Asymptomatic throat carriage rate and antimicrobial resistance pattern of \textit{Streptococcus pyogenes} in Nepalese school children

Dumre SP\textsuperscript{1}, Sapkota K\textsuperscript{2}, Adhikari N\textsuperscript{2}, Acharya D\textsuperscript{2}, Karki M\textsuperscript{3}, Bista S\textsuperscript{2}, Basnyat SR\textsuperscript{2}, Joshi SK\textsuperscript{4}

\textsuperscript{1}National Public Health Laboratory, Teku, Kathmandu, Nepal, \textsuperscript{2}Department of Microbiology, Kantipur College of Medical Science, Kathmandu, Nepal, \textsuperscript{3}Central Department of Microbiology, Tribhuvan University, Kirtipur, Nepal, \textsuperscript{4}Department of Community Medicine, Kathmandu Medical College, Nepal

Abstract

Background: \textit{Streptococcus pyogenes} or Group A streptococcus (GAS) causes several suppurative and non suppurative infections. 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 glomerulonephritis (PSG). GAS frequently colonises in the throat of an asymptomatic person. Pharyngeal carriage rates of GAS among healthy school children vary with geographical location and seasons.

Objectives: We carried out this preliminary study to determine the throat carriage rate and antimicrobial resistance trend of \textit{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 \textit{S. pyogenes} following standard microbiological procedures. Antimicrobial susceptibility testing of the isolates was performed by Kirby Bauer disc diffusion method following CLSI guidelines.

Results: \textit{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 sub-groups, although the rate was slightly higher among girls and age sub-group 9-12 years. No significant difference in carrier rate was observed among different schools (P>0.05). All isolates were susceptible to azithromycin. No resistance was detected for penicillin and its derivative antibiotic ampicillin. Highest resistance rate was observed for cotrimoxazole (71.0\%) followed by chloramphenicol (7.8\%), ciprofloxacin (5.2\%) and erythromycin (5.2\%).

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

Key words: \textit{Streptococcus pyogenes}, Antibiotics Resistance, Throat carriage, Children, Nepal.
Since early 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. Rheumatic heart disease is still prevalent in developing countries, particularly among the children who live in communities that do not have adequate treatment programmes. Beta-lactam antibiotics (mainly Penicillins) and macrolides have been conventionally used to treat *S. pyogenes* infections. The resistance of *S. pyogenes* to macrolides has been reported and is increasing in most of the countries and there were no report of penicillin resistance although isolates with rising minimum inhibitory concentration (MIC) to penicillin are increasing. 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 literature on the carriage rates and resistance patterns of GAS in school children. The present study was undertaken to understand throat carriage rate and antimicrobial susceptibility pattern of GAS among children of different schools within Kathmandu valley. In view of the increasing number of drug resistance pattern among GAS, we also attempted to assess the antibiotic resistance pattern among isolated GAS from the studied samples.

**Materials and methods**

**Study site:** The study population included Nepalese school children from four schools situated at different locations of Kathmandu valley. Laboratory Analysis was performed at Department of Microbiology, Kantipur College of Medical Sciences, Kathmandu, Nepal.

**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. A total of 350 asymptomatic children were included of which 45.7% were boys. The median age of the children was 9.6 years (range, 5–15 years). Children with throat infection or any related sign and symptoms of pharyngitis were excluded from the study. The asymptomatic/healthy and symptomatic children were clinically differentiated by a medical practitioner after clinical examination. Students having past medical history, including antimicrobial therapy and/or hospital attendance, were obtained by formally requesting and collecting medical records from their parents/guardians. Those who received antimicrobial therapy or who had suffered from GAS in the previous three months were excluded from the study. With the recent report of GAS colonisation in lower age group, we divided the age into three sub-groups namely 5 to 8, 9 to 12 and 13 to 15 years. Within this narrow age range, we wished to see if making three sub-groups has any variation in GAS carriage.

**Specimen collection:** Study permission was secured from Department of Microbiology, Kantipur College of Medical Science and the Management Board of participating schools. After institution permission, we forwarded a letter explaining objectives of our study to children’s parents/guardians. After getting written informed consent from guardians/parents or their caretakers with legal custody of the children, pharyngotonsillar specimens were obtained with sterile cotton tipped swab. All the swabs were 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 enriched solid agar plates (5% sheep blood agar, and chocolate agar) and enrichment broth (Brain Heart Infusion, BHI) immediately upon receipt at the microbiology laboratory of Kantipur College of Medical Science. 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 sheep blood agar and chocolate agar plates from the overnight incubated BHI broth. All the plates with beta-haemolytic colonies were microbiologically processed and GAS were identified by conventional methods (colony morphology, haemolysis pattern, catalase test, Gram stain and morphological observation). *Streptococcus pyogenes* was further identified by observing its sensitivity towards 0.04 units of bacitracin disc tested on sheep blood agar plate.

**Antimicrobial susceptibility testing:** Antimicrobial susceptibility testing of the isolates was performed by Kirby Bauer disc diffusion method following clinical and laboratory standards institute (CLSI) recommended interpretive criteria. *Streptococcus pneumoniae* ATCC 49612 was used as the quality control strain. The following antibiotics were tested for all the conforms isolates: Penicillin (10 μU), Ampicillin (10 μg), Ciprofloxacina (5μg), Azithromycin (15 μg); Erythromycin (15 μg), Cotrimoxazole (1.25/23.75 μg) and Chloramphenicol (30 μg).

**Statistical analysis:** Data were coded and sorted in a standard format in MS-Excel sheet. Randomised complete block design (RCBD) and 2 X 3 factorial treatment structures was used to determine the effect of age groups and sex of school children on carriage rate of GAS. There were three age sub-groups and four schools. Age groups and sex were considered as factors and schools were treated as block in Statistical
Analysis System (SAS) design. Statistical analysis was performed using latest SAS software, version 9.2. The relevant SAS outputs are depicted in table 1, 2 and 3.

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 sub-group 9-12 (tables 1, 3), the difference was statistically insignificant (P>0.05).

Similarly, no significant difference in carriage rate was found among the four different schools studied (tables 2, 3).

Of the 38 *S. pyogenes* strains isolated, 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% susceptible.

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

<table>
<thead>
<tr>
<th>Age sub-group</th>
<th>Number of students screened</th>
<th>GAS positive</th>
<th>Mean % of GAS</th>
<th>95% Confidence Interval</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-8</td>
<td>110</td>
<td>12</td>
<td>10.9</td>
<td>7.9</td>
<td>4.5</td>
</tr>
<tr>
<td>9-12</td>
<td>125</td>
<td>15</td>
<td>12.0</td>
<td>9.9</td>
<td>6.5</td>
</tr>
<tr>
<td>13-15</td>
<td>115</td>
<td>11</td>
<td>9.6</td>
<td>7.2</td>
<td>3.9</td>
</tr>
<tr>
<td>Total</td>
<td>350</td>
<td>38</td>
<td>10.9</td>
<td></td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>School</th>
<th>Number of children screened</th>
<th>GAS positive</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Secondary School located at Sitapaila</td>
<td>95</td>
<td>9</td>
<td>9.5</td>
</tr>
<tr>
<td>Secondary School located at Maitidevi</td>
<td>85</td>
<td>8</td>
<td>9.4</td>
</tr>
<tr>
<td>Secondary school located at Swyambhu</td>
<td>80</td>
<td>9</td>
<td>11.2</td>
</tr>
<tr>
<td>Primary school located at Kirtipur</td>
<td>90</td>
<td>12</td>
<td>13.3</td>
</tr>
<tr>
<td>Total</td>
<td>350</td>
<td>38</td>
<td>10.9</td>
</tr>
</tbody>
</table>

Table 3: Analysis of variance (ANOVA) on age subgroups and sex of four schools children

<table>
<thead>
<tr>
<th>Source of variance</th>
<th>DF</th>
<th>F-value</th>
<th>P-value</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>2</td>
<td>0.64</td>
<td>0.64</td>
<td>No significant age effect</td>
</tr>
<tr>
<td>Sex</td>
<td>1</td>
<td>0.99</td>
<td>0.99</td>
<td>No significant sex effect</td>
</tr>
<tr>
<td>Age by sex</td>
<td>2</td>
<td>1.59</td>
<td>1.59</td>
<td>No significant age by sex interaction</td>
</tr>
<tr>
<td>School</td>
<td>3</td>
<td>0.53</td>
<td>0.53</td>
<td>No significant school effect</td>
</tr>
</tbody>
</table>

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 public health issue, as the infection often leads to post streptococcal sequelae and individuals colonised 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 sub-groups (P>0.05), were found to be colonised with GAS. Due to unavailability of published literature on GAS carriage from Nepal, it is difficult to estimate the increasing or decreasing trend of the condition.

The present throat carriage rate of GAS found in Nepalese school children is in accordance with the findings of other studies carried out in many parts of the world. Few studies in different geographical location, settings and countries, however, have shown a different carriage rate. This is because the relative incidence of disease caused by *S. pyogenes* varies throughout the world, in accordance with season and age group. 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, with 8.6% in carriers among healthy children and 12.2% on school children. Another study reports...
low prevalence of GAS in healthy individuals before
the age of 3 years and in adults above 16 years. But on
the contrary, the same study reports highest prevalence
rate of GAS in the age group 3-15 years\textsuperscript{11}. In similar
studies conducted in turkey, the rate of GAS carriage in
asymptomatic school children varied from 2 to 46%\textsuperscript{1,15}.
In the present study, though statistically not significant,
the age sub-group 9-12 years was the most susceptible
group for throat carriage of GAS, followed by age sub-
groups 5-8 and 13-15 years respectively. Age sub-group
wise difference was not significant in our study since
we had a narrow age range and focused only in children
population from 5-15 years of age. Few studies have
reported the age group variation\textsuperscript{1,15} which could be due
to analysis of all age groups including adults.

We found no significant variation of carriage rate with
different schools as well. A study carried out in Turkish
school children showed the variation in the carriage rate
of GAS in two different schools 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\textsuperscript{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.

There are several mechanisms suggested for colonisation
of GAS in pharynx\textsuperscript{17} and the colonisation may turn into
clinical infections under certain circumstances\textsuperscript{18}. A
study on environmental health effects of brick kilns in
Kathmandu valley showed high prevalence of tonsillitis
(4.17%) and acute pharyngitis (4.08%) in school children residing near brick kilns\textsuperscript{19}. We assume that air
pollution may enhance the infection in GAS colonised
children. However, confirmatory relation can only be
established when both epidemiological and etiological
investigations are conducted to show if air pollution
enhances GAS related throat infection in children.

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 (71.0%) 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 microorganisms 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\textsuperscript{8}. However, reports of a rising

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 initiated
and 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
the clear-cut picture, studies among both healthy and
symptomatic children of different geographical locations
needs to be conducted. More detailed study is also
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 wish to thank the participating schools’ teachers and guardians for their consent and cooperation during the entire period of our study. We would also like to thank undergraduate and graduate students of Kantipur College of Medical Science for helping in sample collection and Mr. Raju Lama for assisting in culture and sensitivity tests. Special thanks go to Mr. Achyut Adhikari for providing constructive feedback on research design and statistical analysis using SAS.

References