded the pellets were transferred onto two ml sterile capped tube for DNA extraction.

DNA EXTRACTION:

The DNA from Group A Streptococci was extracted by Bacterial Genomic DNA Purification Kit.

Kit: HIPURA Bacterial Genomic DNA Purification Kit

DNA EXTRACTION PROCEDURE:

1) Lysozyme solution was prepared by using lysozyme from chicken egg white, which is provided in the kit.

2) 1.5 ml of bacterial broth culture was taken in the 2ml capped collection tube by centrifugation for 2 minutes at 13000 rpm. The culture medium was removed completely and discarded.

3) The pellet was re suspended in 200microlittre of lysozyme solution and incubated for 30 minutes at 37^0 C.

4) 20 microlittre of Proteinase K was added to the sample.

5) 200 microlittre of lysis solution was added, vortex done thoroughly for few seconds and incubated at 55^0C for 10 minutes.

6) 200 microlittre of ethanol was added to the lysates prepared already.

7) The lysate obtained was transferred into HiElute Miniprep Spin Column (capped) and centrifuged at 10000 rpm for 1 minute. The flow through liquid was discarded and the spin column was placed in the same 2ml collection tube.

8) **Prewash**: 500microlittre of prewash solution was added to the column and centrifuged at 10 thousand rpm for 1 minute. Flow through liquid was discarded and the same collection tube re-used with the column.

9) **Wash**: 500microlittre wash solution was added to the column and centrifuged for 3 minutes at a maximum speed 13,000 rpm. Flow through liquid was discarded and centrifuged again at same speed for additional 1 minute to dry the column.

10) The HiEilute Miniprep Spin column was transferred into fresh uncapped collection tube.200microlittre of elution buffer was added into the column without spilling to the sides of the tube and incubated for 1 minute at room temperature then centrifuged at 10 thousand rpm for one minute to elute the DNA. The elute was transferred into a fresh capped 2ml collection tube for longer storage at -20^{0}C.

DNA Amplification

The extracted DNA from the isolates were amplified by using Real time PCR-AB Applied biosystems.

Sterile 1.5ml tubes were taken for the preparation of PCR

Master mix- 12.5microlitres

PCR water- 8.5microlitres

DNA lysate- 2micrlittre

Forward primer- 1microlitre (Primer F 5'TATTCGCTTAGAAAATTAA3')

Reverse primer- 1microlitre (Primer R 5'GCAAGTTCTTCAGCTTGTTT3')

The temperature setup was done for the following steps

Holding time- 95^0C for 5 minutes

Denaturation- 95^0C for 30 seconds

Annealing - 46^0C for 45 seconds

Elongation – 72^0C for 59 minutes

Final elongation- 72^0C for 7 minutes

At the end of 30 cycles the amplified products were obtained.

GEL ELECTROPHORESIS

1. Sterile beaker was taken, 1x buffer was prepared, and 80 ml of 1 x buffer was added with 0.76 grams of agarose powder and then kept in the micro wave oven for melting for 3 minutes.
2. After 3 minutes the agarose was cooled to 40^0C, to this 2 microlittre of Ethidium bromide was added
3. In 80ml trough the agarose gel was poured with 15 numbered comb to make wells. After the agar gets solidified the comb was taken out slowly
4. In the first well the 100 base pair ladder was added to the rest of the wells the amplified DNAs were added.

5. 5 .The gel was run at 95 volts for 45 minutes and the appearance of bands were noted under UV light.

6. The bands at 912 base pairs indicates emm gene.

7. The subtypes of emm gene were analysed by EuroFins Genomics (Bangalore) and the results obtained were subjected to BLAST analysis.

Results

A total of 638 samples were collected and processed from school children and PSG Hospitals during the study period January 2015 to July 2016. Out of 638 samples 495 collected from school children and 143 samples collected from patients samples from PSG Hospitals.

Among 495 throat swabs collected from school children 7 children (1.4%) are carriers of Group A Streptococci. 5 children (1.0%) are carriers of Group G Streptococci.

Among 143 samples collected from PSG Hospitals 67 (46%) were found to be Group A Streptococci and 76 (53%) were found to be other serogroups of beta hemolytic streptococci.

Figure 1 shows the distribution of Group A and Group G Streptococci among the total samples collected from school children.

Figure 2 shows the distribution of Group A Streptococci among samples received in Microbiology laboratory.

Distribution of Group A Streptococci among varies samples received in Microbiology laboratory like pus 16 (21%), wound swabs 31 (40%), throat swabs 17(22%), blood 11(14%), ascitic fluid 1(2%), pigtail fluid 1 (1%) is depicted in **Figure 3.**

Representative samples of 50 isolates were tested for emm gene which is responsible for rheumatic fever and molecular study was done for those 50

isolates by RT-PCR and Gel electrophoresis method and the bands were obtained.

Figure 4 show bands which indicate the presence of emm gene in the positive isolates.

Figure 5 shows the subtypes of emm gene from the isolates.

The sequencing pattern of the BLAST analysis is depicted in a graphical pattern in **Figure 6.**

Table 1 shows the comparison of manual nitrous acid extraction method with PLASMATEC commercial strep kit method for sero grouping of streptococci for final confirmation.

Table 2 shows the antibiograms of *S.pyogenes* collected from various clinical samples

Illustration 1 shows the throat swabs collected from school children and sterile sheep blood agar plates used for streaking.

Illustration 2 shows the appearance of Beta hemolytic Group A Streptococci on blood agar

Gram stain of *Streptococcus pyogenes* was confirmed as Gram positive cocci in chains which is shown in **Illustration 3**.

Illustrations 4 -11 show various biochemical tests done to identify *S.pyogenes.*

The isolates of streptococci were preserved in Robertson's cooked meat medium and were subcultured periodically. The isolates were also lyophilized and stored in the vials. (**Illustrations 12 and 13)**

Figure 1: Distribution of Group A & Group G streptococci among total samples collected from School children

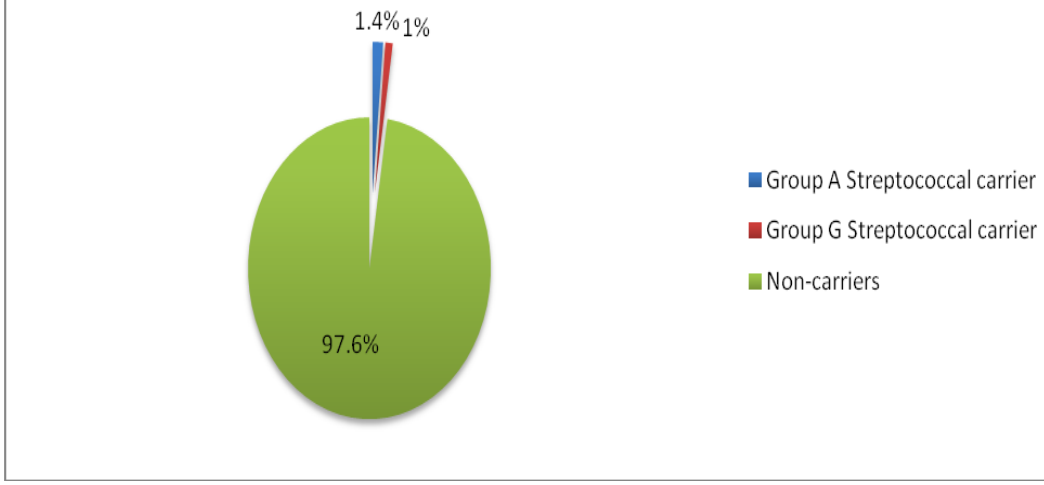
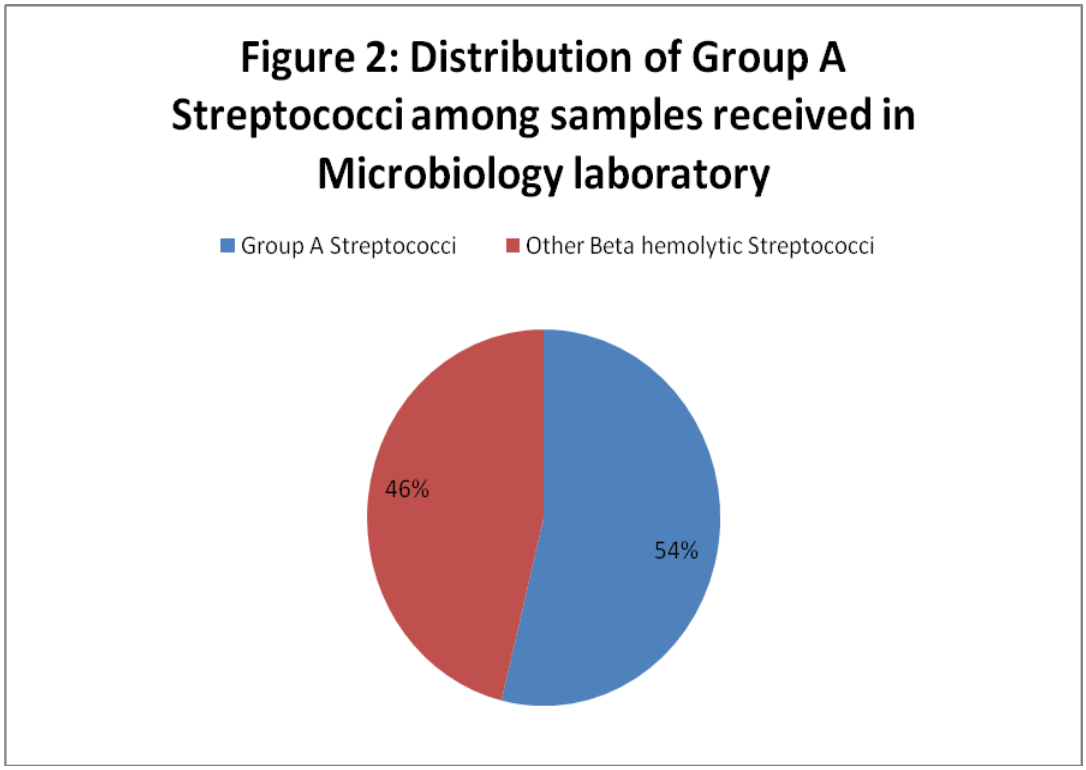


Figure 2: Distribution of Group A Streptococci among samples received in Microbiology laboratory



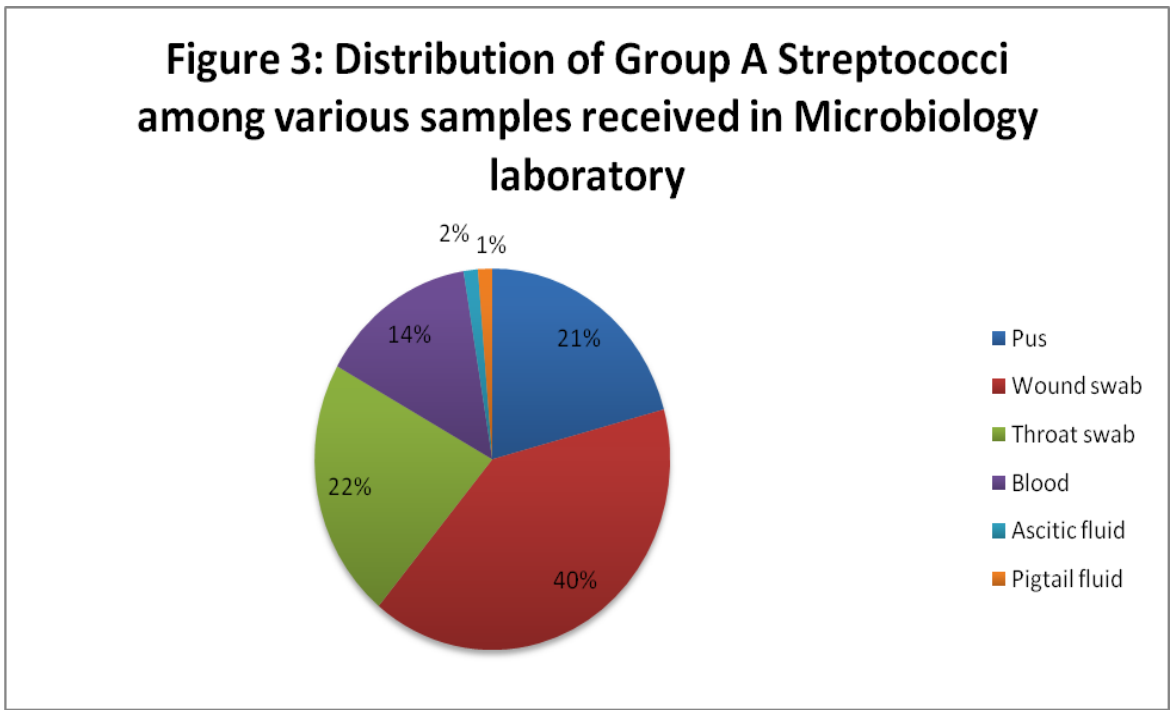


Illustration 1: Sterile throat swabs taken from asymptomatic school children and sheep blood agar plates for streaking

Illustration 2: Beta hemolysis

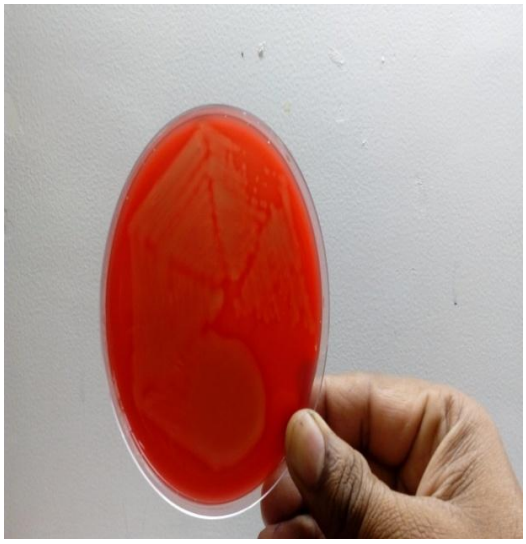


Illustration 3: Gram stain of *S.pyogenes*

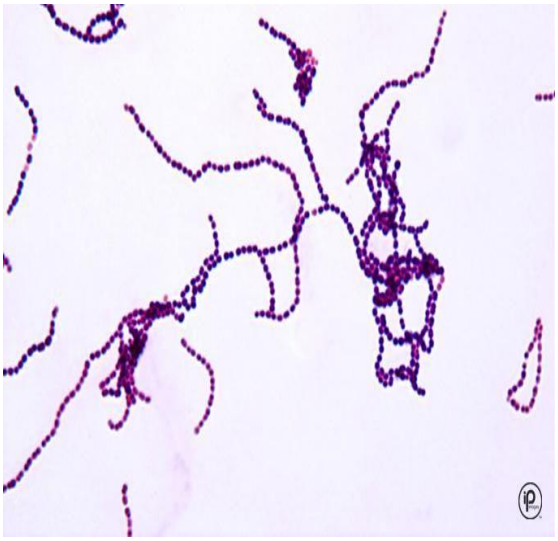


Illustration 4: Catalase test Negative

Illustration 5: Bacitracin sensitivity

Illustration 6: PYR test positive for *Streptococcus pyogenes*

Illustration 7: Ribose fermentation not fermentation negative – *S.pyogenes*

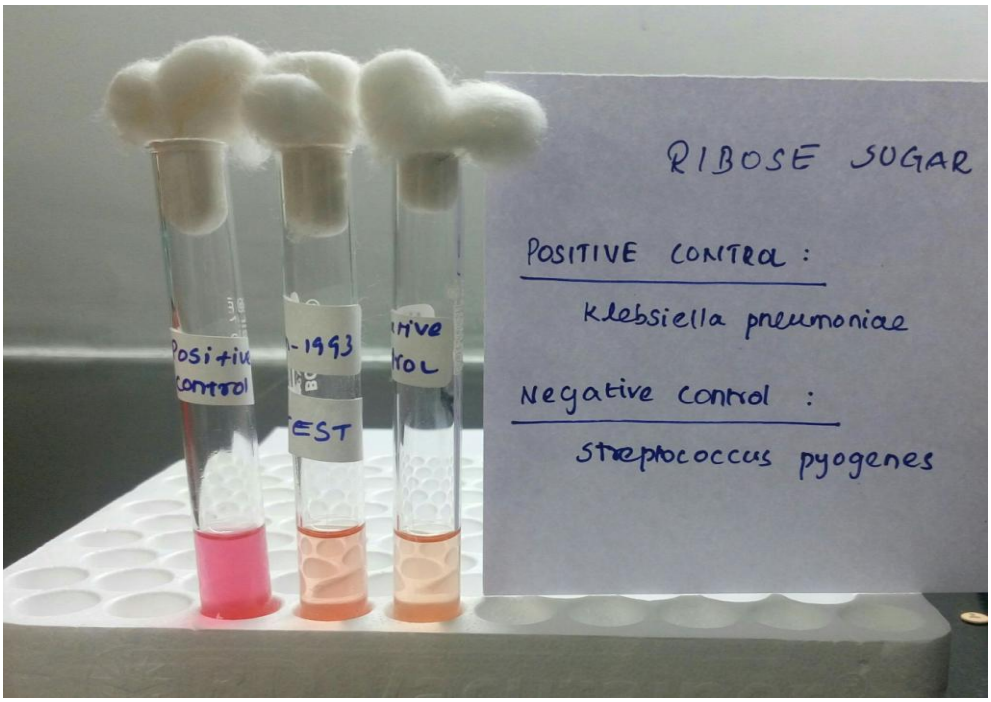


Illustration 8: Manual extraction method for serogrouping of streptococci

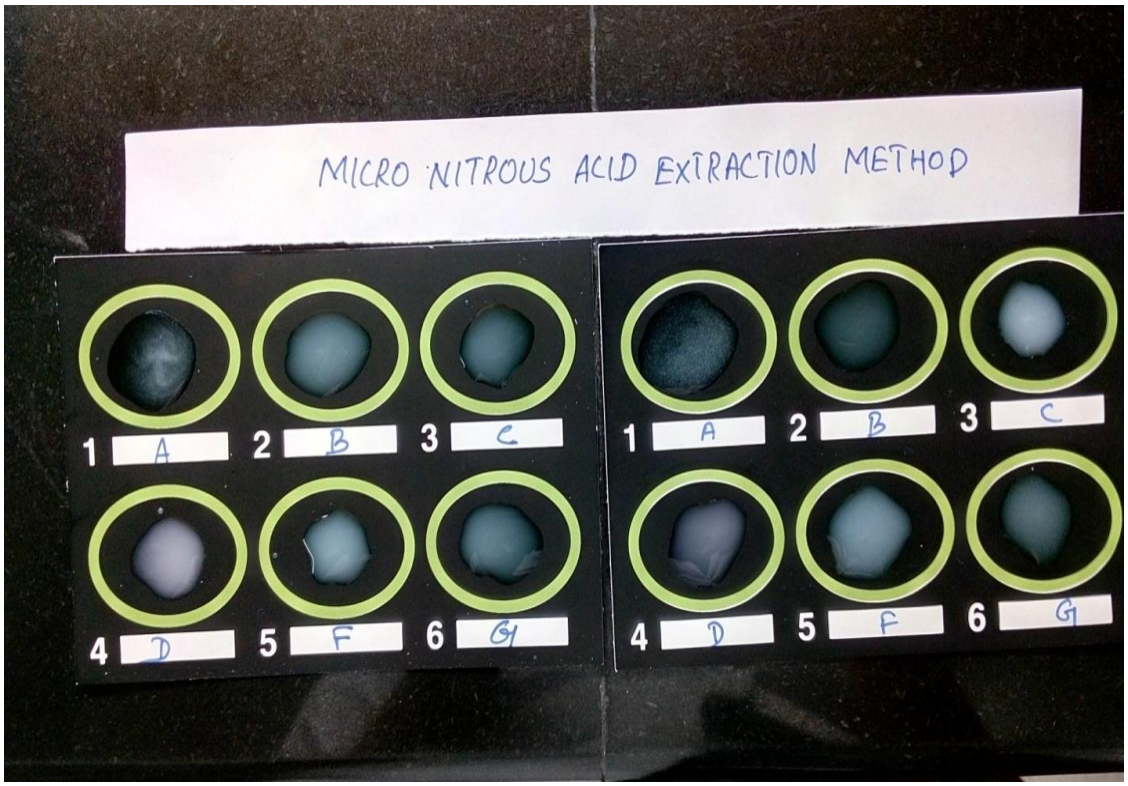


Illustration 9: Serogrouping by Latex kit method

Illustration 10: RTPCR for amplification

Illustration 11: Gel electrophoresis

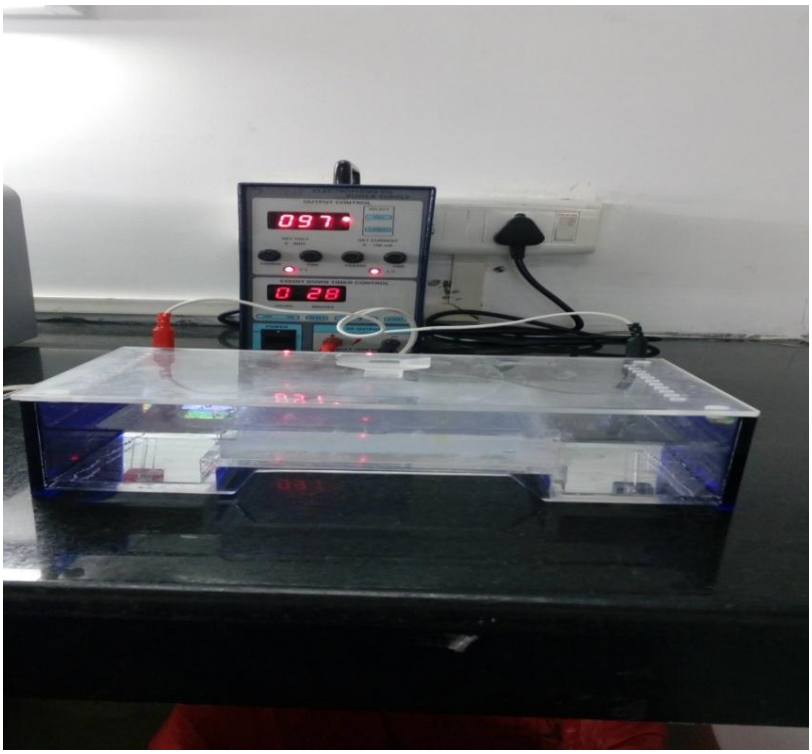
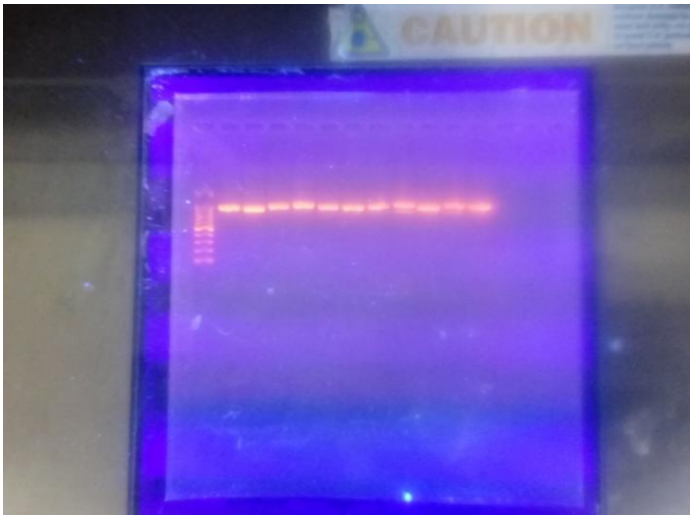


Figure 4 PCR showing emm gene of band 912 bp



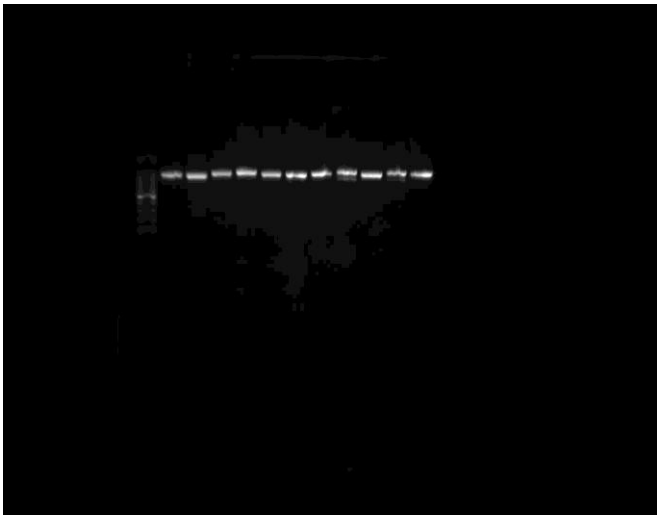


Figure 5 PCR showing emm subtypes

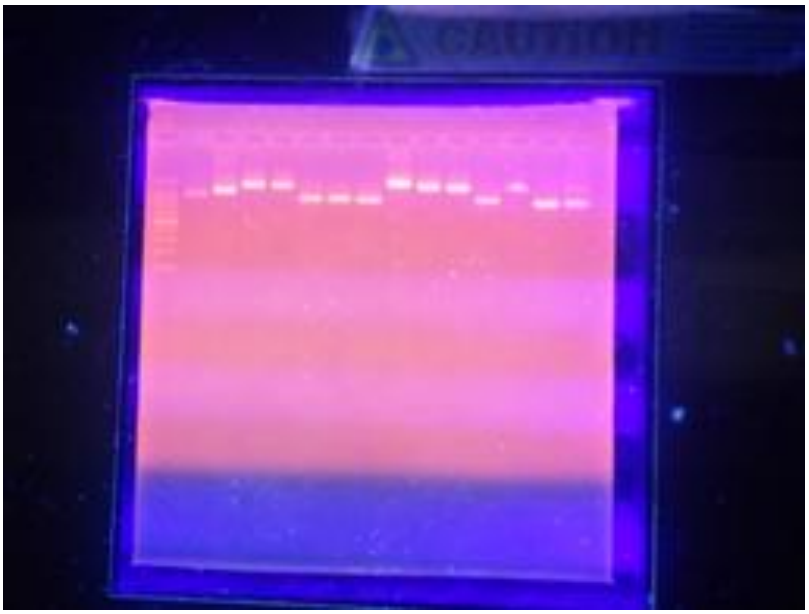


Illustration 12: Robertson cooked meat medium for storage of *S.pyogenes*

Illustration 13: Lyophilization vials – Preservation method for *S.pyogenes*

Table 1: Comparative evaluation of the manual nitrous acid extraction and commercial kit for serogrouping of Streptococci

MANUAL METHOD	PLASMATEC (Commercial kit)	
	POSITIVE	**NEGATIVE**
POSITIVE	78	0
NEGATIVE	6	554

Sensitivity =78/84*100 = 92.85%

Specificity =554/554 =100%

Positive predictive value =78/78+0 = 100%

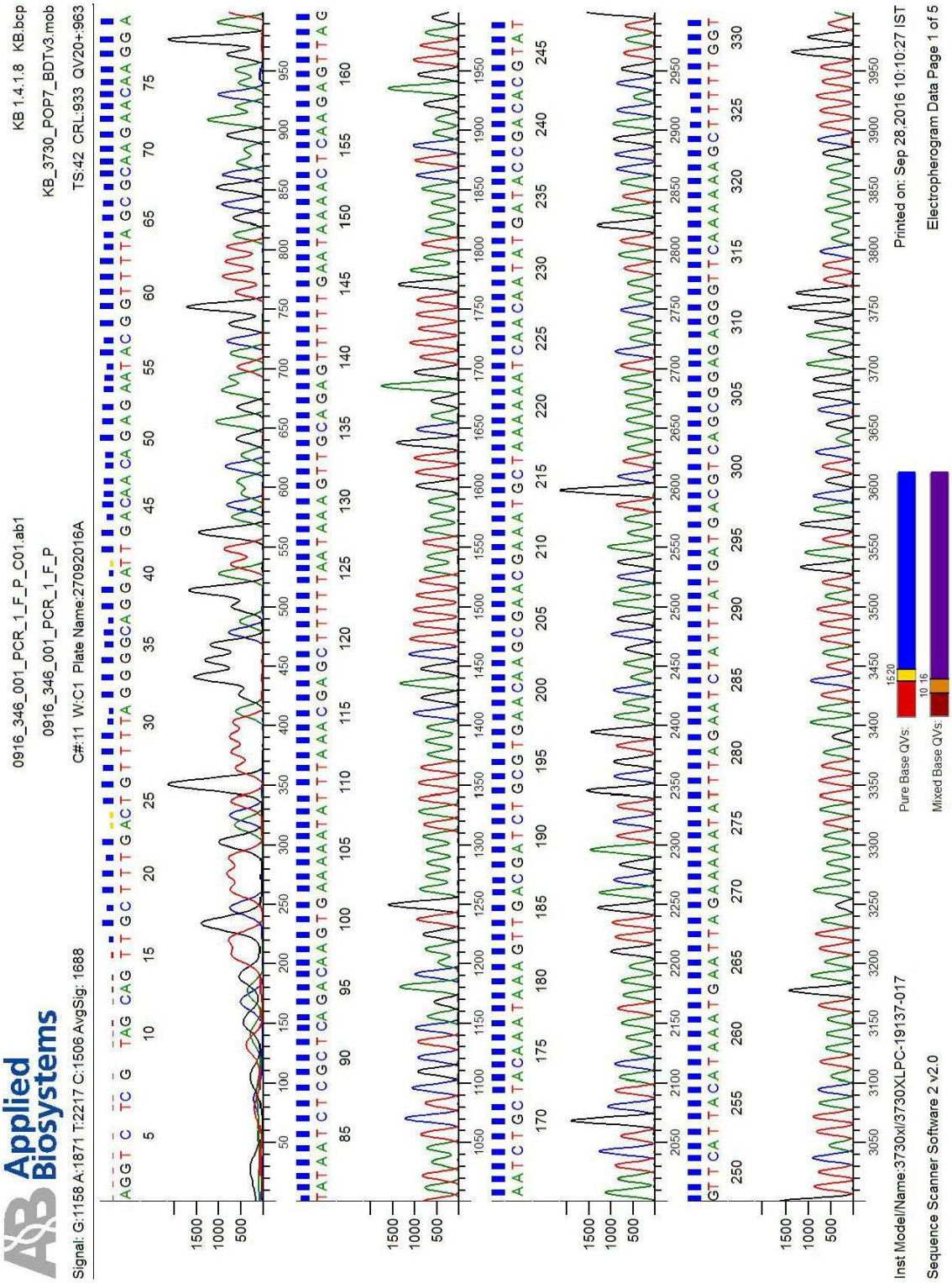
Negative predictive value =554/560 *100 = 98.92%

Table 2: Antibiogram of clinical isolates of *S.pyogenes*

Total number of isolates, n=77

Name of the antibiotic tested	**%Sensitive**	**%Resistance**
Penicillin(10U)	88.32%	11.68%
Cefotoxime (30µg)	100%	0%
Vancomycin (30µg)	100%	0%
Erythromycin (15µg)	64.94%	35.06%
Clindamycin (2µg)	79.23%	20.77%
Co-trimoxazole (25µg)	74.03%	25.9%
Levofloxacin (5µg)	88.32%	11.68%

Figure 6 : Sequencing Pattern of emm Subtypes



Discussion

Group A Streptococci produce a wide range of diseases from pharyngitis to streptococcal toxic shock syndrome. Since the carriers are more prone to spread the infections, they are considered as a major public health problem. To avoid post infection sequlae of GAS, it is mandatory to identify the organism at the earliest[57-59] Group A Streptococci mostly colonizes in the throat of an asymptomatic carriers. Geographical location and season play a major role in the carrier rate of GAS among healthy school children. Several studies reported the carrier rates of 15-20% among healthy school children[39]

In our study the incidence of Group A Streptococci was found to be 0.75 % among clinical samples in our hospital. This is higher when compared with a study conducted in USA which showed 1.5 to 3 cases per 100,000 population[60]. One more study conducted in different time period also reveals incidence around 3.5 cases per 100,000 population[61-63]. A study was conducted in Canada showed the incidence of Group A Streptococci was 1.5 cases per 100,000 population[64.] while another study conducted in Denmark showed the incidence of *Streptococcus pyogenes* infection was 2.6 cases per 100,000 population[65]

The reason for high incidence of Group A Streptococcal infections could be middle and low socio economic status, overcrowding , high population density, the hot and humid climate , air pollution ,more number of students in one class room and poor hygienic practices.[66,67]

Apart from Group A Streptococci the incidence of Group G Streptococci was found to be 0.70% among the clinical isolates. This percentage is much less when compared to a study conducted in Mumbai which showed the prevalence was 22.19%. One more study conducted in New Delhi showed the prevalence of Group G Streptococci was 21% [68]

A study conducted in school children showed that among the beta hemolytic Streptococci, 40% was contributed by Group A and 60% by other groups of Streptococci[75]

Distribution of Group A Streptococci among various samples received in Microbiology laboratory which included pus 16 (21%), wound swabs 31 (40%), throat swabs 17(22%), blood 11(14%), ascitic fluid 1(2%) and pigtail fluid 1 (1%). This distribution is more or less similar to a study conducted which showed 10.6% of throat cultures, 8.7% of urine cultures, and 7% of pus and body fluids.[69] A study conducted in Germany showed that 53.9% isolates were obtained from blood samples and 17.6% isolates were obtained from wound swabs.[70]

Among the 77 isolates of GAS isolated from various clinical samples, 11.68% showed resistant to Penicillin, 25% were resistant to Co-trimoxazole, 11% were resistant to Levofloxacin, 20% were resistant to Clindamycin and 35% of the isolates were resistant to Erythromycin. A study about the antibiotic resistant in Kolkata showed, *S.pyogenes*

were 100% sensitive to Penicillin, Vancomycin, Cefotaxime, Clindamycin and 52% were resistant to Tetracycline, 34.7% were resistant to Erythromycin. There was an increase in MIC of the Penicillin drug was noted in 5% of the isolates [81.]

Since the mild throat and skin infections of GAS can lead to fatal life threatening complications, rapid and accurate diagnose of the GAS infections is important to avoid post infectious immune mediated complications[80] Comparison of manual nitrous acid extraction method with commercial kit (PLASMATEC) for the sero grouping identification of *Streptococcus pyogenes* showed the manual conventional method had a sensitivity of 92% and specificity of 100% with positive predictive value of 100%and negative predictive value of 98.92%. Similar results have been reported that the sensitivity of manual extraction method when compared with commercial Kit method was 90% and specificity was 100%[71,72.] Similar reports have been published the Sensitivity was 97.71% and Specificity was 80.64% for Latex agglutination assay. Commercial kits provide as easy and reliable method for serogrouping of *S.pyogenes.*

The prevalence of asymptomatic carriers of Group A Streptococci among school children screened in our study was found to be 1.4%, this is lesser than a study conducted in Guntur, India which showed the prevalence was around 12%[29]. This might be explained by the fact that the children are from urban areas with middle to higher socioeconomic groups. A study was

conducted in North India about the epidemiology of group A streptococcal pharyngitis which reported the prevalence of 2.8% which is higher than our study[30] Studies in Karnataka about the prevalence of beta hemolytic streptococci in school children between the age group of 5-10 years was 30.1% which is higher compared to our study. A one year prospective study was conducted in Chennai about the prevalence of GAS infection in the age group of 5-15years. It was reported that in various parts of the country, the carriers of Group A Streptococci was 11.2-34 %[73,74]. Group G Streptococci prevalence was found to be 0.25% in few studies[75]

A prospective study conducted in asymptomatic school children in Nepal showed the prevalence of GAS infections was around 10.9%[8.] While another report shows the prevalence Group A Streptococcal infection among school children asymptomatic carrier rate to be 0.8%[76] In our study, apart from Group A streptococci some of the asymptomatic school children also were carriers of Group G Streptococci which was found to be 1.01%. This correlates well with Studies conducted in Turkey where the prevalence of Group G Streptococci in pediatric Age group, was of 1% [77]

A cluster of emm like gene which includes emm, mrp, enn are located in the Vir locus of the GAS chromosome. The M protein of the GAS encoded by emm gene, the hyper variable region of 5' sequence of emm genes gives distinguishable serotypes in M protein. Currently the M protein Serotyping is

done by using specific PCR products. Among the 50 positive isolates of Group A Streptococci, 13 emm gene that is 26% identified at 912 base pairs.

A study about the emm analysis in children with pharyngitis showed that, out of 25 GAS isolate sequenced the emm type 3 was present in 80%, emm 1 was 16% and emm 79 was 4%. emm 3 was mostly associated with severe pharyngitis[78] compared with this study the incidence of emm gene in our study is less. It is of interest that of six emm types more commonly seen in the USA (emm 1, emm 3, emm 28, emm 12, emm 4, and emm 11) only emm 12 was seen in South Indian population. This observation would be of great significance in designing a vaccine based on emm sequences, and would imply that a vaccine designed for a Western population may not be suitable for India[79]

The BLAST analysis of the representative samples sent for sequencing revealed that the subtypes of emm genes identified in our study were emm19,113, STP 2736,121,12(RE 611 strain) st 2460, st 6735,401,17 and st 11014.

Summary and Conclusion

1. The incidence of Group A Streptococci from various clinical samples were found to be 0.75%.

2. The distribution of Group A Streptococci among various samples were pus 16 (21%), wound swabs 31 (40%), throat swabs 17(22%), blood 11(14%), ascitic fluid 1(2%), pigtail fluid 1 (1%).

3. 3. The isolates of *S.pyogenes* were 100% sensitive to Cefotaxime and Vancomycin, 88.32% sensitive to Penicillin and Levofloxacin, 79.23% sensitive to Clindamycin, 74.03% sensitive to Co-trimoxazole and 64.94% sensitive to Erythromycin.

4. The prevalence of asymptomatic carriers of Group A Streptococci was found be 1.4% and Group G Streptococci was found to be 1.0%

5. The prevalence of emm gene was found to be 26%

6. The prevalence of emm gene was found to be 26% and subtypes emm19,113,STP 2736,121,12(RE 611 strain) ,st 2460, st 6735,401,17 and st 11014 were identified by BLAST analysis.

7. The commercial streptococcal grouping kit method was found to be 100% sensitive and specific when compared to conventional method.

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Annexures

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To
Dr S Shanmugapriya
Postgraduate
Department of Microbiology
PSG IMS & R
Coimbatore

Ref: Project No. 14/411

Date: January 2, 2015

Dear Dr Shanmugapriya,

Institutional Human Ethics Committee, PSG IMS&R reviewed and discussed your application dated 05.12.2014 to conduct the research study entitled "*Epidemiology of group A streptococcal infections in and around Coimbatore*" during the IHEC meeting held on 22.12.2014.

The following documents were reviewed and approved:

1. Project Submission form
2. Study protocol
3. Assent form (ver 1.1)
4. Parental consent form (ver 1.1)
5. Application for waiver of consent
6. Confidentiality statement
7. Current CVs of Principal investigator, Co-investigators
8. Budget

The following members of the Institutional Human Ethics Committee (IHEC) were present at the meeting held on 22.12.2014 at IHEC Secretariat, PSG IMS & R between 10.00 am and 11.00 am:

Sl. No.	Name of the Member of IHEC	Qualification	Area of Expertise	Gender	Affiliation to the Institution Yes/No	Present at the meeting Yes/No
1	Dr. P. Sathyan (Chairperson, IHEC)	DO, DNB	Clinician (Ophthalmology)	Male	No	Yes
2	Dr. S. Bhuvaneshwari (Member-Secretary, IHEC)	MD	Clinical Pharmacology	Female	Yes	Yes
3	Dr. S.Shanthakumari	MD	Pathology, Ethicist	Female	Yes	Yes
4	Mrs P Rama	M Pharm	Non-medical (Pharmacy)	Female	Yes	Yes

The study is approved in its presented form. The decision was arrived at through consensus. Neither PI nor any of proposed study team members were present during the decision making of the IHEC. The IHEC functions in accordance with the ICH-GCP/ICMR/Schedule Y guidelines. The approval is valid until one year from the date of sanction. You may make a written request for renewal / extension of the validity, along with the submission of status report as decided by the IHEC.

PSG Institute of Medical Sciences & Research
Institutional Human Ethics Committee
Recognized by The Strategic Initiative for Developing Capacity in Ethical Review (SIDCER)
POST BOX NO. 1674, PEELAMEDU, COIMBATORE 641 004, TAMIL NADU, INDIA
Phone : 91 422 - 2598822, 2570170, Fax : 91 422 - 2594400, Email : ihec@psgimsr.ac.in

Following points must be noted:

1. IHEC should be informed of the date of initiation of the study
2. Status report of the study should be submitted to the IHEC every 12 months
3. PI and other investigators should co-operate fully with IHEC, who will monitor the trial from time to time
4. At the time of PI's retirement/intention to leave the institute, study responsibility should be transferred to a colleague after obtaining clearance from HOD, Status report, including accounts details should be submitted to IHEC and extramural sponsors
5. In case of any new information or any SAE, which could affect any study, must be informed to IHEC and sponsors. The PI should report SAEs occurred for IHEC approved studies within 7 days of the occurrence of the SAE. If the SAE is 'Death', the IHEC Secretariat will receive the SAE reporting form within 24 hours of the occurrence
6. In the event of any protocol amendments, IHEC must be informed and the amendments should be highlighted in clear terms as follows:
   a. The exact alteration/amendment should be specified and indicated where the amendment occurred in the originai project. (Page no. Clause no. etc.)
   b. Alteration in the budgetary status should be clearly indicated and the revised budget form should be submitted
   c. If the amendments require a change in the consent form, the copy of revised Consent Form should be submitted to Ethics Committee for approval
   d. If the amendment demands a re-look at the toxicity or side effects to patients, the same should be documented
   e. If there are any amendments in the trial design, these must be incorporated in the protocol, and other study documents. These revised documents should be submitted for approval of the IHEC and only then can they be implemented
   f. Any deviation-Violation/waiver in the protocol must be informed to the IHEC within the stipulated period for review
7. Final report along with summary of findings and presentations/publications if any on closure of the study should be submitted to IHEC

Kindly note this approval is subject to ratification in the forthcoming full board review meeting of the IHEC.

Thanking You,

Yours Sincerely,

2.1.15

Dr S Bhuvaneshwari
Member-Secretary
Institutional Human Ethics Committee

SECRETARY
PSG IMS&R
COIMBATORE-641004.
INSTITUTIONAL HUMAN ETHICS COMMITTEE

QUESTIONNAIRE

NAME:

AGE:

SEX:

GRADE& SECTION:

NAME OF THE SCHOOL:

ADDRESS:

AVERAGE CLASS SIZE IN THE SCHOOL:

H/O THROAT INFECTION IN PAST TWO WEEKS:

H/O JOINT PAIN:

H/O TAKING MONTHLY INJECTION:

H/O DRUG INTAKE:

SOP 03-V 3.0 / ANX 09-V 2.0

Institutional Human Ethics Committee
PSG Institute of Medical Sciences and Research, Coimbatore

Assent to be in a Research Study
For children between 7-18 years old

Why are we meeting with you?

We want to tell you about something we are doing called a research study. A research study is when doctors collect a lot of information to learn more about something related to health and disease.. .....and some other doctors are doing a study to learn more about Streptococcal infections in the throat After we tell you about it, we will ask if you'd like to be in this study or not.

Why are we doing this study?

We want to find out **Streptococcal throat carrier in school children**
So we are getting information from lots of boys and girls like you.
In the whole study, there will be about ..... children.

What will happen to you if you are in this study?

Only if you agree, two things will happen:

1. A small amount of your blood will be drawn. That means it will be taken by a needle in your arm. This will happen……..times. **NA**

   *[If some or all of blood draws would be done anyway as part of child's clinical care, emphasize here what will be done **extra** for the study.]*

2. The doctors will do some tests . **A throat sawb will be taken from you for the study. It is a noninvasive technique.**
3. You will need to answer some questions about …**Nil**…..

Will this study hurt? NO

The stick from the needle to draw your blood will hurt, but the hurt will go away after awhile.

Will you get better if you are in this study?
No, this study won't make you feel better or get well. But the doctors might find out something that will help other children like you later.

Will everybody come to know about my condition? (Confidentiality) NO
We will not tell other people that you are in this research and we won't share information about you to anyone who does not work in the research study

Is this bad or dangerous for me? (Risks involved)

Do I get anything for being in the research?
[Mention any reimbursements or forms of appreciation that will be provided. Any gifts given to children should be small enough to not be an inducement or reason for participating]

Will you tell me the results?
[Describe to the ability of the child to understand that the research findings will be shared in a timely fashion but that confidential information will remain confidential. If you have a plan and a timeline for the sharing of information, include the details. Also tell the child that the research will be shared more broadly, i.e. in a book, journal, conferences, etc.]

Do you have any questions?
You can ask questions any time. You can ask now. You can ask later. You can talk to me or you can talk to someone else.

Do you have to be in this study?
No, you don't. No one will be mad at you if you don't want to do this. If you don't want to be in this study, just tell us. Or if you do want to be in the study, tell us that. And, remember, you can say yes now and change your mind later. It's up to you. *This will not affect in any way your future treatment in this hospital.*

Who can I talk to or ask questions to?
List and give contact information for those people who the child can contact easily (a local person who can actually be contacted). Tell the child that they can also talk to anyone they want to about this (their own doctor, a family friend, a teacher).

If you don't want to be in this study, just tell us. If you want to be in this study, just tell us. This will not affect in any way your future treatment in this hospital.
The doctor will give you a copy of this form to keep.

SIGNATURE OF PERSON CONDUCTING ASSENT DISCUSSION
I have explained the study to _____(*print name of child here*) in language he/she can understand, and the child has agreed to be in the study.

_____ _____
Signature of Person Conducting Assent Discussion Date

_____
Name of Person Conducting Assent Discussion (*print*)

SOP 03-V 3.0 / ANX 10-V 3.0

Institutional Human Ethics Committee
PSG Institute of Medical Sciences and Research, Coimbatore

Parental Consent Form

Title of Study:

Name of the Principal Investigator:

Department:

Your (son/daughter/child/infant/adolescent youth) is invited to participate in a study of (describe the study).

My name is _____and I am a _____ at PSG Institute of Medical Sciences and Research, Coimbatore. This study is (state how study relates to your program of work or your supervisor's program of work).

I am asking for permission to include your (son/daughter/child/infant/adolescent youth) in this study because

I expect to have …… (Number) participants in the study.
If you allow your child to participate, (state who will actually conduct the research) will (describe the procedures to be followed.)

Any information that is obtained in connection with this study and that can be identified with your (son/daughter/child/infant/adolescent youth) will remain confidential and will be disclosed only with your permission. His or her responses will not be linked to his or her name or your name in any written or verbal report of this research project.

Your decision to allow your (son/daughter/child/infant/adolescent youth) to participate will not affect your or his or her present or future relationship with PSGIMS&R or PSG Hospitals or (include the name of any other institution connected with this project). If you have any questions about the study, please ask me. If you have any questions later, call me at …… If you have any questions or concerns about your (son/daughter/child/infant/adolescent youth)'s participation in this study, call……..

You may keep a copy of this consent form.
You are making a decision about allowing your (son/daughter/child/infant/adolescent youth) to participate in this study. Your signature below indicates that you have read the information provided above and have decided to allow him or her to participate in the study. If you later decide that you wish to withdraw your permission for your (son/daughter/child/infant/adolescent youth) to participate in the study, simply tell me.

You may discontinue his or her participation at any time. *This will not affect in any way your future treatment in this hospital.*

Printed Name of (son/daughter/child/infant/adolescent youth)

Signature of Parent(s) or Legal Guardian with Date

Signature of Investigator with Date

பி.எஸ்.ஜி.மருத்துவக்கல்லூரி, கோயம்புத்தூர்

பெற்றோரின் ஒப்புதல் படிவம்

ஆய்வின் நோக்கம்

தொண்டடையில் கிருமித்தொற்றுதலை கண்டறிதல்.

முதன்மை ஆய்வாளர்

S.சண்முகப்பிரியா

ஆய்வின் வழிகாட்டி

டாக்டர்.S.பார்வதி

ஆய்வின் துறை

நுண்ணுயிரியில் துறை

ஆய்வு மேற்கொள்ளும் இடம் :

பி.எஸ்.ஜி.மருத்துவ கல்லூரி வளாகம்

டாக்டர்.S.சண்முகப்பிரியா ஆகிய நான் பூ.சா.கோ மருத்துவக்கல்லூரியில் நுண்ணுயிரியில் துறையில் முதலாம் ஆண்டு பயில்கிறேன்.

என்னுடைய ஆய்விற்காக தங்களது குழந்தையை உட்படுத்த அனுமதிக்க வேண்டுகிறேன்.

தொண்டடையில் இருக்கும் சளியை எடுத்து பரிசோதனை செய்யப்போகிறேன். இதனால் தங்கள் குழந்தைக்கு எந்தவித பக்க விளைவுகளும் எற்படாது என உறுதி அளிக்கிறேன்.

இந்த பரிசோதனையின் முடிவுகள் எக்காரணத்தை முன்னிட்டு வெளிப்படுத்தப்படாது. இந்த ஆய்வில் இருந்து விலகிக்கொள்ள எந்த நேரத்திலும் உங்களுக்கு உரிமை உண்டு. இந்த ஆய்வினைப்பற்றிய சந்தேகங்களுக்கு 9940749125 என்ற எண்ணுக்குத் தொடர்பு கொள்ளவும்.

குழந்தையின் பெயர் :

பெற்றோர் கையொப்பம் :

ஆய்வாளர்
கையொப்பம்

ஆய்வுக்குட்படுபவரின் ஒப்புதல்

நான் இந்த ஆராயச்சியின் நோக்கம் மற்றும் அதன் பயன்பாட்டினைப் பற்றி தெளிவாகவும், விளக்கமாகவும் தெரியப்படுத்தப்பட்டுள்ளேன். இந்த ஆராய்ச்சியில் பங்கு கொள்ளவும். இந்த ஆய்வில் மருத்துவ ரீதியான குறிப்புகளை வரும் காலத்திலும் உபயோகப்படுத்திக் கொள்ளவும் முழுமனதுடன் சம்மதிக்கிறேன்.

ஆய்வுக்குட்படுபவரின் பெயர் :

முகவரி :

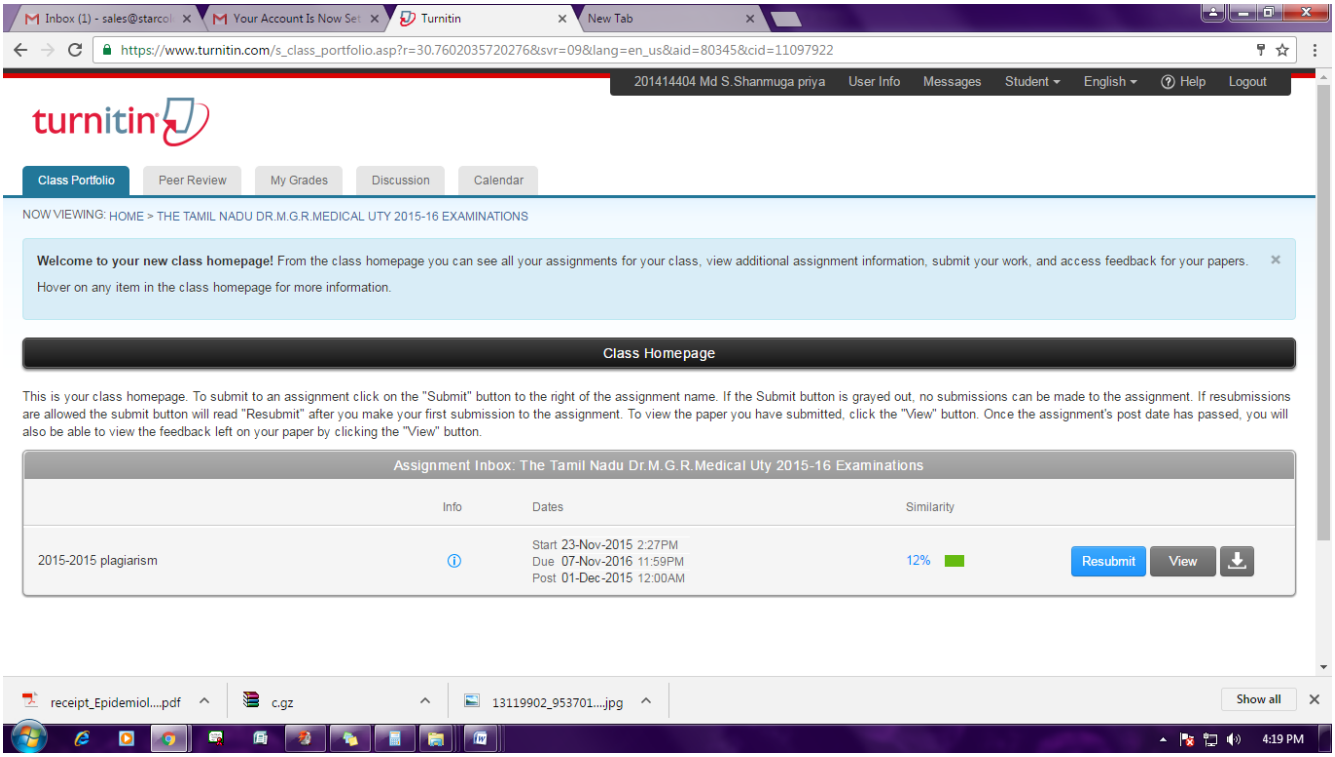
கையொப்பம் :

தேதி :

ஆய்வாளரின் தொலைபேசி எண் : 9940749125

மனித நெறிமுறைக்குழு அலுவலகத்தின் தொலைபேசி எண். : 0422-2570170, Extn : 5811

இந்த ஆய்வுக்கு உதவியதற்கு நன்றி.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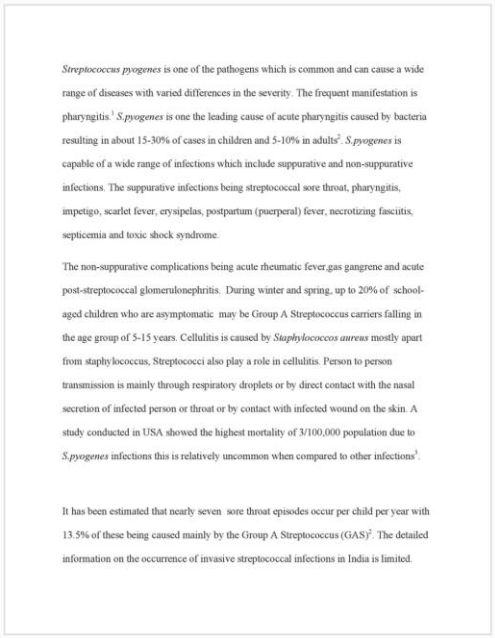

Streptococcus pyogenes is one of the pathogens which is common and can cause a wide range of diseases with varied differences in the severity. The frequent manifestation is pharyngitis.[1] *S.pyogenes* is one the leading cause of acute pharyngitis caused by bacteria resulting in about 15-30% of cases in children and 5-10% in adults[2]. *S.pyogenes* is capable of a wide range of infections which include suppurative and non-suppurative infections. The suppurative infections being streptococcal sore throat, pharyngitis, impetigo, scarlet fever, erysipelas, postpartum (puerperal) fever, necrotizing fasciitis, septicemia and toxic shock syndrome.

The non-suppurative complications being acute rheumatic fever,gas gangrene and acute post-streptococcal glomerulonephritis. During winter and spring, up to 20% of school-aged children who are asymptomatic may be Group A Streptococcus carriers falling in the age group of 5-15 years. Cellulitis is caused by *Staphylococcos aureus* mostly apart from staphylococcus, Streptococci also play a role in cellulitis. Person to person transmission is mainly through respiratory droplets or by direct contact with the nasal secretion of infected person or throat or by contact with infected wound on the skin. A study conducted in USA showed the highest mortality of 3/100,000 population due to *S.pyogenes* infections this is relatively uncommon when compared to other infections[3].

It has been estimated that nearly seven sore throat episodes occur per child per year with 13.5% of these being caused mainly by the Group A Streptococcus (GAS)[2]. The detailed information on the occurrence of invasive streptococcal infections in India is limited.