

Burden of bacterial upper respiratory tract pathogens in school children of Nepal

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ABSTRACT

Introduction Respiratory tract infections are one of the most common human infections in all age group and important cause of mortality and morbidity worldwide. Most bacterial upper respiratory tract infections are vaccine preventable. This study aimed to determine the prevalence of carrier state of bacterial upper respiratory tract pathogens among school children. It also aimed to study their antibiograms.

Methods The specimen from posterior pharyngeal wall and tonsils were collected from 204 participants on calcium alginate coated swabs (HiMedia). Isolates were identified by standard microbiological methods and tested for in vitro antibiotic susceptibility testing by modified Kirby-Bauer disc diffusion method.

Results In this study, *Streptococcus pneumoniae* (16.6%) was the most common bacterial pathogen recovered, followed by *Staphylococcus aureus* (14.7%), β-haemolytic streptococci (non-Group A) (8.8%), *Streptococcus pyogenes* (5.3%) and *Corynebacterium diphtheriae* (3.4%). The Gram negative bacteria were *Klebsiella pneumoniae* (4.9%), *Haemophilus influenzae* (3.4%) and *Neisseria meningitidis* (1.4%). Important findings in antibiogram include high resistance of *Streptococcus pneumoniae* to penicillin (91.17%) and resistance of *S. aureus* to oxacillin (23.3%).

Conclusion Pharyngeal colonisation by *S. pneumoniae* was found high among school children and this calls for an urgent need to include pneumococcal vaccine in routine national immunisation schedule of Nepal given the high burden of invasive pneumococcal disease. Despite expected universal vaccination, pharyngeal colonisation by *C. diphtheriae* is possible and there is possibility of transmission of these respiratory pathogens to other healthy children.

BACKGROUND

Respiratory tract infection refers to any infectious disease involving the respiratory tract. In low-income and middle-income countries, respiratory tract infection is considered as one of the major public health problems. It can lead to severe mortality and morbidity in children as well as adults.¹ In most of the viral upper respiratory tract infections (URTIs), recovery occurs without any complications,

Key messages

- Most of the bacterial pathogens isolated in this study are not only associated with respiratory tract infection but also with systemic infections.
- The school children are potential carriers of respiratory pathogens and are likely to be exposed to respiratory infections during winter.
- Findings of this study would be important in minimising the transmission of respiratory pathogens among school children by effective vaccination, screening and treatment of carriers.

while bacterial infections need specific antibiotic therapy. Secondary bacterial infections are also common particularly in malnourished and young children.² Common bacterial pathogens responsible for URTIs are *Haemophilus influenzae* type b (Hib), *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Corynebacterium diphtheriae*. *Neisseria meningitidis* is not seen as a URTI causative agent. It does not cause URTI but cause central nervous system (CNS) infection. The pharynx is just a reservoir and thus colonisation a risk factor for CNS disease.³

Carriage (also known as colonisation) can lead to transmission of these organisms. The carrier state occurs when a pathogen grows as the normal flora in an individual without causing disease.⁴ Although colonisation itself does no harm to the host, it is a known risk factor for developing and transmitting clinically relevant infections.⁵ However, when the condition of the host is altered, microorganisms may invade adjacent sites and/or invade the blood stream, causing disease.⁶

S. pneumoniae is responsible for an estimated 14.5 million episodes of serious disease and 826 000 deaths annually among children aged 1–59 months.⁷ Hib is a major cause of invasive bacterial infection and pneumonia in childhood. Globally, Hib is estimated to cause

over 3 million cases of serious disease and 400 000 deaths, primarily among children in resource-poor countries.^{8,9} *N. meningitidis*, *C. diphtheriae* and *S. pyogenes* can also have acute effect and sometimes long-term sequel like rheumatic fever.

It is important to study bacterial colonisation in young children with developing immune system since the aetiology of many childhood diseases may originate early in life. The highest transmission and carriage rates have been reported in populations where people live in close contact with one another, for example, students in schools.¹⁰ Several other determinants such as the number of siblings, family size, vaccination status, season and socioeconomic status influence carrier rate in healthy children.¹¹

Most of these infections and diseases can be prevented by vaccination. Vaccination against diphtheria and pertussis is readily available and is given on regular basis. This prevents occurrence of diphtheria and pertussis disease but may not prevent carrier state. Unvaccinated population thus remains at risk of infection and disease from such carriers. Vaccination against Hib has recently been introduced by the Government of Nepal. Vaccination against pneumococcal infections has recently been introduced but not used in regular basis. At present, vaccines for prevention of meningococcal disease are not available in Nepal. The requirement, benefits and success of vaccination programme depends on the prevalence of carrier state and risk factors associated with disease.

Surveillance of respiratory tract infections in defined populations is required to monitor prevailing pathogens, while determination of population groups at risk is important for implementing preventive strategies. Furthermore, monitoring of antimicrobial resistance patterns is needed to guide empirical therapy and also to monitor trend of these infections.¹²

This study intends to determine the prevalence of carrier state of bacterial agents of respiratory tract infections, among school children in Pokhara. It also aims to study their antibiograms. The data are expected to reveal the risk of diseases in children and need for vaccines for the population at risk. Some of the anticipated outcomes of the project can be used to determine the need for introduction of vaccines against meningococcal and pneumococcal infections and to provide baseline data in determining the effectiveness of vaccination programmes.

MATERIALS AND METHODS

This study was carried out in two phases in different seasons (summer and winter) in 2015, over a period of 1 year in the Department of Microbiology, Manipal College of Medical Sciences, Pokhara, Nepal.

Study population

Out of total 232 school children screened, 204 school children were enrolled in this study. The study population

included two sets of school children. Group A comprises 54 students of age group 5–9 years. Group B comprises 150 students of age group 10–14 years. This totals to 204 school children selected from four schools of Pokhara valley.

Government school:

1. Shree Satya Sai School, Nadipur
2. Pardi Government Higher Secondary School, Pardi

Private school

3. Manakamna Boarding School, Phulbari
4. Dynamic Academy, Simalchour.

Inclusion and exclusion criteria

Children of age group <15 years present during the day of sample collection were enrolled in this study. Children suffering from throat infections or already detected and treated for rheumatic diseases/nephritis and having taken antibacterial therapy in preceding 15 days were excluded in this study. Informed consent was obtained from the principal of respective schools, parents/guardians and participants.

Sample collection

A preformed questionnaire was used to collect the demographic and health-related information of the participants selected for this study (online supplementary file 1). The specimens from posterior pharyngeal wall and tonsils were collected on calcium alginate coated swabs (HiMedia) and were labelled. These were protected from light and stored in Stuart Transport Media (HiMedia) for transportation. Each sample was labelled with code number and information including age, gender, location, etc were recorded in relevant form. The sample was transported to the laboratory within 1–2 hours for processing.

Sample processing

Throat swab was inoculated on the same day on 5% sheep blood agar, chocolate agar and potassium tellurite agar. Plates were incubated at 37°C overnight under 5%–10% CO₂. Plates were examined for type of growth. The organisms were subcultured to obtain pure growth and were processed. The plates were observed up to 48 hours. If no growth of relevant bacteria was seen after 48 hours of incubation, the culture was declared negative. Preliminary identification was based on colony morphology and cultural characteristics on selective and differential media and results of Gram's staining.

Identification of bacteria

Isolates were stored for further characterisation/study. All bacterial isolates were identified by conventional microbiological method¹³ and tested for in vitro antibiotics susceptibility by modified Kirby-Bauer disc diffusion

method following the criteria designed by the Clinical and Laboratory Standards Institute.¹⁴

Data analysis

The data were first entered in the Microsoft Excel and were analysed by SPSS V.20. Differences between proportions were assessed by means of χ^2 analysis. Statistical significance was set at 0.05.

RESULTS

This study comprises a total of 204 participants, 108 (52.9%) male and 96 (47.1%) female, 54 (26.5%) belong to age group of 5–9 years (mean age=6.8±1.19 years, median age=7.0 years) and 150 (73.5%) belong to 10–14 years (mean age=12.12±1.1 years, median age=12.0 years) from various schools of Pokhara valley. Among 204 participants, 16 (7.84 %) were non-immunised with BCG and diphtheria–pertussis–tetanus (DPT) vaccination while 188 (92.16%) were immunised. None of the participants were vaccinated with pneumococcal, meningococcal and Hib vaccines. In government school, none of the participants were staying in hostel because there is no facility for hostel at government school but in private school, out of 102 participants, sample was collected from 30 male and 22 female of the age group 10–14 years who were staying at hostel; these children live permanently within accommodation associated with the schools. None of the participants belonging to the age group 5–9 years were staying in hostel (table 1).

This study depicts that majority of the Hindu and Buddhist participants were from private schools, while majority of the Christian participants were from government school. On comparing the proportion of school children going to government and private school based

on their religion, statistical analysis showed significant difference in the trend ($\chi^2=16.25$, $df=2$, $p<0.001$). Majority of Brahmin and Mongol children were from private school as compared with Dalit who were from government schools (see table 1 in the online supplementary file 1).

Table 2 shows that out of 204 participants, 54 (26.5%) were in age group of 5–9 years, while 150 (73.5%) were in age group of 10–14 years, 102 samples each were collected from government and private school in summer and winter. Among the schools, greater number of organisms were isolated from the students of government school (two organisms/sample) compared with private school (1.78 organisms/sample). Out of the total 394 isolates, maximum number (2.28 organisms/sample) were isolated in winter and rest (1.57 organisms/sample) were isolated in the summer (see table 2 in the online supplementary file 2).

This study revealed that greater number of organisms (1.96 organisms/sample) were isolated from participants of age group 5–9 years compared with (1.92 isolates/sample) age group of 10–14 years (table 3). Maximum number of *Viridans streptococci*, *Moraxella* spp, *S. pneumoniae*, *S. aureus*, *S. pyogenes* and *K. pneumoniae* were isolated from students of government school compared with private school (see table 3 in the online supplementary file 4).

Table 4 shows the number of Gram positive and Gram negative bacteria isolated from throat swabs. The history of repeated throat infection was part of the questionnaire which was sent to parents and also verbally asked with children during examination of upper respiratory tract that does he/she suffer from repeated throat infection, any history of repeated throat infection and how many times in a year. In participants with no history of repeated

Table 1 Age-wise and gender-wise distribution of the study groups

Age group	Mean age	Median age	Gender		Immunisation status		Hostel status		Total (%)
			M	F	NI	I	NH	H	
5–9 years	6.8±1.19	7.0	28	26	6	48	54	0	54 (26.47)
10–14 years	12.12±1.1	12.0	80	70	10	140	98	52	150 (73.53)
Total			108 (52.9)	96 (47.1)	16 (7.84)	188 (92.16)	152 (74.5)	5 (25.4)	204 (100)

Figures in parenthesis depict %.

F, female; H, hostellers; I, immunised; M, male; NI, non-immunised; NH, non-hostellers.

Table 2 Samples collected from different age groups from government and private school in summer and winter

Type of school	Season	Number of sample collected		Total number of sample collected	Total number of organisms isolated
		5–9 years	10–14 years		
Government	Summer	15	37	52	97
	Winter	16	34	50	115
Private	Summer	11	39	50	64
	Winter	12	40	52	118
Total		54	150	204	394

Table 3 Organisms isolated in different age groups

Organism	Age group		Total (204 samples)	p Value
	5–9years (54 samples)	10–14years (150 samples)		
Viridans streptococci	43 (79.6)	117 (78)	160 (78.4)	0.82
<i>Streptococcus pneumoniae</i>	10 (18.5)	24 (16)	34 (16.6)	0.86
<i>Staphylococcus pyogenes</i>	3 (5.5)	8 (5.3)	11 (5.3)	0.99
<i>Staphylococcus aureus</i>	14 (9.3)	18 (8.8)	0.90	
<i>Corynebacterium diphtheriae</i>	9 (16.6)	21 (14)	30 (14.7)	0.85
Beta-haemolytic streptococci	2 (3.7)	5 (3.3)	7 (3.4)	0.98
<i>Moraxella</i> spp.	21 (38.8)	72 (48)	93 (45.5)	0.45
<i>Neisseria meningitidis</i>	2 (3.7)	1 (0.6)	3 (1.4)	0.89
<i>Klebsiella pneumoniae</i>	3 (5.5)	7 (4.6)	10 (4.9)	0.95
<i>Pseudomonas aeruginosa</i>	0 (0)	3 (2.0)	3 (1.4)	–
<i>Enterobacter</i> spp.	0 (0)	3 (2.0)	3 (1.4)	–
<i>Citrobacter</i> spp.	1 (1.8)	4 (2.6)	5 (2.4)	0.96
<i>Acinetobacter</i> spp.	1 (1.8)	4 (2.6)	5 (2.4)	0.96
<i>Haemophilus influenzae</i>	2 (1.3)	7 (3.4)	0.73	
<i>Haemophilus parainfluenzae</i>	2 (3.7)	3 (2.0)	5 (2.4)	0.91
Total	106	288	394	

Figures in parenthesis depict %.

Table 4 Number and types of organisms isolated with or without repeated throat infection

Isolates	Number of isolates (N)		Total (204 samples)	p Value
	No history of repeated throat infection (112samples)	History of repeated throat infection (92 samples)		
Gram positive bacteria				
<i>Viridans streptococci</i>	86 (76.7)	74 (80.4)	160 (78.4)	0.57
<i>Streptococcus pneumoniae</i>	6 (5.3)	28 (30.4)	34 (16.6)	0.21
<i>Streptococcus pyogenes</i>	2 (1.7)	9 (8.0)	11 (5.3)	0.76
β-haemolytic streptococci (non-Group A)	8 (7.1)	10 (8.9)	18 (8.8)	0.89
<i>Staphylococcus aureus</i>	6 (5.3)	24 (21.4)	30 (14.7)	0.36
<i>Corynebacterium diphtheriae</i>	2 (1.7)	5 (4.4)	7 (3.4)	0.87
Total	110	150	260	
Gram negative bacteria				
<i>Moraxella</i> spp	53 (47.3)	40 (43.4)	93 (45.5)	0.71
<i>Neisseria meningitidis</i>	0 (0)	3 (2.6)	3 (1.4)	–
<i>Klebsiella pneumoniae</i>	3 (2.6)	7 (6.2)	10 (4.9)	0.82
<i>Pseudomonas aeruginosa</i>	2 (1.7)	1 (0.8)	3 (1.4)	0.95
<i>Enterobacter</i> spp	2 (1.7)	1 (0.8)	3 (1.4)	0.95
<i>Citrobacter</i> spp	2 (1.7)	3 (2.6)	5 (2.4)	0.95
<i>Acinetobacter</i> spp	1 (0.8)	4 (3.5)	5 (2.4)	0.89
<i>Haemophilus influenzae</i>	2 (1.7)	5 (4.4)	7 (3.4)	0.87
<i>Haemophilus parainfluenzae</i>	1 (0.8)	4 (3.5)	5 (2.4)	0.89
Total	66	68	134	

Figures in parenthesis depict %.

throat infection, majority of Gram positive bacteria were isolated (one organisms/sample) compared with Gram negative bacteria (0.58 organisms/sample). Majority of Gram positive bacteria were isolated (1.63 organisms/sample) compared with Gram negative bacteria (1.35 organisms/sample) in participants with history of repeated throat infection. Greater number of organisms were isolated from the participants with history of repeated throat infection (2.36 organisms/sample) compared with participants with no history of repeated throat infection (1.57 organisms/sample).

Table 5 shows high resistance of *S. pneumoniae* to penicillin (91.17%) and resistance of *S. aureus* to penicillin (86.6%) and oxacillin (23.3%). *S. pyogenes* were 100% sensitive to all the antibiotics tested. All the Gram negative isolates were found resistant to ampicillin. *H. influenzae* and *H. parainfluenzae* showed 100% resistance to amoxy-clavulanic acid and cotrimoxazole. All the isolates of *N. meningitidis* were resistant to penicillin.

DISCUSSION

Human oropharynx is colonised by wide spectrum of microorganisms from commensal bacteria to potential pathogens. Few studies have been carried so far which highlight the carrier states of these pathogens in school children of Nepal. Higher number of respiratory pathogens was isolated from participants staying in the hostel, while less pathogens were isolated from non-hostellers. Hostel children are more likely to have closer contacts during most of the activities. Hence, there is higher risk of transmission of respiratory pathogens among hostel children.

In our study, majority of the Hindu and Buddhist children were sent to private schools as compared with Christian children going to government school. Statistical analysis revealed significant association between the religious belief and the type of school ($\chi^2=16.25$, $df=2$, $p<0.001$). This may not reflect the religion as such but socioeconomic status since Christian children are more likely to be from lower socioeconomic status. Majority of Brahmin and Mongol children were sent to private school as compared with Dalit who were sent to government schools. This also highlights the socioeconomic status of the school children based on their ethnicity.

In this study, greater number of organisms (1.96 organisms/sample) was isolated from participants of age group 5–9 years compared with (1.92 isolates/sample) age group of 10–14 years, despite the fact that there were almost three times less participants belonging to this age group. Other studies revealed that greater number of isolates was obtained from school children from this age group.^{15–17} These findings suggest that school children belonging to age groups 5–9 years are at a higher risk most probably because of immature immune system.

This study shows that children studying in government schools are more potential carriers. Greater number of organisms were isolated from the students of government

school (two organisms/sample) compared with private school (1.78 organisms/sample). The reason for these differences could be because of the poor infrastructure of the school and overcrowding. Greater number of organisms (2.28 organisms/sample) was isolated in winter as compared with summer (1.57 organisms/sample). This shows that school children are more likely to be exposed to throat infections during winter. Other studies revealed that more pathogenic organisms were grown in July and also in May and September.¹⁸ Another author reported that pathogenic organisms were grown more in March and also from August to October.¹⁹ Nandi *et al* reported bimodal peak during the months of November to January and in August.²⁰ These differences could be dependent on the prevailing geographical and climatic conditions at different places.

In this study, maximum number of *S. pneumoniae*, *S. aureus*, *S. pyogenes* and *K. pneumoniae* were isolated from participants of government school compared with private school. *St. pneumoniae* (16.6%) was the most common bacterial pathogen recovered followed by *S. aureus* (14.7%), β -haemolytic streptococci (non-Group A) (8.8%), *S. pyogenes* (5.3%) and *C. diphtheriae* (3.4%). The Gram negative bacterial isolates were *K. pneumoniae* (4.9%) followed by *H. influenzae* (3.4%), Acinetobacter species (2.4%) and *N. meningitidis* (1.4%). In previous studies, authors have reported that these organisms were major respiratory pathogens among school children.²¹ Review of literature revealed that results of this study are similar to the studies from China,²² Iran²³ and South Africa.²⁴

The colonisation rates of *S. pneumoniae* varies widely; it ranged from 3.5% in Italy to as high as up to 90% in Gambia as compared with this study which revealed carrier rate of 16.6%. Coles *et al* have shown very high colonisation rate of *S. pneumoniae* up to 80% in healthy children of Sarlahi, Nepal.²⁵ Different carriage rates of *S. pneumoniae* have been reported in different geographical areas.^{26–28} The effect of pneumococcal conjugate vaccines on nasopharyngeal carriage regardless of the carrier protein used lead to reduction in colonisation.²⁹ Interestingly, rate of invasive pneumococcal disease among adults has also declined since the introduction of the vaccine.³⁰ The carrier rate of β -haemolytic streptococci (non-group A) in this study is 8.8% which is comparable with study from India by Devi *et al*.³¹ Beta-haemolytic streptococci can cause local infections but are not linked with chronic sequel.

S. pyogenes was another important pathogen isolated from 5.3% of participants in this study is comparable with studies from India and Turkey by Dhakal *et al* and Gazi *et al*.^{32,33} Rijal *et al*³⁴ found the prevalence of carriers among primary school children to be 9.2%. Bahadur *et al*,³⁵ from Nepal, have reported the prevalence of rheumatic heart disease in school children to be 1.2/1000 population. This could be directly related to higher carrier rate of *S. pyogenes* and needs attention.

H. influenzae was the predominant cause of URTI before the introduction of routine infant immunisation

Table 5 Antibiotic resistance pattern of Gram positive and Gram negative isolates

Isolates	P	E	CIP	CTR	OX	G	COT	CN	AMP	C	AZM	AMC	AK
<i>Streptococcus pneumoniae</i> (34)	91.7	14.7	8.82	5.88	-	-	38.2	-	-	00	-	-	-
<i>Streptococcus pyogenes</i> (11)	00	00	00	00	-	-	00	00	-	-	-	-	-
β -haemolytic streptococci (non-Group A) (18)	00	00	00	00	-	-	00	-	00	-	-	-	-
<i>Staphylococcus aureus</i> (30)	86.6	20	13.3	10	23.3	-	-	00	-	-	-	-	-
<i>Corynebacterium diphtheriae</i> (7)	28.5	42.8	-	28.5	-	00	-	-	-	-	-	-	-
<i>Klebsiella pneumoniae</i> (10)	-	-	00	20	-	00	00	30	100	-	-	-	-
<i>Pseudomonas aeruginosa</i> (3)	100	-	00	00	-	00	100	-	100	-	-	-	-
Enterobacter spp (3)	00	-	-	00	-	33.3	00	33.3	100	-	-	-	-
Citrobacter spp (4)	-	-	00	-	-	00	00	100	100	-	-	-	00
Acinetobacter spp (5)	-	-	00	-	-	00	00	100	100	-	-	-	00
<i>Haemophilus influenzae</i> (7)	-	-	00	00	-	-	100	-	-	00	00	100	-
<i>Haemophilus parainfluenzae</i> (5)	-	-	00	00	-	-	100	-	-	00	00	100	-
<i>Neisseria meningitidis</i> (3)	100	-	00	00	-	-	00	-	-	00	-	00	-

Figures in parenthesis depict %.

AK, amikacin; AMC, amoxy-clavulanic acid; AMP, ampicillin; AZM, azithromycin; C, chloramphenicol; CIP, ciprofloxacin; CN, cephalosporin; COT, cotrimoxazole; CTR, ceftriaxone; CZ, ceftazolin; E, erythromycin; G, gentamicin; OX, oxacillin; P, penicillin; 00, no resistance; -, not tested.

with Hib vaccines with carriage rates of 20%, 57% and 67.9% in Cameroon, India and Saudi Arabia, respectively.³⁶ However, following the Hib vaccine in these and other countries reduced, incidence of *H. influenzae* infection has been documented. Studies from Costa Rica, Israel and USA reported 57%, 95% and 98% decline, respectively.³⁷ Pharyngeal carrier rate of *H. influenzae* varies globally, with high prevalence up to 88% in Costa Rica and low prevalence of 3% in Sweden.³⁸ This study revealed carrier rate of 3.4%. The detection rate for *H. influenzae* is much lower than that of other studies that have used PCR detection likely due to the difficulty in isolating this species by culture. William *et al*³⁹ reported 5% carrier rate of *H. influenzae* among the urban population of Kathmandu, Nepal, which is higher than these results.

Despite routine vaccination, *C. diphtheriae* was isolated from 3.4% of the participants. This result is comparable with the carrier rate of 2.5% reported by Nweze EI *et al*.⁴⁰ Khare *et al* reported the carrier state of *C. diphtheriae* as 13.3%.⁴¹ The vaccination with diphtheria toxoid protects from illness due to diphtheria toxin but does not prevent colonisation by *C. diphtheriae*. This showed that colonisation in vaccinated children is likely and may lead to transmission of the organism to the non-vaccinated population. Only few studies have been conducted to determine the prevalence of carrier state of *C. diphtheriae*. Screening of large population is required to determine geographical prevalence of *C. diphtheriae* among the children. This also emphasises that effective vaccination must continue to prevent occurrence of diphtheria disease. In former USSR, discontinuation of diphtheria vaccination had led to outbreaks of diphtheria disease. The case fatality rate of respiratory diphtheria even with appropriate treatment is 5%–10%.⁴²

In the present study, *N. meningitidis* was isolated in 1.4% of participants. According to the data obtained from the studies on school-age children in other countries, nasopharyngeal carriage rate was found to be 2.3% in Spain, 2.8% in Sweden and 6.2% in Nigeria.⁴³ Asymptomatic carriage of *N. meningitidis* is an important risk factor for developing infection. At present, vaccines for prevention of meningococcal disease are not available in Nepal. Unvaccinated population thus remains at risk of infection and disease from such carriers. Determination of population groups at risk is important for implementing preventive strategies. Prevalence of carrier state of *N. meningitidis* needs to be studied in larger population and in different locations. Any decision regarding introduction of meningococcal vaccine would need to consider this aspect as well.

Majority of Gram positive bacteria (1.63 organisms/sample) were isolated compared with Gram negative bacteria (1.35 organisms/sample) in participants with history of repeated throat infection (3–4 times in a year). Higher number of pathogens (2.36 organisms/sample) was isolated from participants with history of repeated throat infection compared with the participants who had

no history of repeated throat infection (1.57 organisms/sample). Maximum number of *S. pyogenes* was isolated from participants with history of repeated throat infections; early detection of these cases can prevent rheumatic fever and rheumatic heart disease in future. Similarly, maximum number of *C. diphtheriae* was isolated from participants presenting with history of repeated throat infections. If routine immunisation falls below 80% and the herd immunity becomes low, these carriers have the potential to start an epidemic.

This study showed that the colonisation rate of respiratory pathogens varies in different geographical location. The possible reason for this variation in the result of our study compared with other studies could be associated with study population which included both government rural school students (lower socioeconomic group) and private urban school students (higher socioeconomic group), while other studies were conducted on one population, vaccination status, season of sampling, socioeconomic status, standard of living and overcrowding of population.

Among the commonly used antimicrobials in URTI, high resistance of *S. pneumoniae* to penicillin (91.17%) and resistance of *S. aureus* to penicillin (86.6%) and oxacillin (23.3%) were seen. *S. pyogenes* was 100% sensitive to all the antibiotics tested. All the Gram negative isolates were found resistant to ampicillin. *H. influenzae* and *H. parainfluenzae* showed 100% resistance to amoxy-clavulanic acid and cotrimoxazole. All the isolates of *N. meningitidis* were resistant to penicillin.

The strength of this study is that most of the bacteria responsible for URTI were isolated. Important findings were isolation of *C. diphtheriae*, *S. pyogenes*, *S. pneumoniae*, *H. influenzae* and *N. meningitidis*. Several determinants that influence carrier rate in school children such as the vaccination status, hostel stay, season of sampling, socioeconomic status and school type were studied. This study reveals the risk of respiratory infection among school children and the need for introduction of vaccines. It also provides baseline data in determining effectiveness of vaccination programmes. The weakness is that only the univariate analysis was performed given possible missing covariates and likely confounding factors such as socioeconomic factors and siblings and no possible adjustment for multiple testing. Serotyping and molecular characterisation of microorganisms were not studied.

CONCLUSION

Considering various findings of the present study, pharyngeal colonisation by *S. pneumoniae* among school children was found high and there is a need to include pneumococcal vaccines in national immunisation schedule of Nepal. Group A beta-haemolytic streptococcal carriers are potential threat to other healthy children. Hence, a constant monitoring and optimal therapy are necessary to prevent the spread of Group A beta-haemolytic streptococcal pharyngitis, rheumatic fever and carditis among school children. Despite expected universal vaccination,

pharyngeal colonisation by *C. diphtheriae* is possible and there is possibility of transmission. Better understanding of the burden of meningococcal disease will guide next-generation vaccine development and allow accurate assessment of the potential impact of such vaccines if introduced into vaccination calendars. Transmission of respiratory pathogens and occurrence of diseases among school children can be minimised by effective vaccination, screening and treatment of carriers. Surveillance of bacterial infections and monitoring their antimicrobial susceptibility pattern must be carried out in different parts of Nepal. Such data will help in designing and validating the accuracy of guidelines for empirical treatment of URTI.

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Contributors SG conceived and designed the study. ST, SG and ALS prepared the questionnaire. ALS facilitated the study in different schools. ST collected the samples processed the samples. LBS and ST analysed the results. SA, RG, SS and PN prepared the initial draft of the manuscript. ST and SG searched the scientific literature. ST, SG and SA prepared and refined the manuscript.

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REFERENCES

- Bhatta DR, Gokhale S, Sharma AL, et al. Carrier state of *Haemophilus influenzae* type b (Hib), *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Neisseria meningitidis* and *Corynebacterium diphtheriae* among school children in Pokhara, Nepal. *Asian Pac J Trop Dis* 2014;4:45–9.
- Amin R, Hoque AMW, Khan RF, et al. Considering respiratory tract infections and antimicrobial sensitivity: an exploratory analysis. *Malaysian J Microbiol* 2009;5:109–12.
- van der Zalm MM, van Ewijk BE, Wilbrink B, et al. Respiratory pathogens in children with and without respiratory symptoms. *J Pediatr* 2009;154:396–400.
- Casadevall A, Pirofski LA. Host–pathogen interactions: basic concepts of microbial commensalism, colonization, infection, and disease. *Infect Immun* 2000;68:6511–8.
- Wertheim HF, Melles DC, Vos MC, et al. The role of nasal carriage in *Staphylococcus aureus* infections. *Lancet Infect Dis* 2005;5:751–62.
- O'Brien KL, Wolfson LJ, Watt JP, et al. Burden of disease caused by *Streptococcus pneumoniae* in children younger than 5 years: global estimates. *Lancet* 2009;374:893–902.
- Williams EJ, Lewis J, John T, et al. *Haemophilus influenzae* type b carriage and novel bacterial population structure among children in urban Kathmandu, Nepal. *J Clin Microbiol* 2011;49:1323–30.
- Watt JP, Wolfson LJ, O'Brien KL, et al. Hib and Pneumococcal Global Burden of Disease Study Team. Burden of disease caused by *Haemophilus influenzae* type b in children younger than 5 years: global estimates. *Lancet* 2009;374:903–11.
- Faden H, Duffy L, Wasielewski R, et al. Tonawanda/Williamsville Pediatrics. Relationship between nasopharyngeal colonization and the development of otitis media in children. *J Infect Dis* 1997;175:1440–5.
- Principi N, Marchisio P, Schito GC, et al. Risk factors for carriage of respiratory pathogens in the nasopharynx of healthy children. Ascanius Project Collaborative Group in Turkey. *Int J Clin Pract* 2003;57:686–8.
- García-Rodríguez JA, Fresnadillo Martínez MJ. Dynamics of nasopharyngeal colonization by potential respiratory pathogens. *J Antimicrob Chemother* 2002;50 Suppl S2:59–74.
- Shet A, Kaplan E. Addressing the burden of group A streptococcal disease in India. *Indian J Pediatr* 2004;71:41–8.
- Koneman EW, Allen SD, Janda WM. *The enterobacteriaceae*. In *Color atlas and textbook of diagnostic microbiology*. 5th edn. Philadelphia: JB Lippincott Co, 2006:211–302.
- Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 2011;31:42–6.
- Goto H, Shimada K, Ikemoto H, et al. Antimicrobial susceptibility of pathogens isolated from more than 10000 patients with infectious respiratory diseases: a 25-year longitudinal study. *J Infect Chemother* 2009;15:347–60.
- Lebon A, Moll HA, Tavakol M, et al. Correlation of bacterial colonization status between mother and child: the Generation R Study. *J Clin Microbiol* 2010;48:960–2.
- Ayyildiz A, Aktas AE, Yazgi H. Nasopharyngeal carriage rate of *Haemophilus influenzae* in children aged 7–12 years in Turkey. *Int J Clin Pract* 2003;57:686–8.
- Peter G, Smith AL. Group A streptococcal infections of the skin and pharynx (second of two parts). *N Engl J Med* 1977;297–365.
- Jadhav PE, Meshram SA, Borkar RS, et al. A study on pattern of organisms in throat swab culture and their sensitivity to antibiotics in patients of RIMS, Adilabad, Andhra Pradesh. *Int J Biol Med Res* 2013;4:2915–9.
- Nandi S, Kumar R, Ray P, et al. Group A streptococcal sore throat in a periurban population of northern India: a one-year prospective study. *Bull World Health Organ* 2001;79:528–33.
- Ndip RN, Ntiege EA, Ndip LM, et al. Antimicrobial resistance of bacterial agents of the upper respiratory tract of school children in Buea, Cameroon. *J Health Popul Nutr* 2008;26:397–404.
- Wang F, Zhu DM, Hu FP, et al. Surveillance of bacterial resistance among isolates in Shanghai in 1999. *J Infect Chemother* 2001;7:117–20.
- Jonaidi Jafari N, Ranjbar R, Haghi-Ashtiani MT, et al. The study of prevalence and antimicrobial susceptibility of tracheal bacterial strains isolated from pediatric patients. *Pak J Biol Sci* 2009;12:455–8.
- Liebowitz LD, Slabbert M, Huisamen A. National surveillance programme on susceptibility patterns of respiratory pathogens in South Africa: moxifloxacin compared with eight other antimicrobial agents. *J Clin Pathol* 2003;56:344–7.
- Coles CL, Sherchand JB, Khatri SK, et al. Nasopharyngeal carriage of *S. pneumoniae* among young children in rural Nepal. *Trop Med Int Health* 2009;14:1025–33.
- Yadav JK, Farooq U, Begum R, et al. Isolation and identification of organism from throat swab along with sensitivity pattern from the patients in North India. *Ann Int Med Den Res* 2015;1:268–71.
- Lee S, Kim JH, Kim SH, et al. Prevalent multidrug-resistant nonvaccine serotypes in pneumococcal carriage of healthy Korean children associated with the low coverage of the seven-valent pneumococcal conjugate vaccine. *Osong Public Health Res Perspect* 2013;4:316–22.
- Mackenzie GA, Leach AJ, Carapetis JR, et al. Epidemiology of nasopharyngeal carriage of respiratory bacterial pathogens in children and adults: cross-sectional surveys in a population with high rates of pneumococcal disease. *BMC Infect Dis* 2010;10:304.
- Mbelle N, Huebner RE, Wasas AD, et al. Immunogenicity and impact on nasopharyngeal carriage of a nonavalent pneumococcal conjugate vaccine. *J Infect Dis* 1999;180:1171–6.
- Dagan R, Melamed R, Muallem M, et al. Reduction of nasopharyngeal carriage of pneumococci during the second year of life by a heptavalent conjugate pneumococcal vaccine. *J Infect Dis* 1996;174:1271–8.
- Devi U, Borah PK, Mahanta J. The prevalence and antimicrobial susceptibility patterns of beta-hemolytic streptococci colonizing the throats of schoolchildren in Assam, India. *J Infect Dev Ctries* 2011;5:804–8.
- Dhakar R, Sujatha S, Parija SC, et al. Asymptomatic colonization of upper respiratory tract by potential bacterial pathogens. *Indian J Pediatr* 2010;77:775–8.

33. Gazi H, Kurutepe S, Sürücüoğlu S, *et al.* Antimicrobial susceptibility of bacterial pathogens in the oropharynx of healthy school children in Turkey. *Indian J Med Res* 2004;120:489–94.
34. Rijal KR, Dhakal N, Shah RC, *et al.* Antibiotic susceptibility of group A Streptococcus isolated from throat swab culture of school children in Pokhara, Nepal. *Nepal Med Coll J* 2009;11:238–40.
35. Bahadur KC, Sharma D, Shrestha MP, *et al.* Prevalence of rheumatic and congenital heart disease in schoolchildren of Kathmandu valley in Nepal. *Indian Heart J* 2003;55:615–8.
36. Al-Hadramy MS, Al-Tahawy AT, Shafi MS. Acute lower respiratory tract infection in Jeddah. *Saudi Med J* 1988;9:34–9.
37. Howard AJ, Dunkin KT, Millar GW. Nasopharyngeal carriage and antibiotic resistance of Haemophilus influenzae in healthy children. *Epidemiol Infect* 1988;100:193–203.
38. García-Rodríguez JA, Fresnadillo Martínez MJ. Dynamics of nasopharyngeal colonization by potential respiratory pathogens. *J Antimicrob Chemother* 2002;50 Suppl S2:59–74.
39. Williams EJ, Lewis J, John T, *et al.* Haemophilus influenzae type b carriage and novel bacterial population structure among children in urban Kathmandu, Nepal. *J Clin Microbiol* 2011;49:1323–30.
40. Nweze EI, Ezute S, Emeka NCC, *et al.* Bacteria etiological agents causing respiratory tract infections in children and their resistance patterns to a panel of ten antibiotics. *Asian Pac J Trop Dis* 2012;2:18–23.
41. Khare M, Yadav N, Patidar RK, *et al.* Antibigram assessment against upper respiratory tract infection causing bacteria. *IJPSR* 2012;3:4827–30.
42. World Health Organization, Expanded programme on Immunization (EPI). Diphtheria endemic in the newly independent states of the former USSR. *Wkly Epidemiol Rec* 1996;71:245–50.
43. Gazi H, Surucuoglu S, Ozbakkaloglu B, *et al.* Oropharyngeal carriage and penicillin resistance of Neisseria meningitidis in primary school children in Manisa, Turkey. *Ann Acad Med Singapore* 2004;33:758–62.