

Original Research Article

A Bacterio-Immunological Study of Strep Throat in Paediatric Patients Attending OPD of a Tertiary Care Hospital

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Abstract: Streptococcus pyogenes is one of the commonest bacterial pathogen that causes pharyngitis especially among children. Aim: To isolate Streptococcus pyogenes from children attending paediatric and ENT OPD, AIMSRS, Bathinda and to find their antimicrobial susceptibility pattern. Material & Method: 200 children, aged 5-15 years, clinically suspected of acute Streptococcal pharyngitis and tonsillitis were included in the present study. The study was conducted over a period of one year from January 2015 to December 2015. Throat swabs were collected from patients for gram stain and culture. Streptococcus pyogenes was identified by beta hemolytic colonies on blood agar and confirmed by Bacitracin sensitivity and PYR positivity. Results: Among 200 clinically suspected children, Streptococcus pyogenes was isolated from 26 cases, prevalence being 13%. The prevalence was more in the age group 5-10 years. All the isolates were sensitive to Penicillin, Amoxicillin, Cephalexin, Cefuroxime (100%), 95.83% of isolates were sensitive to Azithromycin while 70% were sensitive to Erythromycin. 30.76% of the culture positive cases were also positive for the Antistreptolysin 'O' antibodies.

Keywords: Streptococcus pyogenes, paediatric age group, Culture, ASO.

INTRODUCTION

Streptococcus pyogenes is one of the commonest bacterial pathogens that cause pharyngitis among school aged children [2, 8, 10]. Pharyngitis is predominantly caused by viruses and treatment with antibiotics should be restricted and started only upon strong clinical suspicion or after confirmation by culture [16]. Streptococcus pyogenes is of significant clinical interest because of its association with two non suppurative sequelae: Acute Rheumatic Fever and Acute Glomerulonephritis which are responsible for significant morbidity & mortality [1, 10, 13]. Other post streptococcal syndromes like arthritis, neuropsychiatric disorders also mandates the importance of isolation of S.pyogenes and elevation of ASO and anti DNA ase B antibody titres [12]. The prevalence of group A beta hemolytic streptococci (BHS) carriage in the throat of school children varies from 13 to 50% depending upon

the population studied, season and other factors [2]. This study was done to know the prevalence of Group A beta hemolytic streptococci among school children attending paediatric OPD, AIMSRS, to find their antimicrobial susceptibility pattern and measure ASO titres in culture positive cases.

MATERIALS AND METHODS

The study included 200 school going children suspected clinically of acute streptococcal pharyngitis and tonsillitis, attending outpatient department of paediatrics and ENT, Adesh Medical College, Bathinda. The age of the children included in the study ranged from 5 to 15 years. The study was conducted for a period of one year i.e., January 2015 to December 2015, in the Central Laboratory, AIMSRS, and Bathinda.

Hi Media swabs with Aims transport media were used to collect samples. Two throat swabs were collected from each child. The swabs were processed immediately in the laboratory. Gram stain was prepared from one swab and the second throat swab was cultured on 5% sheep blood agar and on crystal violet blood agar. These inoculated plates were incubated overnight at 37°C [4, 5, 6].

Preliminary identification of *Streptococcus pyogenes* was done by growth of beta hemolytic colonies on blood agar, presence of gram positive cocci in chains on gram stain smear and catalase negative reaction. The provisionally identified beta hemolytic streptococcus (BHS) was subcultured in brain heart infusion broth and incubated at 37°C for 2 to 4 hours. The broth was subcultured on blood agar and tested for Bacitracin (0.04 units) sensitivity. After overnight incubation, the zone of inhibition was measured. An inhibitory zone diameter of > or = 11mm was considered sensitive [4, 5, 6].

Further confirmation was done by conventional bacteriological testing using STREP IDENTIFICATION Kit (Microexpress – Tulip diagnostic Pvt. Ltd.) catalogue no: 20793001, which included panel of 12 tests which are as follows: 1. Esculin hydrolysis (negative) 2. Voges-Proskauer test (negative) 3. Arginine utilization (positive) 4. PYR hydrolysis (positive) 5. ONPG utilization (negative) and Carbohydrates: Glucose (positive), Arabinose (negative), Sorbitol (negative), Mannitol (negative), Sucrose (positive), Raffinose (negative), Ribose (negative).

The antibiotic susceptibility pattern of the test isolates was done by Kirby-Bauer's disc diffusion method on Blood Mueller Hinton Agar. The following antibiotics were used. Penicillin (10 µg), Amoxicillin (10 µg), Cephalexin (30 µg), Cefuroxime (30 µg), Azithromycin (15 µg) and Erythromycin (15 µg). The media and antibiotic discs used in the study were procured from Himedia Laboratories Pvt. Ltd.

Blood samples were also collected from all cases and ASO titres were done only for culture positive cases. The Antistreptolysin 'O' (ASO) titre in the culture positive cases was done by semi-Quantitative method. The upper limit of normal antibody is 200 Todd units/ml. [5] ASO titre was determined by latex agglutination method (RHELAX – ASO Tulip diagnostic Pvt. Ltd.)

RESULTS

The present study was conducted for a period of one year i.e., from January 2015 to December 2015. 200 clinically suspected children were included. Among

these 26 cases were Beta hemolytic streptococci. Percentage of isolation is depicted in table 1.

Table 1: Percentage of isolation of Beta hemolytic streptococcus (BHS)

Total no of cases	No. of BHS isolated	Percentage of isolation
200	26	13%

The age of these children ranged from 5-15 years. In this study, the percentage of isolation of *Streptococcus pyogenes* was found to be more among children of 5-10 years age (73%). The age wise distribution of children is shown in Table 2.

Table 2: Age Distribution

Age in years	BHS positive	% BHS
5-10	19	73.07%
11-15	7	26.92%

All the 26 isolates were sensitive to Bacitracin (0.04 units), zone of inhibition ranging between 12-16 mm in diameter and positive for pyrrolidonyl-beta-naphthylamide test (PYR test).

The confirmed isolates were tested for antibiotic sensitivity pattern by Kirby – Bauer disc diffusion method by using antibiotic discs of Penicillin (10 µg), Amoxicillin (10 µg), Cephalexin (30 µg), Cefuroxime Oral (30 µg), and Erythromycin (15 µg), Azithromycin (15 µg). Percentage sensitivity to Penicillin, Amoxicillin, Cephalexin, and Cefuroxime was 100%. 95.83% of isolates were sensitive to Azithromycin and 70% to Erythromycin. Antibiotic sensitivity pattern of the isolates is shown in table 3.

Table 3: Antibiogram

Antibiotic tested	% sensitive	% Resistant
Penicillin	100	0
Amoxicillin	100	0
Cephalexin	100	0
Cefuroxime	100	0
Erythromycin	70	30
Azithromycin	95.83	4.17

Among the 26 culture confirmed cases, the ASO titre was positive in 8(30.76%) cases. The ASO titre was greater than 400 IU/L in 2 cases (7.69 %) while in 6(23.07 %) cases the titre ranged between 200-400 IU/L. Breakup of ASO is shown in table 4

Table 4: Antistreptolysin O titre among culture positive cases

greater than 400 IU/L	between 200-400 IU/L
2 cases (7.69%)	6 cases (23.07%)

DISCUSSION:

Streptococcal Pharyngitis is one of the most common bacterial infections in children. Acquisition of this infection is rare in infancy due to mother's immunity conferred transplacentally. Infection is uncommon under two years of age. In toddler years Streptococcal infection usually involves only nose. Teenagers and adults acquire immunity over time and hence the most susceptible years are from five to fifteen [14].

In a study in south India by Koshi et al. [3] the prevalence of streptococcal pharyngitis among children was 12%. Sarkar S et al. [9], prevalence in rural Varanasi, India was 13.6%. In another study in 1992 by Gupta et al.; [2], prevalence of streptococcal pharyngitis was 13.7%. According to Sobhan Nandi et al.; [10] the prevalence of streptococcal pharyngitis was 13.5%. In the present study, the prevalence is 13% which is in concordance with other studies.

Quinn Denny and Riley [1] reported that the children of age group 8-10 years showed 59% culture positivity for beta hemolytic streptococci. Myer and Koshi in their study recorded peak prevalence of beta hemolytic streptococcal pharyngitis between 6-10 years age group [8]. Peak prevalence of streptococcal pharyngitis between 5-10 years age group was also observed by Cauwenberge PBV, et al.; [7]. Our study also shows highest prevalence among 5-10 years of age group.

No clinical isolate of Group A streptococcus anywhere in the world has been documented to be resistant to penicillin. (CLSI guidelines, 2014). Sometimes cases that do not respond to penicillin have to be treated alternatively with Amoxicillin. Still penicillins are preferred over cephalosporins. Clindamycin, Clarithromycin or Azithromycin are preferred in cases of penicillin allergy [16]. In the present study, all the isolates were sensitive to Penicillin, Amoxicillin, Cephalexin and Cefuroxime (100%), 95.83% of isolates were sensitive to Azithromycin and 70% of isolates were sensitive to Erythromycin.

S.Nandi et al. [13] mentioned in their publication that in an Egyptian study, the attack rate of Group A beta hemolytic streptococcus infection was defined as positive throat culture with concomitant rise in Antistreptolysin 'O' titre. In the present study, 33.33% of the culture positive cases showed rise in ASO titre.

Although number of studies have been carried out to detect prevalence of Group A streptococcal pharyngitis, there is need for studies for rapid diagnosis using enzyme Immunoassays, Chemiluminescent DNA

probes for RADT (Rapid Antigen Detection Tests). Culture methods to grade streptococcal growth into few, moderate and many colonies should be done so that carrier and pathogen state can be appropriately identified. Further investigations like Anti Streptolysin antibody titre and Anti DNase B antibody titres should be done to correlate post streptococcal syndromes.

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