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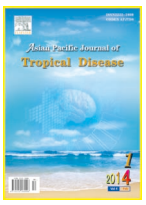
Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd



Document heading doi:10.1016/S2222-1808(14)60312-1

Carrier state of *Haemophilus influenzae* type b (Hib), *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Neisseria meningitidis* and *Corynebacterium diphtheriae* among school children in Pokhara, Nepal

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PEER REVIEW

Peer reviewer

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Comments

This study I think is good, especially for developing countries where vaccination program against all pathogens included in this study are in starting phase. Findings of the study are important for deciding whether vaccines are required or not. Results of the study are interesting and laboratory methods used are standard.
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ABSTRACT

Objective: To determine the incidence of carrier state of *Haemophilus influenzae* type b, *Streptococcus pneumoniae (S. pneumoniae)*, *Streptococcus pyogenes*, *Neisseria meningitidis* and *Corynebacterium diphtheriae* among school children.

Methods: Specimen from posterior pharyngeal wall and tonsils were collected on calcium alginate coated swabs from 102 participants. Processing of specimen and antimicrobial susceptibility testing was done by standard procedures.

Results: Potential pathogens isolated in our study were *S. pneumoniae* (14.7%), *Staphylococcus aureus* (12.7%), *Corynebacterium diphtheriae* (3.9%), *Streptococcus pyogenes* (3.9%) and *Haemophilus influenzae* (1.9%). Important findings in antibiogram include high resistance of *S. pneumoniae* to penicillin (73%) and resistance of *Staphylococcus aureus* to oxacillin (23%).

Conclusions: Pharyngeal colonization by *S. pneumoniae* among school children was found high and there is need of introduction of pneumococcal vaccines among children. Despite expected universal vaccination, pharyngeal colonization by *Corynebacterium diphtheriae* is possible and there is possibility of transmission.

KEYWORDS

Respiratory tract infections, School children, Bacterial colonization, Carriers

1. Introduction

Respiratory tract infections are the most common human infections and involve both upper and lower respiratory tract. Most of the upper respiratory tract infections are of viral etiology in which full recovery occurs without any complications while bacterial infections need specific antibiotic therapy for recovery as well as to prevent complications. Some important bacterial pathogens like *Haemophilus influenzae* type b (Hib), *Streptococcus pneumoniae (S. pneumoniae)*, *Streptococcus pyogenes (S. pyogenes)*, *Neisseria meningitidis (N. meningitidis)* and

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Foundation Project: This study is financially supported by Faculty of Science and Technology, Pokhara University, Kaski, Nepal (Grant No. 498/067/068).

Article history:
Received 14 Oct 2013
Received in revised form 22 Oct, 2nd revised form 28 Oct, 3rd revised form 4 Nov 2013
Accepted 10 Dec 2013
Available online 28 Feb 2014

Corynebacterium diphtheriae (C. diphtheriae) can lead to severe morbidity and mortality in childhood[1].

There are various sources of bacterial respiratory pathogens and one of these with the most importance includes healthy/convalescent carriers. Nasopharyngeal colonization by these pathogens is the first and important step in the pathogenesis.

Pneumonia among the respiratory diseases is considered as one of the leading causes of death among children in developing countries, with *S. pneumoniae* being one of the most important pathogenic species[2]. *S. pneumoniae* is responsible for an estimated 14.5 million episodes of serious disease and 826 000 deaths annually among children aged 1 to 59 months[3]. 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[4,5]. *N. meningitides*, *C. diphtheriae* and *S. pyogenes* can also have acute effects as well as long term sequel like rheumatic fever.

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. At present vaccines for prevention of meningococcal and pneumococcal infections are not available in Nepal. The requirement, benefits and success of vaccination program depends upon the prevalence of carrier state and risk factors associated with disease.

This study was undertaken to determine the incidence of carrier state of Hib, *S. pneumoniae*, *S. pyogenes*, *N. meningitidis* and *C. diphtheriae* among school children. The data was expected to reveal the risk of disease in children and need for vaccines for the population at risk. Some of the anticipated outcomes of the project were used to determine the need for introduction of vaccines against meningococcal and pneumococcal infections and to provide base line data in determining the effectiveness of vaccination programs.

2. Materials and methods

This study was conducted by the Department of Microbiology and Department of Community Medicine, MCOMS, Pokhara, Nepal. The project was supported by Pokhara University, Pokhara, Nepal, with the research grant. Permission of the Institutional Ethics and Research Committee was obtained before commencing this study. Informed consent from the parents/guardian of the participants was obtained.

2.1. Study population

The study population included two sets of school children. Group A comprised of 50 students aged 5-13 residing in hostels of boarding schools. Group B comprised of 52 day scholar students aged 5-14 residing at home.

2.2. Period of study

The study was carried out in two phases in different seasons. Phase 1 started from 19th December 2011 to 30th December 2011 and phase 2 from 28th June 2012 to 10th July 2012.

2.3. Inclusion and exclusion criteria

Students present in the selected schools on the days of specimen collection were randomly enrolled. Students who had any clinical features of infections or had taken any antibiotic treatment in preceding 15 d were excluded from the study.

2.4. Specimen collection and transport

Informed consent was obtained from the parents/guardians of the students. Selected students were examined for any evidence of upper respiratory tract infection. The findings were recorded as per the protocol. Specimens were collected only from the students who did not have any clinical evidence of infection with the above mentioned organisms. Specimen from posterior pharyngeal wall and tonsils were collected on calcium alginate coated swabs (Hi media) and labeled. These were protected from light and stored in Stuart Transport Media (Hi media) for transportation.

2.5. Specimen processing

After collection, swabs were transported to the laboratory within 2 h. The specimens were inoculated on 5% sheep blood agar, chocolate agar and potassium tellurite agar. Plates were incubated at 37 °C overnight in presence of 5%-10% CO_2. Plates were examined every 24 h for type of growth. The organisms were subcultured to obtain pure growth and processed. The plates were observed up to 96 h before discarding. Isolates were identified by standard microbiological methods and stored for further characterization/study[6]. Antibiotic susceptibility testing of all potential pathogens was performed.

3. Results

A total of 102 students from four private schools

participated in this study, of which 62 (60.8%) were male and 40 (39.2%) were female. Participants were in age group of 5–14 years. Majority of the participants were in age group of 10–14 years (79.4%). None of the participants included in our study had any type of illness on the date of specimen collection and none of them had received antibiotic therapy in preceding 15 d. All the participants were found to be vaccinated with Bacille Calmette Guerin which was confirmed by observing the scar at deltoid region and none of them were vaccinated with pneumococcal or *Haemophilus* vaccine.

Details of the isolated organisms from throat swab samples are shown in Table 1. Well known commensal, *Viridans streptococci* and *Moraxella* spp. were isolated from majority of participants. Potential pathogens isolated in our study were *S. pneumoniae* (14.7%), *Staphylococcus aureus* (*S. aureus*) (12.7%), *C. diphtheriae* (3.9%), *S. pyogenes* (3.9%) and *Haemophilus influenzae* (*H. influenzae*) (1.9%). *N. meningitidis* was not found in any of the participants.

Table 1
Organisms isolated.

Organism		Number	Percentage
Gram positive bacteria	*Viridans streptococci*	82	80.4
	S. pneumoniae	15	14.7
	S. pyogenes	4	3.9
	Beta hemolytic streptococci (non group A)	10	9.8
	S. aureus	13	12.7
	C. diphtheriae	4	3.9
Gram negative bacteria	*Moraxella* species	53	60.0
	Klebsiella pneumoniae	4	3.9
	Pseudomonas aeruginosa	1	1.0
	Enterobacter species	1	1.0
	Citrobacter species	2	1.9
	Acinetobacter species	3	2.9
	Haemophilus influenzae	2	1.9
	Haemophilus parainfluenzae	2	1.9

Antibiogram of isolated pathogens is shown in Table 2. Important findings in antibiogram included high resistance of *S. pneumoniae* to penicillin (73%) and resistance of *S. aureus* to oxacillin (23%). *S. pyogenes* were sensitive to all the antibiotics tested. One isolate of *C. diphtheriae* was resistant to penicillin while two isolates were resistant to erythromycin.

Table 2
Antibiotic resistance pattern of *S. aureus*, *Streptococcus* species and *C. diphtheriae* (%).

Organism	P	E	CF	CI	OX	CZ	G
S. pneumoniae	73.0	13.0	6.6	6.6	--	--	--
S. pyogenes	00.0	00.0	00.0	00.0	--	--	--
Beta hemolytic streptococci (non group A)	00.0	00.0	00.0	00.0	--	--	--
S. aureus	92.3	46.1	15.3	15.3	23.0	15.3	15.3
C. diphtheriae	25.0	50.0	--	25.0	--	--	00.0

P: Penicillin, E: Erythromycin, CF: Ciprofloxacin, CI: Ceftriaxone, C: Chloramphenicol, OX: Oxacillin, CZ: Cefazolin, G: Gentamicin.

4. Discussion

Human nasopharynx is colonized by wide spectrum of microorganisms from commensal bacteria to potential and opportunistic pathogens. Review of literature has revealed only few studies about carrier states of these pathogens in Nepal, therefore this study was conducted to determine the prevalence of carrier state of important respiratory bacterial pathogens among school children in Pokhara, Nepal. In our study, *S. pneumoniae* was found to be the commonest bacterial pathogen followed by *S. aureus*, *C. diphtheriae*, *S. pyogenes* and *H. influenzae*. Various studies have demonstrated that these organisms were major respiratory pathogens for school children[7–9].

Review of literature revealed that carrier rate of bacterial agents varies greatly from place to place. Previous similar studies on school children have demonstrated that carrier rate of *S. pneumoniae* ranged from 3.5% in Italy to as high as up to 90% in Gambia[10]. Similar study conducted by Coles *et al.*, which has shown very high colonization rate of *S. pneumoniae* up to 80% in healthy children of Sarlahi, Nepal[11]. This clearly showed great variation in the colonization rate of pathogens in different geographical location. Possible reason for this variation could be associated with socioeconomic status, standard of living and overcrowding of population. Our study population included private urban school students (higher socioeconomic group) while other studies were conducted on rural population (lower economic group). This perhaps is an important reason for the differences in results of our and other studies.

Studies on carrier rate of *H. influenzae* have demonstrated similar results to case of *S. pneumoniae*. Pharyngeal carrier rate of *H. influenzae* varies globally, with low prevalence in Sweden (3%) and high prevalence up to 88% in Costa Rica[12]. William *et al.* reported 5% carrier rate of *H. influenzae* among the urban population of Kathmandu, Nepal, which is higher than our results[4]. The population studied by William *et al.* was from outpatient department patients of the hospital and is perhaps not a reflection of general population but a selected group. This result is comparable with the carrier rate of 3%–7% in United States and United Kingdom before introduction of routine infant immunization of Hib vaccines. Results of our study are comparable with the study by Gazi *et al.*, where the carrier rate of *H. influenzae* was 2.9%[13].

Another important pathogen isolated in our study is *S. pyogenes*, which was found in 3.9% of the participants and carrier rate is comparable with studies from India and Turkey by Dhakal *et al.* and Gazi *et al*[8,13]. Prevalence of rheumatic heart disease in school children in Nepal has been reported to be 1.2/1 000 population by Bahadur *et al.* while prevalence of that in developed countries is much

lower[14]. Primary pharyngeal colonization/infection by group A streptococci in children, if not treated or partially treated, may lead to rheumatic fever and subsequently cardiac complications like rheumatic heart disease. The higher prevalence of rheumatic disease in Nepal could be directly related to higher carrier state of group A streptococci in children. Beta hemolytic streptococci (non group A) carrier rate in our study is 9.8% which is comparable with study from India by Devi *et al*[15]. These organisms can cause local infections but not linked with chronic sequel.

Despite routine vaccination, *C. diphtheriae* was isolated in 3.9% of the cases. The vaccination with diphtheria toxoid protects from illness of diphtheria toxin but does not prevent colonization by *C. diphtheriae*. This showed that colonization in vaccinated children is likely and may lead to transmission of the organism to non-vaccinated population. Only few studies have been conducted to determine the prevalence of carrier state of *C. diphtheriae* and screening of large population is required to know the area wise prevalence of *C. diphtheriae* among the children. This also emphasizes that effective vaccination must continue to prevent occurrence of diphtheria disease. In former USSR, discontinuation of diphtheria vaccination had led to out breaks of diphtheria disease[16].

Interestingly, *N. meningitidis* was conspicuously absent in our study population. 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.

Higher number of respiratory pathogens was isolated from group A children who were staying in the hostel while less number of pathogens were isolated from group B children who were non hostellers. The sample size was small hence the statistical difference was not significant. Hostel children are more likely to have closer contacts in group as compared to non hostellers and there is higher risk of transmission of respiratory pathogens among them.

The authors conducted this study in two different seasons, winter and summer. More number of pathogens were isolated from group A participants during winter while less respiratory pathogens were isolated from group B participants during summer (P=0.32). This may be associated with more prevalence of respiratory pathogens during winter.

Important findings in antibiotic susceptibility pattern of the isolates include high resistance of *S. pneumoniae* to penicillin (73%) and isolation of oxacillin resistant *S. aureus* (23%). *S. pyogenes* and non group A streptococci were uniformly sensitive to all antibiotics including penicillin.

Pharyngeal colonization by *S. pneumoniae* among school children was found high and there is need of introduction of pneumococcal vaccines among children. Pharyngeal colonization by other respiratory pathogens such as *Haemophilus* species, *C. diphtheriae* and Beta hemolytic streptococci were comparatively less. Despite expected universal vaccination, pharyngeal colonization by *C. diphtheriae* is possible and there is possibility of transmission. The meningococci were not detected in our study. Thus, at present it does not seem to be need of introduction of meningococcal vaccine. Transmission of respiratory pathogens and occurrence of diseases among school children can be minimized by effective vaccination, screening and treatment of carriers.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

We would like to thank Pokhara University of their financial support, Department of Community Medicine and all the staffs and post graduate students of Department of Microbiology for their contribution. Last but not the least; we would like to thank the management and the students of the schools for participating in this study. This study was financially supported by Faculty of Science and Technology, Pokhara University, Kaski, Nepal (Grant No. 498/067/068).

Comments

Background

Respiratory tract infections are one of the most common human infections in all age group patients. Severity of respiratory tract infections varies depending upon the type of pathogen. Pharyngeal colonization by pathogenic and non pathogenic bacterial agents is common in children. Bacterial respiratory infections if not treated timely may lead to severe complications like rheumatic heart disease, glomerulonephritis, meningitis *etc*. Many of these infections can be prevented by vaccination.

Research frontiers

This study was done to determine carrier state of

bacterial pathogens from pharynx of school children. Important bacterial pathogens were included in this study which includes Hib, *S. pneumoniae*, *S. pyogenes*, *N. meningitidis* and *C. diphtheriae*.

Related reports

Similar type of study conducted in rural area of Nepal by Coles *et al*. showed very high carrier rate of *S. pneumoniae* (80%). In this study, carrier rate of *S. pneumoniae* (14.7%) is very less as compared to above study; this may be associated with low economic status of rural population. Many studies have included only one pathogen, while this study covered almost all common bacterial respiratory pathogens.

Innovations & breakthroughs

Almost all important respiratory bacterial pathogens were included in this study, which are not only associated with respiratory infection but also with systemic infections. Isolation of *C. diphtheriae*, *S. pyogenes*, *S. pneumoniae* and *H. influenzae* are important findings.

Applications

Findings of this study would be important in minimizing the transmission of these pathogens by screening treatment of carriers. Findings may also be utilized for introducing new vaccines depending upon the carriers rates at different population and determining the effectiveness of vaccination programs.

Peer review

This study I think is good, especially for developing countries where vaccination program against all pathogens included in this study are in starting phase. Findings of this study are important for deciding whether vaccines are required or not. Results of this study are interesting and laboratory methods used are standard.

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