Kathmandu University Medical Journal (2009), Vol. 7, No. 3, Issue 27

Original Article

Throat carriage rate and antimicrobial resistance trend of *Streptococcus pyogenes* among the asymptomatic Nepalese school children

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Abstract

Background: *Streptococcus pyogenes* or Group A streptococcus (GAS) causes several suppurative and non suppurative infections. Since the 1980s there has been re-emergence in the incidence of invasive *S. pyogenes* infections and rheumatic heart disease all over the world and hence throat carriage has assumed of great importance.

Objectives: We carried out this preliminary study to determine the throat carriage rate and antimicrobial resistance trend of *Streptococcus pyogenes* or Group A streptococcus (GAS) among the Nepalese school children.

Materials and methods: Four schools situated at different locations of Kathmandu valley were included in the study. Throat swabs from 350 students of age group 5-15 years were collected, immediately transported to the laboratory and were processed for *S. pyogenes* following standard microbiological procedures. Antimicrobial susceptibility testing of the isolates was performed by Kirby Bauer disc diffusion method following CLSI guidelines.

Results: *S. pyogenes* was isolated from 10.9% (38/350) of the screened children. The GAS colonisation rate was statistically insignificant (P>0.05) with sex and age subgroups, although the rate was slightly higher among girls and age subgroup 9-12 years. No significant difference in carrier rate was observed among different schools (P>0.05). Highest resistance rate was observed for Cotrimoxazole (71.05%) followed by Chloramphenicol (7.8%), Ciprofloxacin (5.2%) and Erythromycin (5.2%). All isolates were susceptible to Azithromycin. No resistance was detected for penicillin and its derivative and Azithromycin.

Conclusion: Antibiotic resistant GAS isolated from asymptomatic Nepalese children is a concern. When screened and appropriately treated with antibiotics, carriers can be prevented from spreading of streptococcal infections in the community. This would ultimately reduce the incidence of life-threatening sequelae which are debilitating and difficult to treat. It is recommended to conduct regular screening programs and GAS surveillance, and maintain rational use of antibiotics to keep GAS carriage/ infections and resistance in check.

Key words: *Streptococcus pyogenes*, Antibiotics Resistance, Children, Throat carriage, Nepal

Streptococcus pyogenes or Group A streptococcus (GAS) causes several suppurative and non suppurative infections and are responsible for a minimally estimated 616 million cases of throat infection (pharyngitis, tonsillitis) worldwide per year, and 111 million cases of skin infection (primarily non-bullous impetigo) in children of less developed countries[1]. Group A streptococci (GAS) are the most frequently isolated pathogens in acute pharyngo-tonsilitis cases in school-aged children causing approximately 20% of the pharyngitis cases[2, 3]. In addition to pharyngitis and skin infections, GAS are also the causative agent of post-streptococcal infection syndromes such as acute rheumatic fever (ARF) and post-streptococcal glumerulonephritis (PSG) and is a major cause of acquired heart disease in children, particularly in the developing countries. GAS frequently colonises in the throat of an asymptomatic person[2]. Pharyngeal carriage rates of GAS among healthy school children vary with geographical location and seasons[1]. Among asymptomatic children, carriage rates of 15-20% have been reported in several studies[4].

Since the 1980s there has been reemergence in the incidence of invasive *S. pyogenes* infections and rheumatic heart disease all over the world and hence throat carriage has assumed of great importance[5]. Rheumatic heart disease is still prevalent in developing countries, particularly among the children who live in communities that do not have adequate treatment

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programmes. Beta-lactam antibiotics (mainly penicillins) and macrolides have been conventionally used to treat *S. pyogenes* infections[6]. The resistance of *S. pyogenes* to macrolides has been reported and is increasing in most of the countries[7, 8] and there were no report of Penicillin resistance although isolates with rising minimum inhibitory concentration (MIC) to Penicillin were increasing[9]. Studies on antibiotic resistance among isolates from pharyngeal carriers could provide important data on resistance profiles of strains circulating in the community.

In Nepal, there is lack of base line information on the carriage rates and resistance patterns of these pathogens in school children. The present study was undertaken to understand throat carriage rate and antimicrobial susceptibility pattern of group A streptococci among school children of different schools within Kathmandu valley. In view of the increasing number of reports on drug resistance among GAS, we also attempted to assess the antibiotic resistance pattern among strains isolated from asymptomatic throat carriage.

Materials and methods

Study site: The study population includes Nepalese school children from four schools situated at different locations of Kathmandu valley. Laboratory analysis was performed at Kantipur College of Medical Sciences, Sitapaila, Kathmandu.

Study population: During February through April 2007, prospective collection of the clinical and microbiological data was completed from healthy school children who were asymptomatic for throat infection. Children with infection or any related sign and symptoms were excluded from the study. The asymptomatic/ healthy and symptomatic children were clinically differentiated by a medical practitioner after clinical examination. A total of 350 asymptomatic children from four different schools were included of which 45.7% were boys. The median age of the children was 9.6 years (range, 5–15 years). Past medical history, including antimicrobial therapy and day-care attendance, was obtained by using a questionnaire and reviewing medical records. Those who received antimicrobial therapy or who had suffered from GABHS in the previous 3 months were excluded.

Specimen collection: After getting the written informed consent from guardians or their care takers with legal custody of the children, pharyngo-tonsillar specimens were obtained with sterile cotton tipped swab. The material from the swab was aseptically transferred to Stuart's transport medium and transported to the laboratory within two hours of collection.

Bacteriological methods: The swabs obtained in transport medium were cultured in agar media plates (5 % sheep blood agar, and chocolate agar) and enrichment broth (BHI broth) immediately upon receipt at the laboratory. Inoculated culture media were incubated at 37 ˚C in 5% carbon dioxide and examined at 24 and 48 hours. Plates without beta haemolytic colonies after 24 hours incubation were re-subcultured on agar plates from BHI broth. All the plates with beta haemolytic colonies were processed and beta haemolytic streptococci were identified by haemolysis pattern, colony morphology and Gram stain. The organisms were further identified by conventional methods and 0.4 µg Bacitracin disc sensitivity.

Antimicrobial susceptibility testing: Antimicrobial susceptibility testing of the isolates was performed by Kirby Bauer disc diffusion method and CLSI recommended interpretive criteria[10]. *Streptococcus pneumoniae* ATCC 49619 was used for quality control. The following antibiotics were tested for all the conformed isolates: Penicillin (10 U), Ampicillin (10 µg), Ciprofloxacin (5µg), Azithromycin (15 µg); Erythromycin (15 µg), Cotrimoxazole (1.25/23.75 µg) and Chloramphenicol (30 µg).

Statistical analysis: Data were managed in a standard format developed in MS-Excel. Statistical analysis was performed using SPSS 11.6. A Chi square test was applied for categorical data.

Results

S. pyogenes was isolated from 10.9% (38/350) of the screened children. Isolation rate was slightly higher among girls (12.5%) than boys (9.4%), however it was statistically insignificant ($p>0.05$). Although the carriage rate was higher in the age subgroup 9-12 than others (Table 1), the difference was statistically insignificant ($p>0.05$). No significant difference in carrier rate was found among the four different schools studied (Table 2).

Of the 38 *S. pyogenes* isolates investigated, 27 (71.0%) were found to be resistant to Cotrimoxazole. Resistance to Chloramphenicol, ciprofloxacin and Erythromycin was in 3 (7.8%), 2 (5.2%), 2 (5.2%) isolates respectively. No resistance was detected among Penicillin and its derivative (Penicillin G and Ampicillin). Similarly, Azithromycin was also found to be 100% effective.

Table 1: Age wise distribution of throat carriage of *S. pyogenes*

Age sub-group	Total population	GAS positive	P value
5-8	110	12 (10.9%)	
9-12	125	15 (12%)	>0.05
12-15	115	11 (9.6%)	
Total	**350**	**38 (10.9%)**	

Table 2: School wise distribution of throat carriage of *S. pyogenes*

School	Total population	GAS positive	P value
Ganesh Secondary school	95	9 (9.5%)	
Suryodaya Secondary school	85	8 (9.4%)	>0.05
Mahamanjushree Secondary school	80	8 (10.0%)	
Golden Rays Secondary school	90	11 (12.2%)	
Total	**350**	**38 (10.9%)**	

Discussion

We report the notable proportion of pharyngeal carriage by group A streptococci among healthy children from different schools of Nepal. GAS throat carriage is an important health issue, as the infection often leads to post streptococcal sequelae and individuals colonized with GAS can serve as a source of spread of infections to other individuals in the community. An overall 10.9% of asymptomatic school children, irrespective of the sex and age groups ($p>0.05$), were found to be colonized with GAS. Due to the lack of information on GAS carriage from Nepal, it is difficult to estimate whether the rate is in an increasing/ decreasing trend or not.

This carriage rate found in Nepalese population is in accordance with the findings of other studies carried out in many parts of the world, although few reports have slightly different rate. This is because the relative incidence of disease caused by *S. pyogenes* varies throughout the world, in accordance with season and age group[1, 11]. In general, the prevalence of carriage of GAS in healthy individuals decreases with age. In a report, the prevalence of streptococci group A was 6% in all the age groups studied[12], 8.6% in carriers among healthy children[13] and 12.2% on school children[14]. Another study reports low the prevalence of GAS in healthy individuals before the age of 3 years and in adults above 16 years and highest in the age group 3-15 years[11]. In similar studies conducted in turkey, the rate of GAS carriage in asymptomatic school children varied from 2 to 46%[1, 5]. Age sub-group wise difference was not significant in our study since we had a narrow age range. Few reports showed the age group variation[1, 11] which could be due to analysis of all age groups including adults; in contrast, we studied only in children population from 5-15 years age range.

We found no significant variation of carriage rate with different schools. A study carried out in Turkish schools showed the variation in the carriage rate of GAS in two different school children, the overall carriage rate was 17%, with 6% in students from school in impoverished area and 28% in students from school in suburban area[16]. No significant variation with schools in our study could be due to the reason that all schools were almost similar in every aspect. Moreover, the schools included in this study were not geographically diverse and hence a similar rate was observed.

Due to the more rapid acquisition of resistance, obtaining appropriate treatment for severe invasive streptococcal infections is now a major challenge in many regions of the world. Our study showed a quite high resistance rate (71.05%) towards Cotrimoxazole which is one of the commonly used drugs to treat children infected with various diseases in Nepal. Such a high level of resistance in a commonly used drug possesses a potential risk for spread of resistance to other organisms as well. None of the strains we isolated was found resistant with Penicillin and ampicillin. Penicillin and its derivative remained the drug of choice for streptococcal pharyngitis with stable Minimum Inhibitory Concentration (MIC) during the last 70 years[9]. However reports of a rising MIC or diminished susceptibility to Penicillin have been published in recent years[9, 17]. In Nepal, Penicillin derivatives are among the easily available antibiotics even in the sub-health post level where culture facility is not available. Although we do not have report of Penicillin resistant GAS yet, empirical therapy based on Penicillin and its derivatives for most of the respiratory infections in the peripheral settings boots up the possible emergence of resistance towards this valuable

drug in the future. Resistance to ciprofloxacin and erythromycin was found in 5.2% isolates. Macrolide resistant *S. pyogenes* isolates are increasing in some parts of the world and 20-30% resistance is being reported in some countries[18]. We found Azithromycin as 100 % susceptible drug against this pathogen in addition to penicillin derivatives. Now-a-days, because of its effectiveness and short course therapy, Azithromycin is among the commonly prescribed drugs in Nepal even though it is more expensive. However, its high frequency of prescription and possible irrational use in the country may push us towards loosing its potency in the future, as erythromycin already became resistant. Therefore, before changing the therapeutic options, it is highly recommended to review the existing antimicrobials and their regimen based on their potency to save the valuable drugs for critical clinical conditions.

Conclusion

The present preliminary study provides the base-line information on the GAS carriage rate and resistance trend among healthy school children. It is highly emphasised that GAS carriage surveillance needs to be established in a large scale population to estimate the national scenario. As the limitation, our study was restricted to schools of Kathmandu valley. Therefore, the carriage rate could be different for the schools of rural areas (outside Kathmandu valley). To draw a clear-cut picture, studies among both healthy and symptomatic children of different geographical locations needs to be conducted. More detailed study also is required to establish the relationship between carriage, acute sore throat, subsequent anti-streptolysin-O titre levels, and their relationship to the post streptococcal sequelae. When screened and appropriately treated with antibiotics, pharyngeal carriers can be prevented from spreading of streptococcal infections in the community. This would ultimately reduce the incidence of life-threatening post-infectious sequelae such as ARF and PSG, which are debilitating and difficult to treat. Spread of resistance could be minimised by appropriate treatment guidelines, rational use of antimicrobials, review of existing antimicrobial therapy with regimen and creating awareness on antimicrobial resistance.

Acknowledgement

We thank the school children and teachers for their consent, students of Kantipur College of Medical Sciences for collecting the specimens and laboratory work, the technical staff of the Kantipur College of Medical Science for laboratory support.

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