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A Bacteriological Study of Acute Pharyngitis in Children Aged 5-15 Years with Special Reference to Streptococcal Grouping

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A B S T R A C T

To know the bacteriological profile of acute pharyngitis in special reference to serological grouping of streptococci in children of 5-15 years age. Study included 100 cases of acute pharyngitis in children between the age group of 5-15 years who attended pediatric outpatient department. Throat swabs were collected from all the 100 cases and subjected to rapid group A streptococcus antigen detection, gram stain, culture and antibiotic sensitivity testing. Isolated β hemolytic streptococcal colonies were grouped using pastorex strep latex kit. 80 cases were found bacterial culture positive among the 100 cases evaluated clinically. Among the 80 culture positive cases, 17 cases (21.25%) were due to β hemolytic streptococci. The grouping of β hemolytic streptococci showed 13 (76.47%) of them belonging to group A, 2 (11.76%) to group C and 2 (11.76%) to group G. The present study showed a 21.5% prevalence of group A β hemolytic streptococcus in the school going children of age 5-15 years suffering from acute pharyngitis. A significant amount of resistance (17%) was seen among some of the commonly used antibiotics like ampicillin, azithromycin, erythromycin and cefuroxime.

Introduction

Acute pharyngitis is the most common upper respiratory tract infection responsible for significant morbidity in the childhood. Even though various bacteria and viruses are incriminated in the etiology, streptococci are of utmost importance worldwide. They are so common in school years that they are called "Occupational diseases of the school children (Kaplan *et al*., 1980). Apart from its high incidence, what makes the streptococci special is not the ability to cause adenitis, otitis media, otitis media, pneumonia or infrequently meningitis.

However, the mysterious pathogenic relationship to its non-suppurative sequelaee like acute rheumatic fever and acute post-streptococcal glomerulonephritis does make the organism unique. These non-suppurative sequelaee are still responsible for significant morbidity and mortality. Rheumatic heart disease remains the major cardiovascular disease in many parts of the world especially in non-developed countries. In India Rheumatic heart disease accounts for 16.5 to 50 percent of the cardiac patients attending OPD and wards in a hospital (OP Ghai 5th

edition). The clinical importance of understanding and preventing streptococcal infections and their sequelae is obvious.

Streptococcal infections of the upper respiratory tract could be of varying degree of severity. Various viral infections of the throat may mimic streptococcal pharyngitis in all respects. Experienced pediatricians are able to distinguish clinically between streptococcal and non-streptococcal pharyngitis with only 50-70% accuracy (Wannamaker *et al*., 1972). Thus, throat culture has become the gold standard in substantiating the diagnosis of streptococcal pharyngitis.

Amongst the groups of streptococci causing human infections group A infections is not only prevalent, but also more severe and is responsible for post streptococcal sequelaee. The other groups produce rather milder diseases possibly there are several factors other than group of organism contributing to the severity of the disease. It has been categorically observed that development of acute glomerulonephritis is more common following skin infections rather than throat infections. Conversely, for rheumatic fever the predisposing infection is found in the throat. Further, the type incidence of streptococci could be responsible for the variations in the sequelaee. Streptococci produce mild upper respiratory infection in hot territories. The exact reason for which is not clearly known. Thus, patient may suffer repeated attacks with possible danger of developing sequelaee. Recently, there are reports of streptococci belonging to group C and G causing primary infection of upper respiratory tract and skin, occasionally group C infection leading to acute glomerulonephritis (Barnham *et al*., 1983). Recently, an entity known as Pediatric Autoimmune Neuropsychiatric Disorders Associated with Strep-Pyogenes (PANDAS) is described (Swedo *et al*., 1998). Hence, surveillance over streptococcal infections by means of microbiological and epidemiological surveys and follow-ups are very much needed in all parts of the world.

Materials and Methods

The study was a cross sectional study carried out at the Department of Microbiology, B.L.D.E.A's Shri B.M.Patil Medical College, Hospital and Research Center, Bijapur.

The study includes 100 cases of acute pharyngitis between the age group of 5-15 years who attended the Pediatric outpatient department of this hospital over a period of 16 months. The following patients were excluded from the study, if they met any one of the following criteria.

Patients who were presently taking antibiotics or had received antibiotics a week before presentation.

Cases of Diphtheria/Candidiasis.

Physical examination revealed evidence of lower respiratory tract infection.

Each case was then subjected to the following investigations:

Blood

1. Hb%, DC, TC, ESR, peripheral smear study.
2. C-reactive protein estimation.
3. Serum ASO titre estimation.

Throat swab

1. Gram stain
2. Antigen detection.
3. Culture and sensitivity.
4. Sero grouping.

Under good light and all aseptic precautions,

the throat swab was collected after exerting light pressure over the tonsillar regions as well as posterior pharyngeal wall. Care was taken to avoid contamination with the oropharyngeal flora.

One of the collected swabs was used for rapid group A streptococcal antigen detection. It is a latex agglutination test. In order to minimize the number of false negative tests, current US guidelines recommend using a throat swab culture to confirm a negative rapid test.

The other swab collected was used for gram stain followed by streaking the third swab collected on blood agar plates containing 5-7% defibrinated sheep blood and incubated under 5% CO_2 aerobic condition for 24 hours.

After overnight incubation, the plates were red for β hemolytic streptococcal colonies. Gram stained smears prepared from typical colonies were studied to confirm the organism.

Antibiotic sensitivity testing was carried out for the β hemolytic colonies by disc diffusion method using penicillin, erythromycin, tetracycline, cephalexin, cefuroxime and azithromycin. The isolated β hemolytic streptococcal colonies were subjected for grouping of group A, B, C, D, F and G using pastorex - strep latex kit. It is a rapid sensitive agglutination test. Streptococcal grouping of β hemolytic colonies require extraction of these antigens from the colonies on blood agar plate. In the presence of antigen, the latex particles coated with homologous antibodies agglutinate very rapidly. 0.3 mL of extraction enzyme solution was taken in a test tube into which 5-6 colonies were added. The above was incubated for 10 minutes at 37^0c. The contents of the vials containing the latex particles were resuspended by shaking them vigorously for few seconds. One drop of each latex suspension was transferred to the corner of the agglutination card to which one drop of extract was added. The contents of each circle were homogenized by using plastic rods. The reaction was positive, if latex particles agglutinated within one minute. The positive reaction was indicated by white clumps on a black background, negative reaction by a uniform brown suspension.

ASO (antistreptolysin-O) test was done. 3 mL of blood was collection and the serum separated. A drop of serum was mixed with one drop of latex reagent on the slide using a disposable stirrer. The test slide was then gently rotated for 2 minutes to see further agglutination. The positive ASO result was indicated by the obvious agglutination pattern of the latex in a clear solution. The test has a detection limit of 200 IU/mL and above.

C-reactive protein estimation was done which is also a latex agglutination test that measures the protein and protein bound molecules. The test is expected to be positive with serum C-reactive protein levels between 6 and 1000 mg/L.

Results and Discussion

Among the 100 patients of acute pharyngitis, 57 were males and 43 were females. Most of the patients belonged to 5-8 years of age (55%) followed by 9-11 years (27%) and 12-15 years age (18%).

63% of the patients had an illness of 0-3 day's duration, followed by 35% with 4-6 days duration. Only 2 patients had a prolonged illness of more than 6 days. 71% of the patients had a history of overcrowding.

Major symptoms were fever followed by cough and sore throat in percentage of 82%, 72% and 60% respectively. Majority of the patients had fever ranging from 38.3 - 39.4^{0}C. Congestion of anterior and posterior pillars of tonsils and posterior pharyngeal wall and tonsillar enlargement was seen in almost all cases. Tender lymph nodes were seen in 44%. Chronic suppurative otitis media and pyoderma associated with acute pharyngitis were seen in significant number followed by vitamin A deficiency, B-complex deficiency and scabies.

Table.1 Organisms isolated on throat culture

Sl. No.	Organism	Number	Percentage
1.	Beta hemolytic streptococci	17	21.25%
2.	Coagulase positive staphylococci	14	17.5%
3.	*Pneumococci*	24	30%
4.	*Streptococcus viridians*	13	16.25%
5.	*Brahmanella catarrhalis*	3	3.75%
6.	Coagulase negative staphylococci	4	5%
7.	Mixed	5	6.25%
Total		80	100%

β hemolytic streptococci were isolated in 17 (21.25%) patients followed by coagulase positive staphylococci 14 (17.5%) and *Streptococcus viridans* 13 (16.25%). Pneumococci were found in 24 cases (30%).

Table.2 Analysis of symptoms

Sl. No.	Symptoms	Non-streptococcal		Streptococcal		P value
		No.	%	No.	%	
1.	Fever	65	78.31	17	100	<0.05
2.	Sore throat	45	54.21	15	88.23	<0.05
3.	Cough	62	74.69	10	12	<0.05
4.	Headache	5	6	13	15.66	<0.05
5.	Swelling in neck	19	22.89	4	23.52	>0.05
6.	Abdominal pain	4	4.81	5	29.41	<0.05
7.	Change in voice	5	6	3	17.64	<0.05
8.	Feeding difficulty	17	20.5	6	35.29	<0.05

All the symptoms were noted in higher percentage in streptococcal pharyngitis except cough, which was noted more commonly in non-streptococcal pharyngitis.

All the isolates of streptococci were sensitive to commonly used antibiotics like penicillin (P), erythromycin (E), tetracycline (Te), gentamycin (Gen), ampicillin (Amp), cefoperazone (Cpz), amoxyclav (Amc), azithromycin (Az), ciprofloxacin (Cip) and cefuroxime (Cf). The antibiotic sensitivity of the organisms is as follows.

Table.3 Antibiotic sensitivity pattern of the isolates on throat culture

Organisms	No. Isolated	P	E	Te	Gen	Amp	Cpz	Amc	Az	Cip	Cf
BHS	17	15	14	14	17	14	14	15	14	12	14
Coagulase Positive staphylococci	14	7	6	6	10	5	3	4	5	8	8
Pneumococci	24	20	20	15	16	18	20	13	11	10	19
Streptococci viridians	13	6	4	6	9	1	11	10	8	8	8
B. catarrhalis	3	-	-	1	2	1	-	1	-	2	2
Coagulase negative staphylococci	4	1	1	1	1	-	1	2	2	1	-
Mixed	5	2	2	-	4	-	3	2	4	2	3

Table.4 Serological grouping of *Streptococci* found on culture N=17

Sl. No.	Group	Number	Percentage
1.	A	13	76.47%
2.	B	-	-
3.	C	2	11.76%
4.	G	2	11.76%

Out of 17 isolates of β hemolytic streptococci 76.47% belong to group A and 11.76% belong to group C and group G each. CRP was positive in 55.5% of cases of streptococcal pharyngitis. In non-streptococcal pharyngitis, the positivity rate was 33.33%. ASO was positive in 59% cases of streptococcal pharyngitis. In non-streptococcal pharyngitis, the positivity rate was 22.7%. These cases may not have been properly picked-up by throat culture or it indicates a recent past streptococcal infection. In non-bacterial pharyngitis, the rate was 18.18% probably indicating recent past streptococcal infection.

Rapid antigen test for group A streptococci was positive in 9 cases among the 17 culture positive cases. Conversely in 3 cases where no growth was observed on culture, antigen for group A was positive. Thus, antigen detection test helps to identify streptococcal infection in culture negative cases and the test can be completed in few minutes. But, since the antigen test also did not detect the antigen in 8 out of 17 positive streptococcal cases, throat culture remains the gold standard for the isolation of streptococci.

Table.5 *Streptococcal* isolation rates in Indian workers

Sl. No.	Author	Year	Ref. No.	Isolation %
1.	Vijay Gulati *et al*	1981	55	33.3
2.	Agarwal S.K. *et al*	1981	48	15.50
3.	Chakrabarti *et al*	1989	56	80
4.	Rajkumar *et al*	1991	57	4
5.	Soban Nandi *et al*	2002	47	13.39
6.	Present study	2010	-	21.25

The streptococcal isolation among Indian workers ranged from a low of 4% to as high as 80% as shown in the above table. Our isolation of 21.25% was slightly higher than 15.5% of Agarwal *et al*., Western workers have isolated streptococci ranging from 24% - 75 % patients as shown in the following table.

Table.6 Streptococcal isolation percentage by different authors

Sl. No.	Author	Year	Ref. No.	Isolation %
1.	Martin C. Randolf *et al*	1985	58	75
2.	Marvin S. Kober *et al*	1985	50	59
3.	Roddey *et al*	1986	60	35.50
4.	Mark C. Stein Hoff	1997	52	24

The following table 7 shows serological grouping of streptococci isolated from cases of streptococcal pharyngitis by different authors.

Table.7 Serological grouping of streptococci isolated from cases of *Streptococcal pharyngitis* by different authors

Sl. No.	Author	Year	Ref. No.	Sero group			
				A	B	C	G
1.	Vijay Gulati *et al*	1981	55	68	1	11	18
2.	S.K. Agarwal *et al*	1981	48	34.3	7.1	4.3	50
3.	Brook l *et al*	1983	62	74	4	9	1
4.	Julia *et al*	1986	54	37.17	0.6	-	1.88
5.	R. Gupta *et al*	1992	64	73.5	2.12	0.7	23.5
6.	Edmond K.M. *et al*	1996	63	21.03	-	4	2
7.	Present study	2010	-	76.47	-	11.76	11.76

All the studies isolated group A in highest percentage symptomatic patients except Grace Koshi (Grace Koshi *et al*., 1977) and S.K. Agarwal (Agarwal *et al*., 1981) who isolated group G in highest percentage.

In the present study group A streptococcus were isolated in number constituting 76.47% of streptococcal isolates followed by group C and group G in 11.76% each. No group B streptococci were isolated.

The following table shows the distributions of subgroups of streptococci among healthy children by different authors. All these studies isolated group G streptococci in highest numbers in asymptomatic carriers ranging from 43.2% to 60%. In our study, we isolated group A and group G equally from the carriers in 42.8% followed by 14.2% of group C streptococci.

Table.8 Serogroups observed by different authors

Sl. No.	Author	Year	Ref. No.	BH	Sero groups in %					
					A	B	C	G	F	NG
1.	Agarwal *et al*	1981	48	7.9	12	2	8	60	-	8
2.	Gulati *et al*	1981	55	6	33.3	-	16.6	50	-	-
3.	Srivastava *et al*	1981	68	28.5	25.4	9.8	4.1	53.3	-	7.4
4.	Pati *et al*	1989	69	8.8	26.4	2.6	18.4	52.6	-	-
5.	Navaneeth *et al*	1998	66	21.6	28.8	1.9	11.5	43.2	0.9	13.4
6.	Present study	2010	-	14	42.8	-	14.2	42.8	-	-

Table.9 Comparison of ASO positivity in *Streptococcal pharyngitis*

Sl. No.	Author	Percentage
1.	Grace Koshi *et al*	48.4
2.	Vijay Gulati *et al*	45.3
3.	Present study	58.8

In our study, we found 59% of ASO positivity in streptococcal pharyngitis, which was slightly high, compared to studies by Grace Koshi (Grace Koshi *et al*., 1977) 8.4% and Vijay Gulati (Vijay Gulati *et al*., 1981) 45.3%.

Table.10 Comparision of C-reactive protein in *Streptococcal pharyngitis*

Sl. No.	Author	Percentage
1.	P. Padmini *et al*	59.4
2.	Edward L. Kaplan	78
3.	Present study	55.55

In our study, the positive percentage of C-reactive protein in streptococcal pharyngitis was 55.55%. P Padmini (Padmini *et al*., 1987) found 59.4% of positivity and Edward L. Kaplan (Edwar Kaplan *et al*., 1977) found the percentage to be as high as 78%.

Acute pharyngitis is among the most common conditions responsible for significant morbidity in childhood. Even though majority of the cases are caused by viruses, it is important to know the bacteriologic etiologies with the treatment and complications point of view. β hemolytic streptococci are not only common pathogens, but they are also important because of the severity of pharyngitis and the complications. Rheumatic heart disease as the common sequelae of streptococcal pharyngitis remains the major cardiovascular disease in many parts of the world especially in non-developed countries. In India, it is responsible for 33-50% of inpatient and outpatient attendance in cardiac clinics. Thus, streptococcal infections represent an important health and economic problem in our country. The importance of understanding the clinical profile and their sequelae is obvious. Various workers have carried out studies on streptococcal pharyngitis.

Robert T. Rowe (Robert Rowe *et al*., 1977) and Robert T. Stone (Robert Rowe *et al*., 1977) reported the incidence of streptococcal pharyngitis as 33% in 2-5 years age group, 45% in 6-10 years and 13% in 11-16 years of age (Robert Rowe *et al*., 1977). Sobhan Nandi (Sobhan Nandi *et al*., 2002) found out that 15% group A streptococcus positivity was seen in 9-12 years of age.

In the present study, we found higher incidence of 64.7% in 5-8 years age group followed by 17.6%, in both 9-11 and 12-15 years age group. Thus, the peak age is 5-8 years.

Study conducted by Agarwal *et al.*, found streptococcal pharyngitis of 16.1% in males and 14.4% in females. This was not significantly different. J.F. Bach (Bach *et al*., 1996) showed a slight female preponderance 11.2% as compared to 9.1% in males. Similar to it, N.B. Mathur also showed a female preponderance of 38.9% as to 15% in males. Sobhan Nandi (Sobhan Nandi *et al*., 2002) found 14.7% positivity in males compared to 11.7% among females. The present study shows slight preponderance of males - 58.8% to females - 41.2% among the streptococcal isolates.

Michael A. Gerber (Michael Gerber *et al*., 1997) performed a study on streptococcal pharyngitis in Connecut and noted fever in 65%, sore throat in 89%, abdominal pain in 28%, cervical adenitis 36%, tonsillar exudates 22%, enlarged tonsils in 33% and pharyngeal congestion in 79% of patients. James M. T. (James *et al*., 1993) in his study of streptococcal pharyngitis found 91% positivity for enlarged tonsils, 72% for fever, 60% tonsillar exudates and headache in 51% cases. Mark C. Stein Hoff (Stein Hoff *et al*., 1997) noted fever in 87.2% and tonsillar exudates in 22% pharyngitis cases.

In a study by Sobhan Nandi (Sobhan Nandi *et al*., 2002) most common symptoms of group A streptococcus sore throat was pain in the throat (86.2%) and major signs were erythema of pharynx (92.7%) enlarged tonsils (86.9%) and lymphadenopathy (87%).

Among the signs, congestion of anterior pillar, posterior pillar and posterior pharyngeal wall and tonsillar enlargement are almost same in both streptococcal and non-streptococcal pharyngitis.

Streptococcal isolation rates from symptomatic patients vary widely among various workers.

In conclusion, the present study showed a 21.5% prevalence of group A β hemolytic streptococcus in the school going children of age 5-15 years suffering from acute pharyngitis. A significant amount of resistance (17%) was seen among some of the commonly used antibiotics like ampicillin, azithromycin, erythromycin and cefuroxime.

This study emphasis the importance for screening of pathogens like streptococcus pyogenes and other β hemolytic streptococci in acute tonsillitis in children to avoid further complications and better outcome of the patient. Prompt screening and performing routine culture and sensitivity testing can help in avoiding the drug resistance to the commonly used antibiotics which may develop due to excessive and injudicious use of antibiotics without proper antibiotic policy.

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