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Pharyngeal carriage of Group A streptococci among school children

Aiswarya Mukundan[1], Vijayakumar S[2]

Abstract:

Objectives: The aims of the study were to determine the pharyngeal carriage rate of Group A streptococci among school children at Thrissur, Kerala and to determine the antibiotic sensitivity pattern of the above isolates.

Materials and methods: Throat swabs were collected from a total of 260 asymptomatic children(130 each from rural and urban areas) of eighth and ninth standard classes during a period between July 2012 and July 2013. Presumptively identified Group A Streptococci (GAS/*Streptococcus pyogenes)* were serogrouped by agglutination tests using specific antisera. Antibiotic sensitivity was performed by disc diffusion method following CLSI guidelines.

Results: The GAS carriage rate noted in the study was 23.1%. Carriage distribution was found to be independent of age, sex and locality with more number of cases during winter season. All the isolates were found to be sensitive to Penicillin and Vancomycin. Five isolates each were found to be resistant to Erythromycin and Clindamycin respectively. Inducible Clindamycin resistance was demonstrated in three of them. GAS was isolated from the throat swab of one child with past history of joint pains and another child with past history of skin infection- both of them presently had findings of heart disease.

Conclusion: When screened and appropriately treated with antibiotics, carriers can be prevented from spreading streptococcal infections in the school environment and the community. Combined efforts by clinicians, community health personnel and clinical microbiologists are required for the early detection and control of streptococcal infections.

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Key words: carrier, children, Group A streptococci, pharyngitis

Introduction:

Pharyngitis is a common disorder in adults and children. The National Ambulatory Medical Care Survey and the National Hospital Ambulatory Medical Care Survey have documented between 6.2 and 9.7 million visits to primary care physicians, clinics and emergency departments each year for children with pharyngitis, and more than five million visits per year for adults.[1,2,3] Group A streptococci (GAS/*Streptococcus pyogenes*) are responsible for approximately 10 to 15% of cases of pharyngitis in adults and 15 to 30% of cases in children.[4] Group A streptococcal pharyngitis is one of the most common bacterial infections of childhood, accounting for 20–40% of all cases of exudative pharyngitis in children; it is rare among those under the age of 3. The most common age group is 5-15 years.[5] Group A streptococci produce suppurative and non-suppurative infections in humans.[6] The suppurative infections include pharyngitis, pyoderma, erysipelas, cellulitis, necrotizing fasciitis, scarlet fever, puerperal sepsis, pneumonia, meningitis and streptococcal toxic shock syndrome. The two non-suppurative sequelae of Streptococcal infections are acute rheumatic fever (ARF) and post-streptococcal glomerulonephritis.

The prevalence of asymptomatic carriage of GAS in different parts of India has been reported to lie in the range of 11.2-34%.[7] GAS colonization of the upper respiratory tract of children play an important role in the spread of infection in household and in

community settings such as schools, day care centres and orphanages.[6] The prediction rules for the diagnosis of GAS pharyngitis are limited because the signs and symptoms of many viral causes of acute pharyngitis overlap with infection caused by GAS. For these reasons, the guidelines from the Infectious Disease Society of America, the Committee on Infectious Diseases of the American Academy of Pediatrics and the American Heart Association recommend confirmation of GAS infection by throat culture, Rapid Antigen-Detection Test (RADT) or both.[8,9,10] There are no definitive guidelines for the management of asymptomatic carriers. A reasonable course of action is to give a 10 day course of penicillin. The combination of Penicillin V (500mg four times daily for 10 days) and Rifampin (600mg twice daily for the last 4 days) has also been used to eliminate pharyngeal carriage. [11]

The objective of the study to determine the prevalence of pharyngeal carriage of *Streptococcus pyogenes* among children in the age group13-15 years in a rural and an urban school in Thrissur, Kerala and to determine the antibiotic sensitivity pattern of the above isolates.

Materials and Methods:

Study design: Cross-sectional study.
Study period: July 2012- July 2013.
Apparently healthy boys and girls of eighth and ninth standard classes of a rural and an urban school at Thrissur were selected. Children who had taken a course of antibiotic within the last four weeks and those with any nasopharyngeal infections such as pharyngitis and tonsillitis were excluded. A total of 260 children were included in the study out of which 130 were from a rural school and 130 were from an urban school. Ethical clearance was obtained. Written consent was obtained from the concerned school authorities. Detailed explanation was given to the parents of the children who were included in the study and informed consent was obtained from them. The children were asked for past history and symptoms of sore throat, skin disease, heart disease and antibiotic usage. Detailed physical examination was done to determine signs of heart disease and the tonsils and pharynx were examined to rule out signs of inflammation.

Methodology: The specimen collected was throat swab. Commercially available sterile cotton swabs were used and each batch was subjected to sterility check by plating onto blood agar. The swabs were placed in sterile test tubes and transported to the lab without delay. The swabs were then inoculated on 5% sheep blood agar and chocolate agar and incubated at 37ºC with 5- 10% CO_2 in a candle jar . After overnight incubation, each plate was checked for colonies with β-hemolytic characteristics. Culture plates negative for beta-hemolytic colonies were incubated for additional 24 hours for the recovery and detection of slow growers. Gram staining and catalase test were done for the beta-hemolytic colonies. Susceptibility to Bacitracin (0.04U) and Trimethoprim-Sulfamethoxazole (SXT) were also tested. Colonies which showed Gram positive cocci in chains on Gram staining, negative catalase test, bacitracin susceptibility, resistance to SXT, positive PYR test and negative CAMP test were presumptively identified as Group A streptococci. Antibiotic susceptibility testing was done for the presumptively identified group using disc-diffusion method following CLSI guidelines. The discs used were Penicillin (10U), Erythromycin (15µg), Clindamycin (2µg) and Vancomycin (30µg). Inducible Clindamycin resistance was tested by D-zone method.[12] 15µg Erythromycin disc and 2µg Clindamycin disc spaced 12 mm apart were placed on sheep blood agar plate and incubated overnight at 37ºC in the presence of 5% CO_2. Flattening of the zone of inhibition was seen adjacent to the Erythromycin disc (referred to as the D-zone).

Confirmation of identification was done by two commercially available Latex agglutination kits (SLIDEX Streptoplus and Streptex). All suspected isolates gave positive agglutination with both the kits.

Results:

Total pharyngeal carriage rate of GAS is shown in **Figure I**. The comparison of GAS carriage based on age, gender and locality are as seen in **Tables I**, **II** and **III**. Seasonal distribution is evident in **Figure II**. **Figure III** shows past history of symptoms in carriers of GAS. The antibiotic sensitivity pattern of the isolates is indicated in **Table IV**. All the 60 isolates were uniformly sensitive to Penicillin and Vancomycin.

Erythromycin and clindamycin resistance were 8.3% each.

Figure I: Total carriage rate of *Streptococcus pyogenes*

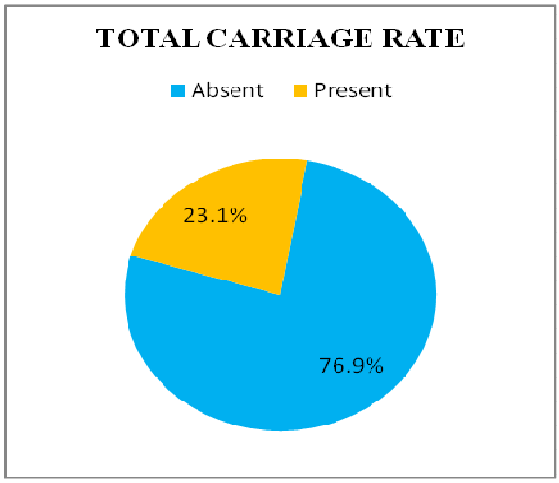


Figure II: Seasonal distribution of *Streptococcus pyogenes* carriage

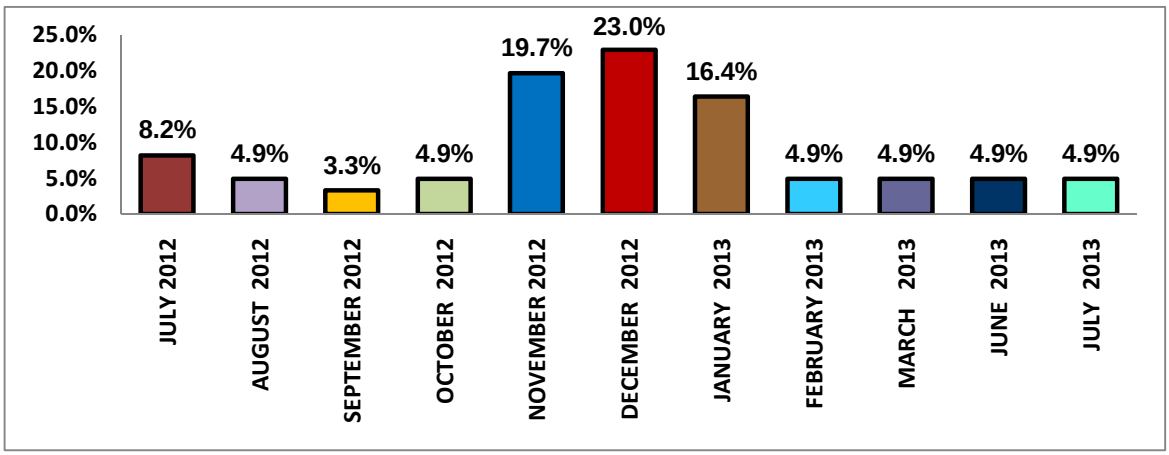
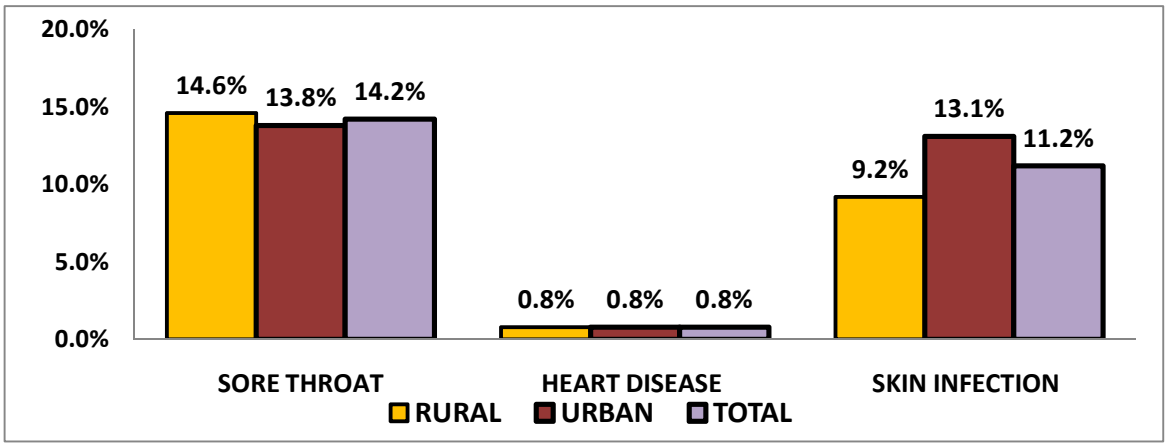


Figure III: Past history of symptoms in carriers of *Streptococcus pyogenes*



According to seasonal distribution, maximum number of cases were noted in the months of November (12), December (14) and January (10) contributing to 19.7%, 23% and 16.4% respectively. The samples were not collected during the months of April and May when the children had vacation.

In the rural area, 19 and 12 students respectively gave a past history of sore throat and skin disease (14.6% and 9.2% respectively). In the urban area, 18 and 17 respectively gave a past history of sore throat and skin infection (13.8% and 13.1% respectively). Out of the 260 students, 37 (14.2%) and 29(11.2%) respectively had past history of sore throat or skin disease. *Streptococcus pyogenes* was isolated from two children in the rural area with past history of sore throat and from one child in urban area with past history of skin disease. *Streptococcus pyogenes* was isolated from the throat of one child in the rural area with past history of joint pain and presently with findings of heart disease and from another child in the urban area with past history of skin infection and presently having physical findings of heart disease.

Table I: Comparison of *Streptococcus pyogenes* carriage based on age

Streptococcus pyogenes		Age						χ^2	P
		13		14		15			
		Number	Percent	Number	Percent	Number	Percent		
Rural	Absent	34	70.8	48	80.0	16	72.7	1.30	0.520
	Present	14	29.2	12	20.0	6	27.3		
Urban	Absent	37	84.1	50	76.9	15	71.4	1.51	0.465
	Present	7	15.9	15	23.1	6	28.6		

'p' value calculated by Chi-square test turned out to be 0.520 and 0.465 in rural and urban areas respectively

Table II: Comparison of *Streptococcus pyogenes* carriage based on gender

Streptococcus pyogenes	Gender				χ^2	p
	Male		Female			
	Number	Percent	Number	Percent		
Absent	97	72.4	103	81.7	3.20	0.073
Present	37	27.6	23	18.3		

'p' value calculated from Chi-square test was 0.073.

Table III: Comparison of *Streptococcus pyogenes* carriage based on locale

Streptococcus pyogenes	Locale				χ^2	p
	Rural		Urban			
	Number	Percent	Number	Percent		
Absent	98	75.4	102	78.5	0.347	0.556
Present	32	24.6	28	21.5		

'p' value was calculated by Chi-square test and was found to be 0.556 i.e., greater than 0.05

Table IV: Antibiotic sensitivity pattern of the isolates

Antibiotic Sensitivity Testing		Rural		Urban		Total	
		Count	Percent	Count	Percent	Count	Percent
Penicillin	Sensitive	32	100.0	28	100.0	60	100.0
	Resistant	0	0.0	0	0.0	0	0.0
Erythromycin	Sensitive	30	93.8	25	89.3	55	91.7
	Resistant	2	6.3	3	10.7	5	8.3
Clindamycin	Sensitive	30	93.8	25	89.3	55	91.7
	Resistant	2	6.3	3	10.7	5	8.3
Vancomycin	Sensitive	32	100.0	28	100.0	60	100.0
	Resistant	0	0.0	0	0.0	0	0.0

Discussion:

Group A streptococci have remained a significant pathogen for centuries. They cause a wide variety of infections ranging from upper respiratory tract infections to life threatening invasive illnesses. Of major concern is the occurrence of post infectious sequelae- acute rheumatic fever and post-streptococcal glomerulonephritis which continue to occur despite tremendous efforts by clinicians, microbiologists and public health officials to control the spread of infection.[13]

Carriage rate: The prevalence of asymptomatic carriage of GAS in different parts of India has been reported to lie in the range of 11.2 to 34 %.[7] The carrier rate in Coimbatore was 5.09% in the study by Dheepa et al, in 2012.[14] In a study by Kalpana et al, in 2012, GAS was isolated in 36% of the children in the age group 5 to 15 years in Chennai.[15] Carriage rate of 8.4% in Chennai was noted in the studies of Lloyd et al in 2006.[16] In Vellore, the carriage rate noted was 2.3% in 2006 by Brahmadathan et al.[13] Koshi et al reported the carrier rates between 2.5% and 14.3% in South India in 1971.[17] The carriage rate was 13.6% in a rural area of Varanasi in the study by Sarkar et al.[18] In school going children in Delhi, the prevalence of GAS pharyngitis was 13.7% as observed by Gupta et al.[7] In the present study, 60 isolates of the total 260 samples were identified as Group A streptococci which constitutes 23.1%. There is not much literature available on the carriage rate in Kerala and hence it is difficult to comment whether the rate is high or low. The rate is somewhat high compared to those in nearby places. However, the above mentioned studies show marked difference in distribution.

Age-wise distribution: Age has been reported to be an important factor in the microbiological etiology of pharyngitis, the peak incidence of GAS pharyngitis occurring in children aged 5–15 years.[19] The present study was conducted in the age group 13 to 15 years. More cases were found in 14 year old children. The 'p' value determined by Chi-square test showed values of 0.520 and 0.465 (greater than 0.05) in rural and urban areas respectively and hence the distribution of streptococcal carriage was found to be independent of age.

Gender-wise distribution: In the present study, the carriage rate was higher among boys compared to girls. Chi-square test was done to assess gender wise correlation of Streptococcus pyogenes. The 'p' value calculated was 0.073.Since the value was greater than 0.05, it can be concluded that the prevalence of *Streptococcus pyogenes* is independent of gender. This finding was also observed in another study by Nandi et al.[20]

Area-wise distribution: The carriage rate was 24.6% in the rural area and 21.5% in the urban area in the present study. The 'p'

value calculated was more than 0.05 (0.556), which showed that the distribution of streptococcal carriage is independent of locality. This was probably because the schools included in the study were not geographically diverse. Studies by Dumre et al in Nepalese school children also showed similar observation.[21] Altindis et al, compared the rate of GAS carriage of children studying at two different schools.[22] They found that the rate of carriage in healthy children in an impoverished region was 6%, and that the rate in more affluent, suburban students was 28% in Turkey.[22]

Seasonal distribution: The incidence of GAS sore throat exhibited a bimodal peak, being higher in the wet summer months and during the winter. During the rainy season and in winter, children mostly live indoors in crowded conditions, which may increase the transmission of infection. Higher carriage rate has been observed during winter and rainy season among children in peri-urban slum areas in North India by Nandi et al.[20] In an Indian community near Varanasi, the highest point prevalence was seen in winter in the study by Sarkar et al.[18] In the present study also, higher rates were found in the winter season (November to January). The total number of cases obtained were 12, 14 and 10 in the months of November, December and January respectively.

Diagnostic methods: Culture of a throat swab on a sheep blood agar plate remains the standard for the documentation of the presence of Group A streptococci in the upper respiratory tract and for the confirmation of the clinical diagnosis of acute streptococcal pharyngitis.[22] If done correctly, culture of a single throat swab on a blood agar plate has a sensitivity of 90%-95% for the detection of GAS in the pharynx.[23] Probably, the most widely used test for the differentiation of Group A streptococci from other beta- hemolytic streptococci is the bacitracin disc test. This test provides a presumptive identification on the basis of observation that more than 95% of GAS demonstrate a zone of inhibition around a disc containing 0.04 U of bacitracin, whereas 83-97% of non-Group A streptococci do not demonstrate this.[24] An alternative and highly specific method of identifying streptococcal serogroups is by detection of the group specific cell wall carbohydrate antigen directly in isolated bacterial colonies. Commercial kits containing group-specific antisera are available for this purpose. However, for children and adolescents, a negative RADT should be confirmed with a throat culture result.[25] In the present study also, throat swab culture on sheep blood agar and Bacitracin susceptibility tests were done for presumptive identification. All the presumptively identified isolates gave positive agglutination with two commercially available kits. Thus, it is evident that positive throat cultures along with RADTs are useful for the identification of Group A streptococci.

Complications: Although ARF and RHD have declined in many parts of the world, they continue to be a major cause of morbidity and mortality in India accounting for around 40% of the cardiac cases in hospitals.[26] Vaishnava et al, have reported carditis in 90% of cases of ARF from South India, congestive heart failure in 45% and mortality in 16% of the cases.[27] Community studies among symptomatic children with clinical evidence of pharyngitis from India reveal that only 4-13% have GAS isolated from their throat whereas the prevalence of asymptomatic GAS carriers range from 3.7 to 20%.[28] In the present study also, complications were seen. Majority of the children were asymptomatic carriers. Although 14.2% (37 out of 260), had past history of sore throat, GAS was isolated from only two of them and they belonged to the rural area. The rest of the children might have suffered from viral pharyngitis. One child in the rural area had history of joint pain. *Streptococcus pyogenes* was isolated from the throat swab and the child

had physical findings of heart disease. Simultaneously, heart disease was diagnosed and the child was advised treatment with Penicillin. Although, 29 students of 260 (11.2%) gave past history of skin infection, *Streptococcus pyogenes* was isolated from only one child who belonged to the urban area. The child gave history of skin infection 5 months ago which subsided after medications. *Streptococcus pyogenes* was isolated from the throat swab of this child and features of heart disease were also present. The child was referred for expert opinion.

Antibiotic resistance: Increasing antimicrobial resistance of GAS has been observed during the last decade in Europe and worldwide.[29] In 1992, Seppala et al, reported a high rate of erythromycin resistance(>45%) in Finland.[30] High frequencies of Erythromycin resistance have also been reported from Spain (21.3%), Italy (22.6%), Greece (24%), France (22.4%), Portugal (26.6%)and USA(32%).[31] Although there have been recent isolated reports of macrolide resistance in the United States, there is no evidence that this is widespread at the present time, as less than 5% of Group A streptococci isolates in the United States have been shown to be resistant to Erythromycin.[6,32] High rates of resistance have also been observed in Australia(17%) and UK(22.8%).[33] On the other hand, resistance rates as low as 0.5% and 1.6% have been found in Netherlands and South-western Germany respectively.[32,13,16] Resistance to Erythromycin was found in 5.2% isolates in studies by Dumre et al, in Nepal.[22] The resistance to Erythromycin was 3.8% in Ankara as per the studies of Horu et al.[34] In the present study, all the 60 isolates were found to be uniformly susceptible to Penicillin and Vancomycin. Five isolates each were found to be resistant to Erythromycin and Clindamycin (8.3% each). Three of them showed inducible Clindamycin resistance by the D zone test.

Conclusion:

Clinicians should re-evaluate continually the clinical signs and symptoms associated with *Streptococcus pyogenes* for preventing further complications. Hence, the present study emphasizes the need for conducting periodic school surveys. Also, well planned longitudinal studies and implementation of effective public health measures have to be undertaken to decrease the streptococcal disease burden in the community**.**

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Authors details:

1. **Corresponding author:** Assistant Professor, Department of Microbiology, Government Medical College, Konni, Pathanamthitta district, Kerala- 689691, India; E-mail: aiswaryamukundan1001@gmail.com

2. Additional Professor, Department of Microbiology, Government Medical College, Konni, Pathanamthitta district, Kerala- 689691, India.