

Antibiotic susceptibility of Group A *Streptococcus* isolated from throat swab culture of school children in Pokhara, Nepal

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ABSTRACT

Group A Streptococcus (GAS) or *Streptococcus pyogenes* is estimated to be present in 5.0-15.0% of normal individual in the respiratory tract, vagina, skin and anus without any sign of disease. This study was carried out to find out the rate of asymptomatic throat carriage of *S. pyogenes* and antibiotic susceptibility of the isolates in school children of Pokhara, Western Nepal. A total of 487 randomly selected children younger than 16 years were included in the study. Throat swabs collected were subjected to 5.0% Sheep blood agar supplemented with crystal violet (CVBA). GAS was identified by α -haemolytic colonies, bacitracin sensitivity, cotrimoxazole resistivity, catalase negativity and PYR positivity. Antibiotic susceptibility test was performed on Muller Hinton agar (MHA) containing 5% sheep blood by modified Kirby-Bauer disc diffusion method. Out of total 487 throat swabs, GAS was isolated in 9.2% (n=45). Among the isolates, 46.6% (n=21) were from male children where as 53.4% (n=24) from female children. There was no significant sex difference in colonization of GAS (p>0.05). Out of 45 isolates, 100.0% isolates were sensitive to antibiotic penicillin-G and amoxycillin where as 15.6%, 6.6%, and 2.2% isolates were resistant to antibiotic erythromycin, tetracycline and azithromycin respectively.

Keywords: GAS, CVBA, α -haemolytic, modified kirby-bauer method.

INTRODUCTION

Group A Streptococcus (GAS) consists of a single species, *Streptococcus pyogenes*.¹ It belongs to Lancefield group A and is beta-hemolytic, hence called Group A beta-hemolytic Streptococci (GABHS).² Streptococcal carriage has been defined as the recovery of GAS from the nasopharynx or oropharynx in the absence of any evidence of acute infection.³ The pathogenesis of GAS is mediated by a variety of factors. One of them is Streptolysin 'O' toxin, which damages cell membranes and accounts for the hemolysis demonstrated on sheep blood agar.¹ Disease spectrum of Group A Streptococcus (GAS) or *S. pyogenes* ranges from mild infectious as pharyngitis, tonsillitis and impetigo to life threatening infections like necrotizing fasciitis and toxic-shock like syndrome. These are often followed by post infective sequelae of rheumatic fever, rheumatic heart disease and post streptococcal acute glomerulonephritis.⁴ GAS infection is ordinarily spread by direct person-to-person contact, most likely via drops of saliva, nasal secretions, contaminated fingers, dust or fomites.⁵ All beta-hemolytic Group A Streptococcus are sensitive to penicillin G, and most are sensitive to erythromycin. A high frequency of resistance to erythromycin in GAS has been reported, particularly in countries where antibiotics are overused.⁶ In Nepal control programs for streptococci does not exist,

moreover, such programs are not even in the row. Little information is known about the prevalence of *S. pyogenes* from the throat swab of school children in Nepal. So, this study was performed outside Kathmandu valley at Western Region, Pokhara. This study provides information on prevalence, distribution and antibiotic susceptibility pattern of *S. pyogenes* isolates.

MATERIALS AND METHODS

Sample collection

Throat swabs of 487 school children within age limit of 5-16 years were collected for the purpose of the study during June to October 2008. The specimens were collected with the help of sterile throat swabs available commercially. The swab was rubbed with rotation over one tonsillar area, then the arch of the soft palate and uvula, the other tonsillar area and finally the posterior pharyngeal wall. Each sample was labeled with code number and various other information including age, sex, location etc were also recorded. The sample was transported to the laboratory of the School of Pharmaceutical and Biomedical sciences in sterile condition within 1-2 hours for processing as have been described.^{7,8}

Sample processing

The throat swab was rubbed, while being rotated over a large well area about one third of the surface on a blood

Table-1: Isolation of *S. pyogenes* from school children

Age group (yrs)	Male			Female			Total		
	No. of throat swabs	No. of <i>S. pyogenes</i> isolates	%	No. of throat swabs	No. of <i>S. pyogenes</i> isolates	%	throat swabs	No. of isolates	%
5-8	85	9	10.6	76	10	13.1	161	19	11.8
9-12	94	7	7.4	98	8	8.2	192	15	7.8
13-16	64	5	7.9	70	6	8.6	134	11	8.2
Total	243	21	8.7	244	24	9.9	487	45	9.2

agar plate and the well was streaked out with a loop over the remainder of the plate. The plate was incubated at 37°C for 24 hours in candle jar. *S. pyogenes* produced beta-hemolytic colonies i.e. the colonies were surrounded by a zone of complete hemolysis with decolorization of the hemoglobin. The suspected colony taken out from primary culture on blood agar plates, were subcultured on crystal violet blood agar plate by placing a bacitracin disc (0.05units) over initial streaked area and cotrimoxazole disc in secondary streaked area and incubated at 37°C for 24 hours supplemented with 5-10% CO₂. α-haemolytic colonies showing bacitracin sensitivity, cotrimoxazole resistivity on CVBA was further identified by gram staining, catalase test and PYR test.⁹

Antibiotic susceptibility test

All the identified GAS isolates from throat swabs were subjected to in-vitro susceptibility test by modified Kirby-Bauer disc diffusion method. The antibiotics used in the study were tetracycline (30µg), erythromycin (15µg), cephalixin (30µg), penicillin-G (10µg), gentamycin (10µg), ciprofloxacin (5 µg), amoxicillin (30 µg) and azithromycin(15µg).

RESULTS

Among 487 school children, 243 (49.9%) were male and 244 (50.1%) were female. Out of 487 throat swabs studied, *S. pyogenes* was isolated from 45 samples (9.2%). Among the isolates, 21 (46.6%) were from male whereas 24 (53.4%) were from female. There was no significant sex difference in colonization of *S. pyogenes* (p>0.05). Highest colonization of *S. pyogenes* was found in the age group 5-8 years (11.8%) (Table-1).

S. pyogenes isolates indicated a relative high rate of resistance towards erythromycin (15.6%) followed by tetracycline (6.6%) and azithromycin (2.2%). According to our study, penicillin G and amoxicillin showed 100% sensitivity towards the isolates (Table-2).

DISCUSSION

Group A beta Hemolytic Streptococci (GABHS) is among the most prevalent bacterial childhood infection and constitutes 20.0%-40.0% of all cases of exudative

pharyngitis. The condition is most prevalent in the age group of 5 to 15 years, the highest prevalence occurring in 7 years old children and rarely occurring in those under 3 years of age. Males are equally affected by GABHS as females. GABHS frequently colonizes the pharynx of asymptomatic individuals, as 15.0%-20.0% of school-age children are asymptomatic carriers. In our study, 9.2% of school children were colonized by GAS in their throat. Similar study done by Tavakkoli *et al*¹⁰ found the prevalence of carriers among primary school children was 4.9%. Bogovac *et al*¹¹ reported 6.0% prevalence in all age groups and 11.7% prevalence in 6-13 years old children from Croatia. Durmaz *et al*¹² showed the prevalence of *S. pyogenes* nasopharyngeal carriage in 14.3% healthy school children and children in an orphanage in Turkey. The study done in Pittsburgh, Pennsylvania reported the prevalence of carriage of group A streptococci in school children to be 27.0-32.0%.¹³ The isolation rate of GAS was 25.9% according to Ozturk *et al*¹ in asymptomatic school children in the study done in Turkey.

In our study, the frequency of GAS was similar in all age groups of school children, but it was slightly higher in children aged 5-8 years. The study done by Durmaz *et al*¹² in Turkey, showed that the rates of carriers for boys and girls were similar and the frequency was similar in all age groups of school children, but it was significantly higher in children aged 4-6 years living in the orphanage.

Macrolides including erythromycin and clindamycin have been widely used for treatment of acute pharyngitis and invasive infection of GAS respectively. Kim *et al*⁶ recently reported a high frequency of resistance to erythromycin in GAS, particularly in countries where antibiotics are overused. Of all throat isolates, 95.0% were predominantly resistant to erythromycin, 70.0% to clindamycin, 56.0% to azithromycin and 24.0% to clarithromycin according to the study done in Sindh.¹⁴ Tamayo *et al*¹⁵ reported the erythromycin resistance rate to be 21.7% in the study done in Spain in 2004. Ciftci *et al*¹⁶ reported resistance to erythromycin, clarithromycin, azithromycin and clindamycin as 3.8%,

Table-2: Antibiotic susceptibility pattern of *S. pyogenes* isolates from throat swabs

Antibiotics	Total isolates	Sensitive		Intermediate		Resistant	
		n	%	n	%	n	%
Amoxycillin	45	45	100	-	-	-	-
Azithromycin		42	93.3	2	4.5	1	2.2
Cephalexin		41	91.1	4	8.9	-	-
Ciprofloxacin		40	88.9	5	11.1	-	-
Erythromycin		28	62.2	10	22.2	7	15.6
Gentamycin		41	91.1	4	8.9	-	-
Penicillin-G		45	100	-	-	-	-
Tetracycline		38	84.4	4	9.0	3	6.6

5.2%, 4.2% and 3.0% respectively. Alberti *et al*¹⁷ reported increased resistance of *S. pyogenes* to ciprofloxacin in Spain at the highest rate ever published and it is 63.3%. Of all the isolates analyzed in our study, 15.6% were resistant to erythromycin, 6.6% to tetracycline and 2.2% to azithromycin. All the isolates were sensitive to beta lactam antibiotics (penicillin and amoxycillin). In a large survey done in Iran by Jasir *et al*¹⁸ found no penicillin resistance strains of *S. pyogenes* and only a few erythromycin resistance strains. Another study done in France by Binjen *et al*¹⁹ found all isolates of *S. pyogenes* were susceptible to amoxycillin. our findings demonstrate that antibiotic resistance of *S. pyogenes* is not clinically significant problem in our country. However, the results of our preliminary study highlights the importance of regular surveillance programs to monitor the rate of GAS carriage and the antibiotic susceptibility of GAS isolates in the community. We, therefore, emphasize the need to carry out this type of study in large sample size with a wide range of geographical and seasonal variations through out the country.

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