



Burden of Group A Streptococci among Children Aged 5-15 years with Symptoms of Pharyngotonsillitis: A Study in a Tertiary Care Hospital, Bangalore

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Abstract

Background: Rheumatic fever and Rheumatic heart disease continue to be major problems in most developing countries including India where prevalence rate is 4-6/1000 children per year. Clinical presentations of streptococcal and non streptococcal pharyngitis overlap broadly, so laboratory diagnosis is required to support clinical diagnosis, minimise the potential adverse effect of inappropriate antimicrobial therapy and to prevent RF/RHD.

Objective: To isolate and determine the prevalence of Group A Streptococci in symptomatic children and detect their antibiotic sensitivity pattern.

Materials and Methods: Throat swabs from 100 symptomatic children were collected and cultured on 5% sheep blood agar. Isolated beta haemolytic colonies were identified by conventional techniques as Group A Streptococci and was confirmed by Streptex Kit. Then DNA was extracted and molecular weight confirmed with standards, thus determining the prevalence rate of 6%. Surface M Protein was extracted and molecular weight analysed using SDS PAGE showing different M Protein types. Antibiotic susceptibility was done to show the sensitivity to penicillin and to macrolides like Erythromycin.

Conclusion: Study revealed prevalence of Group A Streptococci in only 6% of symptomatic children. Protein analysis suggests the existence of more than one M type responsible for infection. None of the isolates showed Erythromycin resistance.

INTRODUCTION

Group A Streptococci cause pharyngitis, tonsillitis and erysipelas leading to suppurative complications like otitis media, quinsy and mastoiditis and non suppurative sequelae like rheumatic fever and rheumatic heart disease (Bramhadathan K N and Gladstone P, 2006)¹.

According to World Health Organization at least 517,000 deaths are attributed to Group A Streptococci annually across the globe and approximately one third of these deaths (163,000) are related to invasive group A Streptococci disease and remaining 3,54000 are related to non

suppurative sequelae of Group A Streptococci infections (Brono A I et.al, 2005)².

Rheumatic fever and rheumatic heart disease continue to be a major problem in most developing countries including India (Kaplan E L, 2005; Padmavathi S, 2001; Kumar R et.al, 2004)^{3,4,5}. In India, prevalence of Group A streptococcal pharyngitis and its carriage is estimated to be 4.2-13.7% and 11.2- 34% respectively (Shet A et.al, 2004; Rajkumar S et.al, 2001)^{6,7}.

Since the clinical presentation of streptococcal pharyngitis overlaps broadly with viral and other pharyngitis, It is impossible to diagnose through clinical observation alone. Hence diagnostic support from laboratory is required, to minimise the potential adverse effects of inappropriate antimicrobial therapy (Bisno A L et.al, 1997)⁸.

Surface M protein of streptococcus pyogenes acts as an important virulence factor which helps antiphagocytic activity. There is also strong association between autoimmune sequelae and particular M types. ASO antibodies in serum are useful for retrospective diagnosis of recent streptococcal infections. Timely diagnosis and treatment of streptococcal pharyngotonsillitis with Benzathine Penicillin help prevention of rheumatic fever and rheumatic heart disease (Nandi S et.al, 2002, 2001)^{9,10}.

AIM

To detect the prevalence of streptococcal pharyngitis among all symptomatic cases of pharyngotonsillitis.

OBJECTIVES

1. To isolate beta haemolytic streptococci from throat swabs of symptomatic children aged 5-15 years.
2. To identify the isolate by conventional and commercially available Streptex kit as Group A Streptococci
3. To compare the molecular weight of extracted DNA from the test strain with that of standards.

4. To extract surface M protein and determine the M protein variation by molecular weight.
5. To determine the sensitivity pattern with special reference to Erythromycin.

MATERIALS AND METHODS

Study Design: Cross Sectional study

Study Period: One year, From January 2007 to December 2007.

Study setting: M S Ramaiah Medical College and Teaching Hospital.

Inclusion Criteria

Children aged 5-15 years with symptoms of acute or chronic pharyngotonsillitis.

Exclusion Criteria

Children already started on antibiotics. The children whose parents did not give consent.

Standard strains used as controls

The Microbial Type Culture Collection and Gene Bank Streptococcus pyogenes MTCC 442 and The American Type Culture Collection Streptococcus pyogenes ATCC19615.

Collection and processing of Samples

Written consent was obtained from parents after explaining the study in detail. Throat swabs were collected from symptomatic children aged 5-15 years attending ENT outpatient department of MSRMTH with clinical features of pharyngotonsillitis aseptically using standard techniques (Bisno A L et.al, 1997)⁸.

Swabs were transported in the stuart's media and were cultured on 5% Sheep blood agar and incubated at 5-10% CO₂ overnight (Nandi S et.al, 2002)⁹ for beta haemolytic colonies. Presence of more than 20 colonies along with clinical features were considered as cases of streptococcal pharyngitis (Nandi S et.al, 2001)¹⁰. Gram stain of suspected colonies was done before processing.

All beta haemolytic colonies showing Gram positive cocci arranged on chains which were catalase negative with Bacitracin (0.04U) susceptibility zone size 10 mm, and pyrrolidonyl-arylamidase test (PYR) positive were presumptively identified as Group A haemolytic

Streptococci. It was confirmed by latex agglutination test using commercially available streptex Kit (Kumar N et.al, 2008)⁶.

Antibiotic susceptibility was done on Muller hinton sheep blood agar plate, Kirby Bauer disc diffusion method using antibiotics Penicillin, Amoxycillin, Erythromycin, Azithromycin, Cefadroxil. ASO titre was done for all patients using commercially available latex agglutination procedure (Span Diagnostics). Stocks were maintained in Todd Hewitt slope, Robertson cooked meat medium and Filter paper method for further nucleic acid and protein analysis.

Genomic DNA was extracted by rupturing the cell wall and nuclear membrane using Tris EDTA and 10% SDS followed by deproteinization, using phenol chloroform mixture and precipitation of nucleic acid using ethanol. Extracted DNA was quantified using UV Mini1240 (Shimadzu) spectrophotometer at 260 nm and the nucleic acid concentration was calculated using Beer Lambert law.

Extracted DNA was demonstrated using Agarose gel electrophoresis and molecular weight of isolated genomic DNA was determined using the DGelDAS (Digital Gel Documentation & Analysis Software) supplied by Biotech R&D laboratories Yercaud. The documented photograph was used in the software to analyse the same (Photo 1).

Cell surface antigenic protein was extracted using Tris- Hcl and 2% SDS and was quantified using Lowry's method. Protein was demonstrated using Sodium Dodecyl Sulphate Polyacrylamide–Slab gel Electrophoresis (SDS PAGE) at 10-15 mA current for 10-15 minutes initially followed by 30 mA for about 3 hours. The cell surface antigenic protein extracts run in 12% polyacrylamide gel along with medium size protein marker (Aristogene).

Molecular weight of extracted cell surface protein were measured using the DGelDAS supplied by Biotech R&D Laboratories, Yercaud. The documented photograph from SDS PAGE was used in the software to analyse the same (Photo 2).

RESULTS

Among hundred children, six were culture positive for Group A beta haemolytic Streptococci. And out of the six, five were Females and one Male. All children who were culture positive were in the age group of 10-15 years.

All the six culture positives showed symptoms of fever, throat pain, difficulty in swallowing, temperature 101-104°F, posterior wall congestion with or without follicles, anterior cervical lymph node palpable with or without congestion.

Among the six children, four were ASO positive with 200 IU, thus 66.6% of the culture positives showing ASO positivity.

Among 94 children who were culture negative, two were ASO positive.

All culture positive cases were obtained in the month of November.

All the six children who were culture positive belong to low socio economic group as per Kuppuswamy's classification (Kumar N et.al, 2007)¹¹.

All the six isolates identified presumptively by Bcitracin 0.04U susceptibility and PYR test positive as Group A Streptococci were confirmed as Group A by Streptex Kit, thus showing 100% correlation between different methods.

All the six isolates were sensitive to Penicillin, Amoxycillin, Erythromycin, Azithromycin and Cefadroxil.

Results of Nucleic acid analysis of all the six isolates were the same as the standard strains used and the molecular weight of genomic DNA was calculated by the software to be around 16000bp thus confirming the isolates as Group A Streptococci.

Cell surface antigenic protein extracted from the isolates and that of standards showed two bands on SDS PAGE. First band between 68000-70000Da with no variation and the second band between 20000-30000 Da showing slight variation in molecular weight.

PHOTO 1: Agarose Gel Electrophoresis showing Molecular Weights of Test and Standard strains DNA.

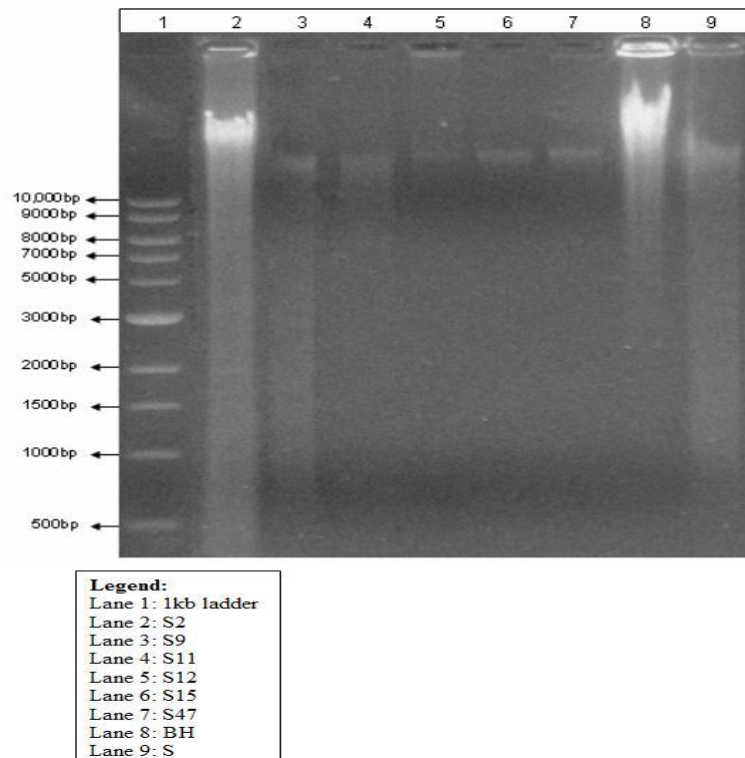
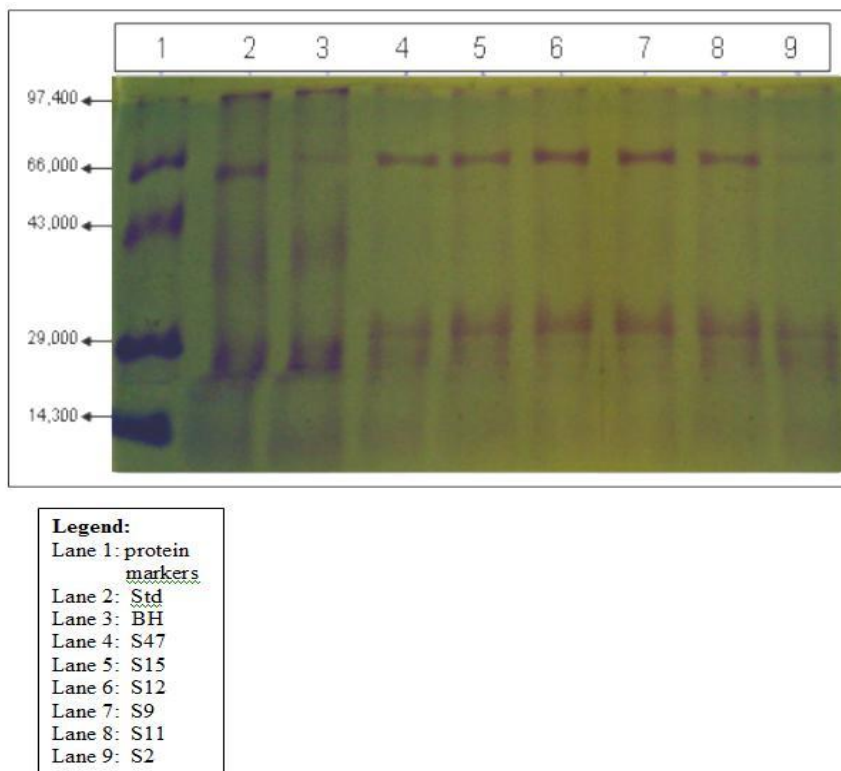


PHOTO 2: SDS PAGE of Extracted Cell Surface Proteins of Test Strains and that of Standards.



DISCUSSION

In this study, all the six children with culture positive for Group A Streptococci present with fever, throat pain, difficulty in swallowing,

temperature 101°-104°F, posterior pharyngeal wall congestion with or without follicles and palpable anterior cervical lymph node with or without congestion.

According to American Heart Association Committee on Rheumatic Fever, Endocarditis and Kawasaki Disease, the children with group A Streptococci present with pain on swallowing fever, fever, headache, nausea, vomiting and abdominal pain, in addition to tonsillopharyngeal erythema with or without exudates and tender enlarged anterior lymph nodes, beefy red swollen uvula and petechiae on the palate. Whereas Siegal et.al have given less importance to enlarged lymph nodes in the differential diagnosis suggesting that exudative pharyngitis is frequently associated with viral infections (Nandi S et.al, 2002)⁹.

The present study is correlating with earlier studies that show isolation of Group A Streptococci in symptomatic children most commonly seen in low socioeconomic status as per Kuppaswamy's classification (Kumar N, et al 2007)¹¹.

In the current study, presumptive identification of beta haemolytic colonies as Group A Streptococci by gram stain, Catalase test, Bacitracin susceptibility and PYR positivity was 100% when confirmed by Streptex agglutination test thus correlating with (Brown et.al, 1979)¹².

In the present study, the prevalence of Group A Streptococci in symptomatic children aged 5-15 years is 6% which is correlating with the previous study (Sindulina C et. al, 2007)¹³.

According to the study carried out by Rajkumar et.al in 1991-1992 at Chennai among symptomatic children prevalence was 4% while it was only 3.7% among asymptomatic. In the study by Nandi et al in 1995-1996 at Chandigarh prevalence was 13.5% among symptomatic. The study by Navneeth et al 1998 at Salem showed prevalence of 6.2% among asymptomatic (Rajkumar S et.al, 2001)⁷.

In the present study, among six culture positives, four serum samples were ASO positive which accounts for 66.6% where as in the previous study by Rajkumar et. al 1991-1992 at Chennai showed 46% ASO positive among symptomatic (Shet A, Kaplan E, 2004)⁶. In another study by Chakrabarti

et al 1989-1991 at Darjeeling ASO positive was 2% among symptomatic(Rajkumar S et.al, 2001)⁷.

In the present study, none of the isolates were showing Erythromycin resistance. Whereas in the study conducted by C.A.C Llyod et.al reported 16.2% resistance and that by Brahmadathan et.al at Vellore 2002 reported 13.8% of Erythromycin resistance among Group A Streptococci isolates (Llyod C A C et.al, 2007)¹⁴.

Overnight Todd Hewitt Broth cultures were used in the present study for total DNA extraction and detergent lysis of cell wall was achieved using SDS followed by phenol chloroform extraction. Fiscetti et. al in similar study have used alternate methods like phage lysis followed by phenol chloroform method (Fiscetti et. al, 1976)¹⁵.

In the present study, the antigenic cell surface M protein was extracted from the cell wall using Tris HCl and SDS PAGE. In other studies, M protein was extracted using heat/pH shock, Peptic Digestion method by Edwin H et.al in 1974 (Beachey E H et.al, 1974)¹⁶ Nitric acid method by Khalil et al 1981 (Russel D H et. al, 1975)¹⁷ and Guanidine extraction method by Harold R et. al in 1975 (Hafez K et. al, 1981)¹⁸.

In the present study, protein analysis revealed the presence of two distinct cell surface antigenic proteins in the clinical as well as standard strains. In the study by Vosti et. al in 1978 (Vosti K L et. al, 1978)¹⁹, the molecular weight of M protein in hydroxyapatite column fractions were determined by electrophoresis in polyacrylamide disc gels with SDS continuous buffer system ranged between 22000-73000Da. The molecular weight of cell surface antigenic proteins extracted in the present study was in accordance with previous studies; the 1st antigenic protein band in SDS PAGE was same around 69000 Da, the 2nd band was between 20000-30000 Da.

In the study conducted by Fiscetti et. al Streptococcal M protein extraction by non-ionic detergents demonstrated by SDS PAGE was around 28000-35000 Da (Fiscetti et.al, 1974)¹⁵. The variation in molecular weight of 2nd antigenic

protein may attribute to the difference in the virulence of different isolates.

In the recent studies conducted by Nandi et.al, Jain et.al, Kushwaha et. al and Singh A K et. al prevalence of GAS isolates among symptomatic children were 18.8%, 12.6%, 39.1% and 4.7% respectively (Singh A K et.al, 2015)²⁰.lk

CONCLUSION

The present study shows a prevalence of Group A Streptococci to be 6% among symptomatic patients with pharyngitis. It is more common in the 10-15 year age group. It is more commonly seen during winters and among low socio-economic group.

Further, evaluation of this six percent of isolates for any variation in the cell surface. M protein by SDS PAGE assay revealed changes in 2nd band which suggest for existence of more than one M types being responsible for infection in the population.

However, inspite of the presence of more than one M type of the Group A Streptococci, all the isolates were found to be sensitive to Penicillin.

No Erythromycin resistance was seen among the isolates.

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