SUPPLEMENT ARTICLE

Hospital-Based Surveillance of Invasive Pneumococcal Disease among Young Children in Urban Nepal

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Background. *Streptococcus pneumoniae* is a leading cause of pneumonia and meningitis in young children. Before implementation of the pneumococcal conjugate vaccine in developing countries, there is an urgent need to provide regional epidemiological data on pneumococcal disease. The aims of this study were to determine the prevalence and serotype distribution of invasive pneumococcal disease among young children hospitalized in urban Nepal.

Methods. Children aged 2 months to 5 years who were admitted to Patan Hospital, Kathmandu, with fever and/or suspected pneumonia, meningitis, or bacteremia were recruited. Blood culture specimens were collected from all participants. In cases of suspected meningitis, cerebrospinal fluid specimens were cultured and were tested for *S. pneumoniae* antigen.

Results. A total of 885 children were recruited during the 21-month study period. Of these, 76 (9%) had meningitis and 498 (56%) had pneumonia, on the basis of clinical criteria. Radiographically confirmed pneumonia occurred in 354 (40%), and probable or definite meningitis occurred in 47 (5%). *S. pneumoniae* was isolated in specimens from 17 (2%) of the children. Serotypes 1 and 12A were isolated most frequently, and only 1 of 17 isolates had a serotype contained in the currently available 7-valent pneumococcal conjugate vaccine.

Conclusions. More than 60% of children aged <5 years who were admitted with fever and/or suspected invasive bacterial disease in urban Nepal had the clinical syndromes of meningitis and/or pneumonia. A new generation of pneumococcal vaccines that prevent infection with a broader range of serotypes may be necessary to most effectively control pneumococcal disease in young children in Kathmandu.

Pneumonia is the leading cause of death in children worldwide, accounting for >19% of the 10 million childhood deaths each year [1], and the majority of cases occur in resource-poor countries. *Streptococcus pneumoniae* is the most common cause of pneumonia in infants and young children and is also a major cause of meningitis and bacteremia in this age group. Since the development of the pneumococcal conjugate vaccine, there has been a global effort to provide local data to plan vaccine-implementation strategies, led by the GAVI Alliance's Pneumococcal Vaccines Accelerated Development and Introduction Plan (PneumoADIP) [2]. In 2007, the World Health Organization recommended that pneumococcal conjugate vaccine should be a priority for inclusion in national childhood immunization programs [3]. Active surveillance of preventable pneumococcal disease was encouraged but should be combined with serotype identification, to guide the choice of pneumococcal vaccine.

Nepal, one of the world's poorest nations, has a very high mortality rate for children aged <5 years, estimated to be 61 deaths per 1000 live births [4]. Pneumonia is among the top 3 causes of death for this age group. Although the importance of pneumococcal disease has been demonstrated in febrile adults in Nepal [5], there are limited published data on the etiology of serious bacterial infections in Nepalese children. Although studies have identified *S. pneumoniae* as a cause of childhood meningitis [6, 7], the local epidemiology of disease caused by this organism is unknown.

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In Nepal, pneumococcal conjugate vaccine is not currently part of the local Extended Programme of Immunization. Although the vaccine is currently available in the private market in most developing countries, only a very small number of families are able to afford it, and at the time of the study, it was unavailable in Nepal. There is growing international support for expansion of the use of vaccines globally, and Nepal is listed as 1 of the 72 countries eligible for financial assistance from the GAVI Alliance. Accurate data concerning the clinical syndromes of pneumonia and meningitis in children and the local epidemiology of disease that is caused by vaccine-preventable infection are required to aid policy makers in their decisions concerning local implementation of the vaccine in Nepal.

The aims of this study were to determine the prevalence of invasive pneumococcal disease among young children hospitalized in urban Nepal and to describe the local serotype distribution and antibiotic-susceptibility profiles of invasive pneumococcal isolates.

METHODS

Setting. Patan Hospital is 1 of only 2 large hospitals in Kathmandu Valley that accepts pediatric referrals and has pediatric inpatient facilities. In 2001, Kathmandu Valley had a total population of 1,645,091, and ~129,784 (7.9%) were children aged <5 years [8]. The other pediatric hospital (Kanti Children's Hospital) admits ~3 times more children than does Patan Hospital. Therefore, the estimated population of children aged <5 years served by Patan Hospital is ~32,500 children. Although this population is mainly urban, Patan Hospital admission records from 2006 showed that 10% of all admissions were from outside the Valley, mostly from rural communities with limited access to health care.

Nepal is a low-income country where about one-third of people are estimated to be living below the poverty line [9, 10]. Access to care differs considerably with geographical setting and other factors, but only one-quarter of children aged <5 years who have pneumonia are taken to a health care facility [11]. The prevalence of malnutrition among children is high; ~50% of children have height and weight measurements 2 SDs below the expected values for their age [12]. HIV antibody seroprevalence among the adult population is low (estimated to be 0.5% in 2005 [13]). No information is available for the prevalence among children.

Sample population. All children aged 2–59 months (inclusive) who were admitted to the pediatric ward at Patan Hospital from April 2005 through December 2006 with fever (temperature, ⩾38°C) and/or a possible clinical diagnosis of meningitis, pneumonia, or septicemia were considered for entry into the study. Demographic and clinical data were collected by local clinical research officers after informed consent was obtained and then were entered into a standardized database. Exclusion criteria were the absence of blood culture specimens collected at admission, hospitalization for any illness within the previous 10 days, and presentation with recurrent wheezing or acute gastroenteritis alone.

Laboratory methods. Full blood counts were performed and blood culture specimens were obtained from all eligible children 24 h per day, 7 days per week, by hospital personnel and study staff. Blood samples were collected before antibiotic administration, whenever possible. The vast majority of blood culture specimens were collected into commercially prepared blood culture bottles (BACTEC Peds Plus/F; Becton Dickinson), but some patients were admitted after a blood culture specimen had already been collected using locally prepared culture bottles. Lumbar punctures were performed at the discretion of the admitting clinician. However, the routine practice at Patan Hospital is to perform lumbar punctures for all children with suspected meningitis, unless the child was medically unstable or there was evidence of increased intracranial pressure. Study patients received standard medical care from the hospital pediatric team.

Blood culture bottles were incubated at 35°C and were inspected twice daily for turbidity. All samples had subcultures performed, irrespective of turbidity, at 12–24 h and 7 days of incubation. CSF samples were directly plated onto sheep blood and chocolate agar and were incubated at 35°C in a carbon dioxide–enriched environment for 24 h. If a CSF sample had a WBC count >5 cells/mm^3, then an immunochromatographic test of pneumococcal antigen (NOW *Streptococcus pneumoniae* Antigen Test; Binax) was performed. Samples from other sterile sites were cultured according to routine hospital practice. Isolation and identification of *S. pneumoniae* was performed using standard microbiological tests. All *S. pneumoniae* isolates were sent to the microbiology laboratory at Christian Medical College (Vellore, India) for confirmation, serotype identification, and antibiotic-susceptibility testing by MIC determination. Serotyping was performed using established methods [14], and Clinical and Laboratory Standards Institute guidelines were used for antibiotic-susceptibility testing [15].

Case definitions. The definitions of clinical syndromes and final diagnoses are listed in table 1. These definitions are based on criteria established by PneumoADIP investigators (see the Appendix in this supplement), with minor alterations for the data collected among our population. Chest radiograph findings were reported by the local clinical team or radiologists. Data on recruitment and consent were collected during the last 12 months of the study.

Ethics approval. Ethics approval was obtained from Oxford Tropical Research Ethics Committee (OXTREC 032–06) and the Nepal Health Research Council.

Table 1. Definitions of clinical syndromes, final diagnoses, and modifications from PneumoADIP definitions.

Syndrome or diagnosis, definition	Modification from PneumoADIP
Meningitis clinical syndrome	Prostration and toxic appearance are not included in the definition
Sudden onset of fever (at <48 h) and ⩾1 of the following:	
Stiff neck	
Altered/reduced level of consciousness	
Bulging fontanelle (for children aged <12 months)	
Lethargy	
Convulsions	
Any convulsion (for children aged <6 months)	
Focal, prolonged, or recurrent seizures (for children aged <6 months)	
Petechial or purpuric rash	
Pneumonia clinical syndrome	History of cough or difficulty breathing are not included in the definition
Tachypnea	
Respiration rate >50 breaths/min for children aged 2 months to <12 months	
Respiration rate >40 breaths/min for children aged 12 months to <5 years	
Purulent CSF	
One of the following criteria:	
Turbid CSF	
WBC count ⩾100 cells/mm^3	
WBC count 10–99 cells/mm^3 and glucose level <40 mg/dL or protein level >100 mg/dL	
Abnormal CSF	
WBC count 10–99 cells/mm^3 and glucose and protein levels normal or not measured	
Meningitis	
Definite	
One of the following criteria:	
Positive CSF culture	
Purulent CSF and positive blood culture or positive Gram stain	
Abnormal/purulent CSF and positive results of antigen test (NOW *Streptococcus pneumoniae* Antigen Test; Binax)	
Probable	
One of the following criteria and not definite meningitis:	
Purulent CSF	

Normal CSF and positive results of antigen test (NOW *Streptococcus pneumoniae* Antigen Test; Binax)	
Abnormal CSF and positive blood culture or positive CSF Gram stain	
Suspected	
Meningitis clinical syndrome and not definite or probable meningitis	
Pneumonia	
Tachypnea or positive findings of chest radiograph and no danger signs of very severe pneumonia and no indrawing	
Very severe pneumonia	Inability to breast-feed or drink, vomiting everything, and severe respiratory distress/ poor respiratory effort are not included in the definition
Tachypnea and ⩾1 of the following:	
Central cyanosis	
Convulsions	
Lethargy	
Severe pneumonia	
Tachypnea and chest indrawing but no danger signs of very severe pneumonia	
Very severe disease	Inability to breast-feed or drink and vomiting everything are not included in the definition
Presence of ⩾2 of the following danger signs, without a diagnosis of meningitis or pneumonia:	
Convulsions	
Lethargy	
Severe malnutrition (identified by weight-for-age *z* score less than −3.00)	
Hypothermia (temperature, ⩽36°C)	
Bacteremia	
Pathogenic bacteria isolated from blood but child does not have meningitis, pneumonia, or very severe disease	

Table 2. Number of cases caused by various organisms, with use of standard case definitions.

Case type	Total no. of cases (no. of deaths)	No. of cases (no. of deaths) with no culture growth or with contaminant	No. of cases (no. of deaths) with *Streptococcus pneumoniae* identified in blood or CSF	No. of cases with other organisms identified in blood
Clinical syndrome[a]				
Meningitis	76 (1)	71 (1)	3	2
Pneumonia	498 (8)	486 (8)	4	8
Diagnosis[a]				
Meningitis				
All	110 (2)	97 (1)	8 (1)	5
Definite	9 (1)	0	8 (1)	1
Probable	38	36	0	2
Suspected	63 (1)	61 (1)	0	2
Pneumonia				
All	498 (8)	486 (8)	4	8
CXR confirmed, very severe	28 (2)	27 (2)	0	1
Probable, very severe	25	25	0	0
CXR confirmed, severe	229 (3)	225 (3)	2	2
Probable, severe	78 (3)	77 (3)	0	1
CXR confirmed	97	92	2	3
Probable	41	40	0	1
Very severe disease	8 (1)	5 (1)	0	3
Bacteremia	19	0	5	14
Other	250 (1)	250 (1)	0	0
Total	885 (12)	838 (11)	17 (1)	30

NOTE. CXR, chest radiograph.

[a] By modified PneumoADIP criteria.

RESULTS

During the 21-month study period, 3903 patients were admitted to the Patan Hospital pediatric ward. Of these, 1106 (28%) were eligible for recruitment and, of the 1106 eligible, 885 (80%) were recruited for enrollment in the study. The reasons that 20% of eligible children were not recruited include refusal of consent, blood culture specimens not collected or lost in transport, and death before recruitment took place. Blood culture specimens were obtained from all 885 children, CSF samples were obtained from 199 children, and culture samples from other sterile sites were obtained from 10 children (5 pleural fluid, 2 subdural empyemas, and 3 other pus samples). The age range of the 885 recruited children was 2 months to <5 years (median age, 12 months; 47% were aged <12 months, and 71% were aged <24 months), and 497 (56%) were male. With a weight-for-age z score cutoff of -2 (Epi Info, version 2002; Centers for Disease Control and Prevention), 324 (37%) of 885 children for whom data were available were underweight. On the basis of parental reporting, 348 (39%) of the children had received antibiotic therapy in the 48 h before admission.

Clinical syndromes and diagnoses. The clinical syndromes and final diagnoses are listed in table 2. Of the 885 patients, 76 (9%) had meningitis clinical syndrome, of whom 13 (17%) had a final diagnosis of definite or probable meningitis with use of the defined criteria. Of note, 34 (72%) of the 47 with a final diagnosis of probable or definite meningitis were missed by the meningitis clinical syndrome criteria, mostly because they had a history of fever lasting >2 days. Pneumonia clinical syndrome was more common, with 498 patients (56%) presenting with tachypnea. Of the CSF samples obtained, 66 (33%) were purulent with a WBC count $\geqslant$10 cells/mm^3. Positive findings of chest radiograph were confirmed for 354 (71%) of patients with pneumonia and were found for 40% of the entire study population of children. Although this finding was 7.5 times more frequent than that of probable or definite meningitis, a pathogen was found for the patients with meningitis 8 times more often than for patients with radiographically confirmed pneumonia (23% vs. 3%).

Microbiologically confirmed pneumococcal disease. Table 3 shows the results of microbiological investigations according to sample type. Of the 885 children, 44 (5%) had blood cultures positive for a pathogenic organism; 16 (36%) of the 44 cultures were positive for *S. pneumoniae.* Of the 199 CSF

samples cultured, 9 (4.5%) yielded a pathogenic organism, 7 (78%) of which were positive for *S. pneumoniae*. The CSF sample from 1 child was culture negative but had positive results of the immunochromatographic antigen test, and *S. pneumoniae* was isolated from the blood culture. There were no additional cases of pneumococcal meningitis identified by the immunochromatographic antigen test alone, which was performed on 58% of CSF specimens.

Among the 17 children with culture-confirmed pneumococcal disease, there was an almost-even sex distribution (8 females and 9 males). The majority (47%) of children with confirmed pneumococcal disease were aged <1 year (figure 1). On the basis of census data for Kathmandu Valley and some additional assumptions (i.e., an estimated 10% of patients come from outside the valley, and 80% of eligible patients were recruited), the estimated crude annual incidence rate of confirmed pneumococcal disease was 34 cases per 100,000 children aged <5 years.

Twelve children who were enrolled in this study died during their hospital stay (table 2). One of them had confirmed pneumococcal meningitis; none of the others had any pathological organisms identified.

The most common serotypes identified were 1 and 12A (figure 2). All the isolates were susceptible to penicillin, whereas only 52% were susceptible to trimethoprim-sulfamethoxazole (cotrimoxazole) (figure 3).

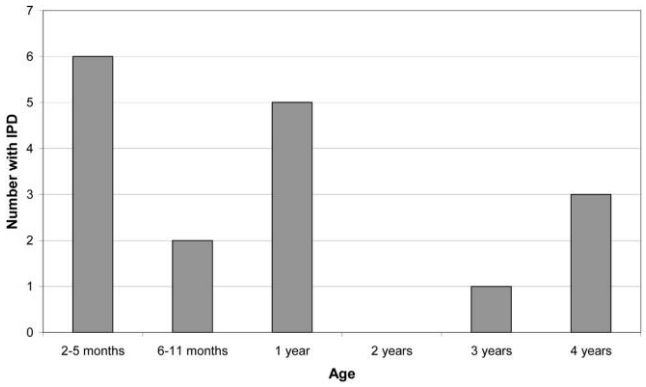


Figure 1. Age distribution among children with confirmed invasive pneumococcal disease (IPD).

DISCUSSION

This study has provided new data about the local epidemiology and serotype distribution of invasive pneumococcal disease at an urban hospital in Kathmandu and is relevant to vaccine policy in Nepal. The vast majority of young children admitted to Patan Hospital with febrile illness and/or suspected pneumonia, meningitis, or septicemia had clinical syndromes of pneumonia or meningitis. *S. pneumoniae* was the most commonly identified organism, causing both pneumonia and meningitis, and despite a high rate of antibiotic use before hospital admission, was identified in specimens from 1.9% of all patients in this study. These findings are broadly similar to those from Kanti Children's Hospital that are reported elsewhere in this supplement [16]. Notable differences between study findings include higher proportions of patients with documented pneumococcal bacteremia and reported prehospital antibiotic use and lower proportions of patients with pneumonia clinical syndrome and with definite meningitis at Patan Hospital, compared with at Kanti Children's Hospital. The lower proportion of children who received a diagnosis of definite meningitis at Patan Hospital reflects, in part, the lower threshold for performance of lumbar punctures at this hospital (22% of patients, compared with 12% of patients at Kanti Children's Hospital). Other differences likely reflect differences in the patient pathway and handling of samples (i.e., blood culture media) at the 2 institutions.

The percentage of children with confirmed pneumococcal bacteremia in our study is similar to that reported for rural Kenya, where 1.8% of hospitalized children aged >60 days had *S. pneumoniae* isolated from blood cultures [17]. However, the Kenyan study included all pediatric admissions and had rates of plasma antimicrobial activity lower than our reported rate

Table 3. Pathogen identification, stratified by identification method.

Specimen, method	Total no. of isolates	No. (%) of specimens positive for any pathogen	No. (%) of specimens positive for *Streptococcus pneumoniae*	No. (%) of specimens positive for contaminant[a]
Blood, culture	885	44 (5.0)	16 (1.8)	66 (7.5)
CSF				
Culture	199	9 (4.5)	7 (3.5)	2 (1.0)
Antigen test[b]	115	8 (6.9)	8 (6.9)	…
Other fluids	10	3 (30)	2 (20)	…

[a] Organisms were considered to be a contaminant if they were unlikely to have caused illness in the clinical setting—that is, multiple organisms, coagulase-negative staphylococcus, and micrococcus.
[b] An immunochromatographic test of pneumococcal antigen (NOW *S. pneumoniae* Antigen Test; Binax).

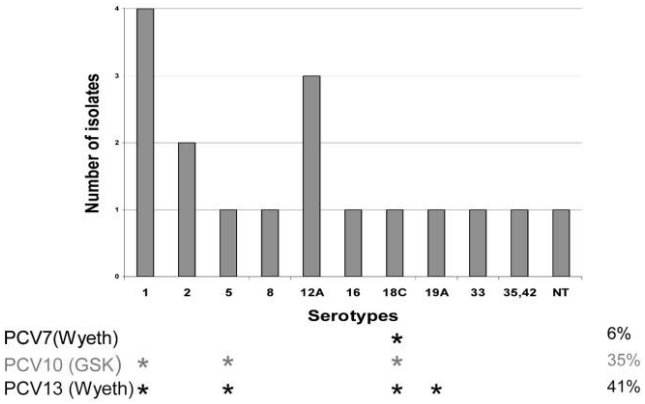


Figure 2. Distribution of the 17 serotypes identified and the percentage of serotypes covered by 7-valent (PCV7), 10-valent (PCV10), and 13-valent (PCV13) pneumococcal conjugate vaccines. NT, nontypeable.

of prehospital antibiotic use. In our study, only 3% of children who received a diagnosis of radiographically confirmed pneumonia and 23% of the children who received a diagnosis of definite or probable meningitis had identifiable causative organisms.

In an attempt to estimate the epidemiology of disease caused by these encapsulated bacteria, standardized syndromic definitions have been developed, such as those established by PneumoADIP investigators. We found that the vast majority of our patients presented with defined pneumonia or meningitis clinical syndromes. Respiratory syndrome, defined as pneumonia clinical syndrome, was seen in more than half of the children in this study. One in 12 children in the study population was classified as having meningitis clinical syndrome on clinical review. However, we experienced limitations in using these standardized clinical definitions for these syndromes. For example, we found that 121 (26%) of the children with chest radiograph changes consistent with pneumonia did not fulfill the criteria for pneumonia clinical syndrome because they did not have tachypnea and that 34 (72%) of the 47 probable or definite meningitis diagnoses were not identified by the meningitis syndromic definition because the patients were not febrile at presentation.

Very severe disease occurred relatively infrequently (1% of admissions), but 38% of the patients with very severe disease had confirmed bacteremia. Lumbar puncture was not included as the sole criterion for very severe disease because there was a low threshold for this investigation in our study setting. A total of 59 additional children (7% of admissions) would have been placed in this category by this criterion alone if it had been included. Despite the fact that 110 children (12%) were admitted with meningitis, very severe pneumonia, or very severe disease, only 12 (1.4%) of the children died. However, because some hospitalized children with suspected sepsis presented at night or in extremis, some children died before recruitment could occur; this may have resulted in substantial underreporting. Although only 1 child who died had *S. pneumoniae* identified microbiologically, the majority (83%) of the children who died had a study diagnosis of meningitis or pneumonia that could reflect pneumococcal disease.

Studies of pneumococcal conjugate vaccine use in infancy show a 20%–30% reduction in the number of cases of radiographically confirmed pneumonia in various settings [18–20]. Such studies show a greater reduction in incidence of disease defined by clinical criteria (such as clinical or radiographic findings of pneumonia) than in the incidence of disease confirmed by culture. In our study, although only 1% of the cases of radiographically confirmed pneumonia had a blood culture positive for *S. pneumoniae,* data from a pneumococcal intervention trial in The Gambia suggest that the true prevalence of pneumonia caused by pneumococcus is likely to be at least 7 times higher [19]. Our reported rates of culture-positive pneumococcal disease are also likely an underestimation of the true rates in Kathmandu, in view of the high rate of prehospital antibiotic use.

The majority of the pneumococcal isolates in this study were serotypes 1 and 12A. Neither of these is covered by the currently available 7-valent pneumococcal conjugate vaccine, which would cover only 6% of the serotypes identified in this study. The newer vaccines that are currently in development provide broader coverage, but even the 13-valent vaccine in advanced development would cover just above 40% of the serotypes of pneumococci isolated at Patan Hospital during the study period. This rate of vaccine coverage of *S. pneumoniae* serotypes

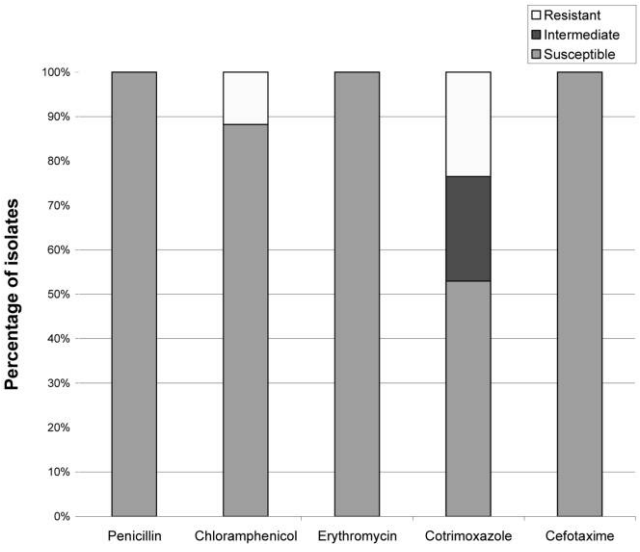


Figure 3. Antibiotic-susceptibility patterns of the pneumococcal isolates.

is much lower than that found in studies in Africa [17, 19, 20]. However, our isolate population is currently small and will be combined with other local data for the determination of vaccine policy in the region. It is also possible that the high rate of prehospital antibiotic use may have selectively influenced the serotype distribution of cultured isolates.

Of the small number of *S. pneumoniae* isolates tested for antibiotic susceptilibity, all were fully susceptible to penicillin, whereas only half were susceptible to cotrimoxazole, which is a current first-line antibiotic treatment for childhood pneumonia in Nepal. This pattern is similar to the antibiotic-susceptibility profile of pneumococci found in Kanti Children's Hospital [16] and reflects a trend of increasing antimicrobial resistance that has been reported in other Asian studies [21–23]. The use of cotrimoxazole as a first-line antibiotic treatment for pneumonia in this setting should be reconsidered.

As in similar studies, we were able to confirm only a minority of cases of pneumococcal disease, because of the limitations of diagnostic tests. Consequently, we have almost certainly underestimated the incidence of pneumococcal disease in this population. We studied a hospitalized population in an urban setting. Only 15% of the population of Nepal live in urban areas [12], and the epidemiology of pneumococcal disease may be different in remote rural areas of the country. The incidence of pneumonia is likely to be higher among children in rural areas of Nepal; among children aged <5 years, an estimated 30%–40% of deaths are due to pneumonia, and the annual expected rate of cases of pneumonia is calculated to be 30% of the population [24].

This study and the other contemporaneous study at Kanti Children's Hospital [16] have clearly shown that there is a significant burden of pneumonia, meningitis, and pneumococcal disease among young children hospitalized in urban Nepal. Although our sample size is limited, the serotypes of the pneumococcal strains isolated differ substantially from those in the currently available 7-valent pneumococcal conjugate vaccine. If this observation is confirmed in larger studies, a new generation of higher-valency pneumococcal vaccines may be required for there to be a significant impact on rates of disease in Nepal.

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