

Molecular and Clinical Diagnosis of Group-A Streptococcus Infection among School Children Throat Samples.

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Abstract: To study Group-A Beta hemolytic streptococcus in school children throat samples. To compare between affected anemic Government school children and private school children to maintain the hygienic to detect the throat sample. Symptomatic pharyngeal carriage of group A streptococci in school children may lead to spread of respiratory infection in the community. To study to screen the school children from various parts of school, to detect pharyngeal carriage of Group-A Streptococcus. To isolate Group-A Beta hemolytic streptococcus in school children throat sample. To identify the human pathogen Group-A streptococcus bacteria Grams staining. Human pathogens Group-A streptococcus were characterized. The Biochemical analysis and 16s rRNA gene sequencing were analyzed. To antimicrobial Susceptibility testing was performed on blood agar by the Kirby-Bauer disc diffusion method. The anti-biogram assay showed that the streptococcal species are particularly sensitive to Penicillin erythromycin, Tetracyclin, clindamycin, linezolid, amikacin, vancomycin, gentamycin cotrimoxazole, and chloramphenicol.

Keywords: streptococcus; throat samples; Bacterial isolates; Hemolytic.

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I. INTRODUCTION

Anemia in childhood is defined as a hemoglobin concentration below. Anemia can cause a variety of complications, including fatigue (tiredness) and stress on the body's organs. The body is destroying red blood cells. Red blood cells are being lost. The body is producing red blood cells too slowly. Anemia is fairly common blood disorder with many causes. They include inherited disorders, nutritional problems (such as an iron or vitamin deficiency), infections, some kinds of cancer, and exposure to a drug or toxin. Screening is recommended only for high-risk children.

Childhood anemia poses a major public health issue leading to an increased risk of child mortality, as well as the negative consequences of iron-deficiency anemia on cognitive and physical development (Janus, et al., 2010). The likely cause of childhood anemia varies depending on the area of the world that the child lives in. Overall, iron deficiency (usually because of diet) is the most common disease such as malaria, helminths infection, HIV and tuberculosis are also important. (Kassebaum et al., 2014).

Congenital hypoplastic anemia that usually presents in infancy (an average of 7 babies born each year) (Vlachos, et al., 2010). Certain racial groups are more likely to have inherited anemia than others for example, sickle cell disease is more common in people of central African origin whilst beta thalassaemias are more common in Mediterranean, Middle Eastern and Southeast Asian populations (Harris, 2004, Anna Seale et al., (2016) To determine the extent of group-A Streptococcus (GAS) infections in sub-Saharan Africa and the serotypes that cause disease, we analyzed surveillance data for 64,741 hospital admissions in Kilifi, Kenya, during 1998–2011. We evaluated incidence, clinical presentations, and emmtypes that cause invasive GAS infection. We detected 370 cases; of the 369 for which we had data, most were skin and soft tissue infections (70%), severe pneumonia (23%), and primary bacteremia (14%). Gaurav Kwatra et al., (2016) study at the most important risk factor for early-onset (babies younger than 7 days) invasive group B Streptococcal disease is rectovaginal colonization of the mother at delivery. Nirmal Kushwaha et al., (2014) Group-A Streptococcus, tonsillitis pharyngitis, school children. Streptococcus pyogenic. The most important gram positive cocci is Streptococcus pyogenes causing pyogenic infections, Group-A beta hemolytic streptococci are the most frequently isolated pathogen in pharyngitis among school going children. The objective of the study was to find the prevalence of Group-A Streptococcus infection among the Government Higher primary school children of an urban community.

Sanjeeb Sharma et al., (2014) To study the prevalence of Group-A beta haemolytic streptococcal (GABHS) infection among children aged between 5 to 15 years suffering from acute tonsillopharyngitis and its

antibiogram. This was a cross sectional prospective study done over two years, by procuring data from the total 123 patient records of OPD patients.

II. MATERIALS AND METHODS

Study area:

A cross sectional study was conducted in Thirupattur, (Sivagangai district, Tamil nadu.) Government and Private school children under the M.sc project work catchment of J.J college of Arts and Science College in microbiology lab for a three month from January to March - 2018.

Sample collection:

A total of 100 children were enrolled in this from 4 different school children. School name: APSA higher secondary school, BABA matriculation school (Government & private school children) in ThirupatturSivagangai District.After obtaining permission from the school authorities, an informed consent was sent to the parents of all the children who are including in the study. The consent explained about the study and the throat swabs which were intended to be taken from the parents.

A throat swabs was taken by depressing the tongue and 2 swabs were passed well over the tonsils, the tonsillar fossa and over the posterior pharyngeal wall[Ross PW,2011]. The swabs were placed in sterile test tubes with the ends sticking outside to facilitate handing and they were immediately transported to the lab.

Blood agar

Blood agar is used for isolation and cultivation of many types of fastidious bacteria. It is also used to differentiate bacteria based on their hemolytic characteristics especially within the genera Streptococcus Enterococcus and Aerococcus. Several species of Gram- positive cocci produce exotoxins called hemolysis able to destroy red blood cells (RBCs) and hemoglobin. Blood agar, which is a mixture of tryptic soy agar and sheep blood, allows differentiation of bacteria based on their ability to hemolysis RBCs .The three major types of hemolysis;

Preparation of blood agar medium:

Isolation of Bacterial Isolates

Ingredients	Grams/ litre
Peptic digest of animal Tissue	5.0
Sodium Chloride	5.0
Beef extract	1.5
Yeast extract	1.5
Agar agar	15.0
Defibrinated blood	70 ml
Final pH (at 25° C)	7.4 ± 0.2.

They were then plated on 5% sheep blood agar.The plates were incubated at 37⁰C in CO₂ and they were read after 24 hours and 48 hours. After incubation beta hemolytic colonies (clear zone) were identified and streptococcus was confirmed by microscopic examination of the gram stained smear from these colonies and biochemical test[Mathur NB, 1992&MacFaddin JF2000].

Kirby Bauer antibiotic sensitivity test:

Dip a sterile cotton swab into a well-mixed saline test culture and remove excess inoculums by pressing the saturated swab against the inter wall of the culture tube. Using the swab, streak the entire agar surface horizontally, vertically and around the outer edge of the plate to ensure a heavy growth over the entire surface.Allow all culture plates to dry for 5minutes.Apply the antibiotic discs, and gently press with help of for caps dipped in alcohol and flamed.Incubate all plates' culture in an inverted position for 24-48 hours at 37°c.

Antibiotic susceptibility test:

Antibiotic susceptibility testing was performed on blood agar by the Kirby-Bauer disc diffusion method by using disc that was obtained from Hi-Media Laboratories Pvt. Ltd. according to the CLSI standards. The antibiotic which was tested included Penicillin erythromycin, Tetracyclin, clindamycin, linezolid, amikacin, vancomycin, gentamycin, cotrimoxazole and chloramphenicol [Shet A, Kaplan E,2004][Pichichero ME,1995].

Bacterial Identification- 16 S rRNA Sequencing

Purification of 16S rRNA PCR product

The partial 16S rRNA gene fragments were amplified from the Cultures. The PCR products were precipitated with 9 μ l of 3 M sodium acetate (pH 5.2) and 60 μ l of isopropanol and resuspended in 50 μ l of distilled water. Extraction of the PCR products with chloroform was carried out [Akhteret al., 2013]. BLAST provides a method for rapid searching of nucleotide and protein databases.

Phylogenetic analysis

The reference sequences required for comparison were down loaded from the BLAST database. All the sequences were aligned using the multiple sequence alignment programme. The aligned sequences were then checked for gaps manually and arranged in a block of 250 bp in each row and saved. The bootstrapped data set was used directly for constructing phylogenetic tree using the multiple distance matrix obtained was then used to construct phylogenetic tree using Neighbour-Joining method of Saitou and Nei (1987).

III. RESULT

A total of 50 school children were enrolled in this study from two Government school children. Throat samples are collected from APSA higher secondary school, BABA matriculation school Thirupattur, surrounding Thirupattur schools. That who were aged between 5-10, 11-13, 14-15, 16-17 years out of 100 school children 60 for female and 40 for male. Out of 60 female throat samples 32(53.3%) female are infected by β – hemolytic Group-A streptococcus. In male out of 40 throat samples 12(30%) throat samples were found to streptococcal infection. A total of 50 children were enrolled in this study from 2 Private school children. Throat sample collected from APSA Higher Secondary School in Thirupattur and BABA primary school in Thirupattur.

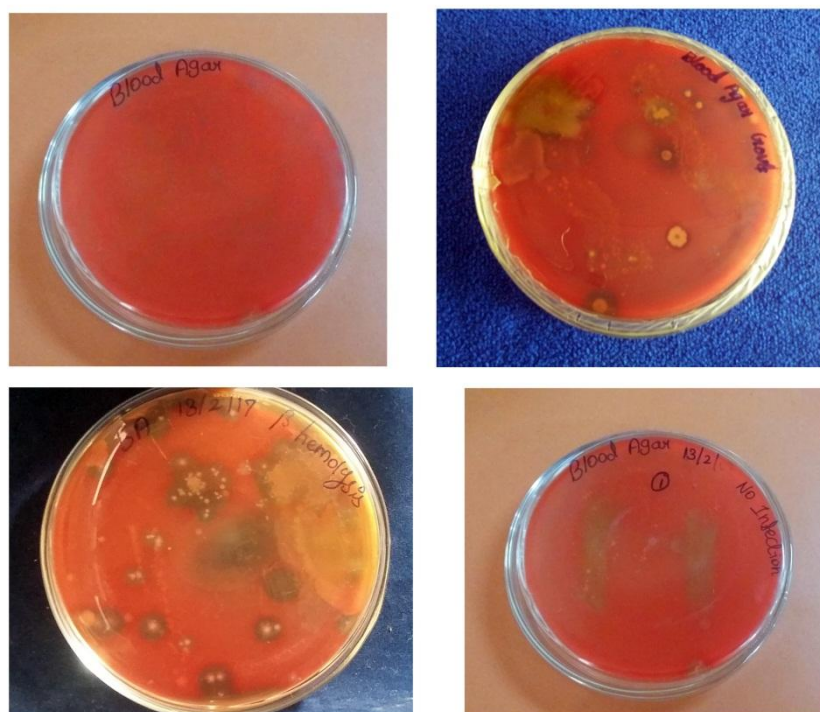


Figure1:Bacterial colonies on Blood Agar Plates after collection of Throat Samples.

Streptococcal produce two types of infection Symptomatic and Asymptomatic. Symptomatic means posterior sides of tongue region appear red spot and look like Strawberry tongue.

Asymptomatic means without any symptoms. But they affect β – hemolytic Streptococcal infection. The throat swabs were inoculated on 5% of sheep blood agar. After incubation at 35 -37^oCor 24 hours, cultures were evaluated. After incubation three types of hemolytic streptococcal species appear on the blood agar plates [Fig-2]. β – hemolytic Group-A streptococcal Species are complete destruction of RBCs and hemoglobin, and result in a clear zone around the colonies. i.e., streptococcus pyogenes. α – hemolytic streptococcal was exhibit incomplete hemolysis, which imparts green appearances to the hemoglobin. These species are called viridian. γ –hemolytic streptococcal are non- hemolytic. The streptococcal included in this group are non-pathogens.

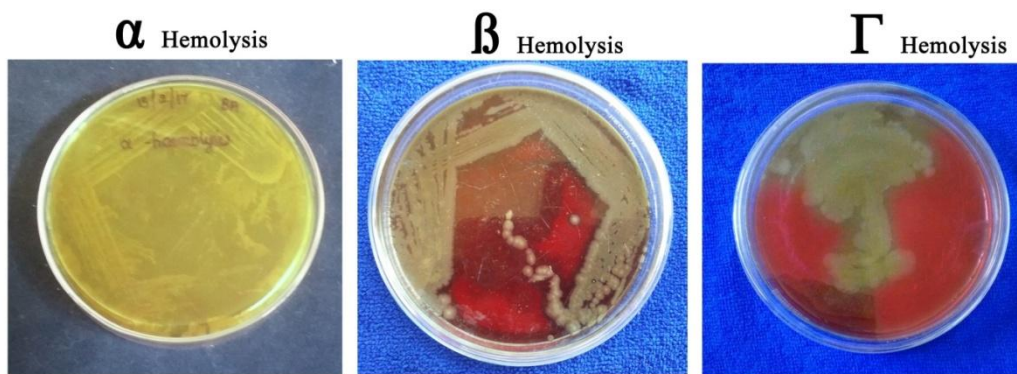
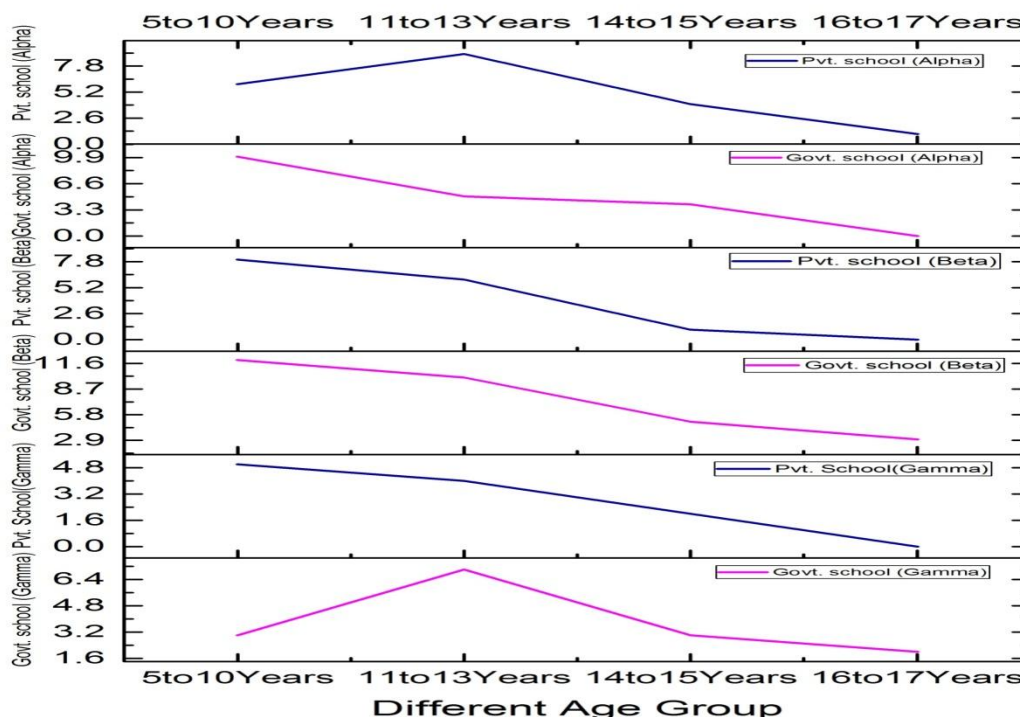


Figure2: Isolated Alpha Hemolytic Streptococcus, Beta Hemolytic Streptococcus and Gamma Hemolytic Streptococcus on BAM.

Beta hemolysis is the complete destruction of RBCs and hemoglobin, and result in a clearing of the medium around the colonies. They are account for the majority of diseases.eg. Streptococcus pyogenes (group-A) and Group-B beta hemolytic streptococci - Streptococcus agalactiae. Alpha hemolysis is the partial distribution of RBCs and produces a greenish discoloration of the agar around the colonies. α hemolysis is due to the reduction of RBC hemoglobin to methemoglobin in the medium surrounding the colony. Many of the α hemolytic streptococci are part of the normal body flora. Eg. Streptococcus pneumoniae. These species are called viridians. Gama (γ) hemolysis is actually non hemolytic and appears as simple growth with no change to the medium. Streptococci included in this group are non-pathogens.

(Table: 1) Among the total 100 school children were enrolled in this studies from 4 different schools around the Thirupattur. Overall 100 school children 60 for male and 40 for Female. They are 4 different categories in age wise. 5-10 years children, 11-13 years children, 14-15 years children, 16-17 years children. After obtaining permission from the school authorities, an -informed consent was sent to the A total of 100 children were enrolled in this from 4 different school children. [School name: APSA high school, BABA matriculation school. (Government & private school children) in Thirupattur].



Graph1: Comparative study of Government and private school children affected by Alpha Hemolytic Streptococcus, Beta Hemolytic Streptococcus and Gamma Hemolytic Streptococcus on BAM.

β – hemolytic Group-A Streptococcus appear in 45 school children out of 100. The infected 45 school children 9 student are Symptomatic and 36 students are Asymptomatic. 20 students in 5-10 years children affected Group-A Streptococcus infection. 16 students in 11-13 years children affected GAS infection. 6 students in 14 -15 years school children affected GAS infection, and 3 students in 16 -17 years school children affected GAS infection [Graph-1].

The overall prevalence of Group-A Streptococcus infection was estimated as 45% higher prevalence was found in 5-13 years of age. The Prevalence percentage declined with increase in age. The prevalence was higher in female as compared to male. In this study overall prevalence Group-A Streptococcus infection affected by Government school children then compared to private school children.

Table: 1. Results of symptomatic and Asymptomatic (Government school and Private school children)

No.of children	Symptomatic	Asymptomatic	No infections
100	9	36	55

Among the 100 throat swabs which were collected from four different school going children who were aged between 5-17 years. Out of 100 school children, 45 children are affected by Streptococcus infection. In 45 affected children 9 for symptomatic and 36 for Asymptomatic.

Sequencing of the 16s r-RNA gene is revealed that organism isolates were streptococcus constellatus [Fig-3]. sequence analysis of the 16s r-RNA gene is considered a more reliable means for species identification and most consider a minimum of 97 -98% sequence similarity as adequate for identification.

phylogeny analysis of *Streptococcus constellatus*

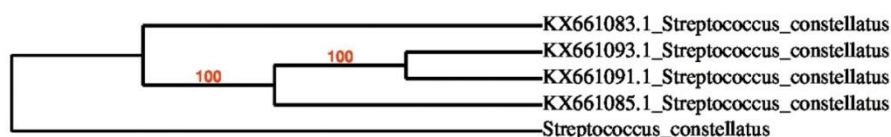


Fig3: Phylogenetic position of isolated *Streptococcus constellatus*

IV. DISCUSSION

Throat samples were collected from different age group of school children. (5-10 years, 11-13 years, 13-15 years and 15-17 years). A similar carrier rate of 8.3% was reported among 1,796 asymptomatic patients in an urban hospital in Croatia. The throat swab samples were analyzed for streptococcal Group-A infection by β – hemolytic test. A study was conducted in around Thirupattur. Among the 100 throat swabs which were collected from four different school going children who were aged between 5-17 years. They are aged between 5-10, 11-13, 14-15, 16-17 years out of 100 school children 60 for female and 40 for male. Out of 60 female throat samples 32(53.3%) female are infected by β – hemolytic Group-A streptococcus. In male out of 40 throat samples 12(30%) throat samples were found to streptococcal infection [Fig-1]. Airport from Chennai however was different where BHS isolated was high (53.5%). [Kalpana S, Sundar JS]. prevalence of β - hemolytic streptococcal pharyngitis was higher among female than compared to male. In Government school children out of 50 school children throat samples 30 samples were found to GAS produce a sharply defined clear colorless zone of hemolysis appear in blood agar plate Menon T reported that among 80 symptomatic children 20% grew beta-hemolytic and among that 50% isolates were Group-A, 19.5% of 230 Asymptomatic children grew beta-hemolytic and among them 40% were Group-A and rest were other groups of Streptococci [Menon et al]. Apart from this other types of hemolytic streptococcal sps. We also isolated and identified. In Government school children out of 50 samples. 19 samples were found to Alpha(α) hemolytic streptococcal infection exhibit incomplete hemolysis, which imparts green appearance to the hemolobin containing blood agar plate and 15 samples were found to Gama(γ) hemolytic streptococcal infection are non- hemolytic the streptococci included in this Group are non-pathogenic [Graph-1]. sequence analysis of the 16s r-RNA gene is considered a more reliable means for species identification and most consider a minimum of 97 -98% sequence similarity as adequate for identification [Fig 3].

V. CONCLUSION

It is concluded the carrier rate of Group-A streptococcus was the highest in the Government school children then compare to private school children. Because of the number of students per classroom may be an important risk factor for the transmission of GAS. Group-A Streptococcal out breaks can occur in day-care centers in some periods despite strict hygiene and appropriate antibiotic remains the treatment of choice for penicillin allergic individuals macrolides are accepted as an alternative. Government school children should follow proper hygienic and take healthy food.

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