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The burden of vaccine-preventable invasive bacterial infections and pneumonia in children admitted to hospital in urban Nepal

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SUMMARY

Background: Protein–polysaccharide vaccines have made a significant impact on the burden of disease caused by encapsulated bacteria such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Neisseria meningitidis*, and have the potential to do so for Salmonella Typhi. Nepal is one of many resource-poor nations with limited information on the epidemiology of childhood infections caused by these pathogens.
Methods: Over a 21-month period, we studied children aged ≤12 years admitted to an urban hospital in Nepal with suspected bacteremia, meningitis, or pneumonia. Patan Hospital is a non-profit hospital with the second largest pediatric unit in the Kathmandu Valley.
Results: Of 2039 children enrolled in the study, 142 (7.5%) included in the analysis had positive blood cultures. The agents of enteric fever, Salmonella Typhi and Salmonella Paratyphi, accounted for 59/142 (42%) of all bacteremias and were the most frequently cultured pathogens in children ≥1 year of age. *S. pneumoniae* was isolated in 16% of positive blood cultures and was the most common cause of bacteremia in children <1 year of age. Pneumonia accounted for 51% of admissions in children ≥2 months, with 44% of these children having radiographically defined primary endpoint pneumonia. *S. pneumoniae* was the most commonly identified pathogen in cases of pneumonia and meningitis. The *S. pneumoniae* serotype distribution indicated that the 10-valent and 13-valent pneumococcal conjugate vaccines would cover 44% and 47%, respectively, of all *S. pneumoniae* cultured from blood or cerebrospinal fluid (CSF) isolates and 62% and 67%, respectively, of isolates associated with pneumonia. *H. influenzae* type b was isolated infrequently from blood or CSF cultures, but is likely to be more important as a cause of pneumonia.
Conclusions: The data on the burden of invasive bacterial infections and pneumonia from this study suggest that vaccines in development against Salmonella Typhi and the pneumococcus have the potential to significantly improve the health of children in Nepal.

1. Introduction

Encapsulated bacteria, such as *Streptococcus pneumoniae*, *Haemophilus influenzae* type b (Hib), *Neisseria meningitidis*, and *Salmonella* species, are principal causes of bacteremia, pneumonia, and meningitis in childhood. Although meningitis and bacteremia are diseases with high rates of mortality, they are relatively low incidence. In contrast, pneumonia is one of the most important causes of child death, causing over 2 million deaths annually in children under 5 years of age.[1,2]

The development of efficacious protein–polysaccharide conjugate vaccines against *S. pneumoniae*, Hib, *N. meningitidis*, and against enteric (typhoid) fever provides an opportunity to reduce the burden of these diseases in children. The World Health Organization (WHO) has recommended that vaccines against *S. pneumoniae* and Hib should be a priority for introduction into

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Table 1
Clinical features used to identify afebrile children with suspected pneumonia or invasive bacterial disease for inclusion in the study. Derived from South Asian Pneumococcal Alliance (SAPNA) clinical standard operating procedures

Pneumonia	Severe illness/sepsis	Meningitis
• rapid breathing • lower chest wall indrawing • cyanosis/hypoxemia • physician diagnosis of pneumonia clinically *or* • X-ray	• inability to drink • lethargic or unconscious • hypothermia <36 °C • physician diagnosis of septicemia	• bulging fontanelle • stiff neck • reduced level of consciousness • convulsion • physician diagnosis of meningitis

immunization schedules for all countries,[3,4] and that large-scale vaccination of children may be required to control enteric fever in endemic areas.[5] The Global Alliance for Vaccines and Immunisation (GAVI) has initiated specific programs and new funding mechanisms in order to accelerate the introduction of both *S. pneumoniae* and Hib vaccines into the developing world.[6] Studies of invasive bacterial infection in children have shown geographic variation in the most prevalent pathogens,[7–9] making the acquisition of accurate regional data on disease burden a key component in directing public health priorities.

Nepal is a low income country in South Asia with a population of 25.8 million people and an estimated infant mortality rate of 61/1000 live births.[10] Access to health care varies considerably within the country, but as few as 18% of children under 5 years of age with pneumonia are taken to health care facilities.[11] There are only limited data on the relative burden of disease due to bacterial pathogens in Nepali children.

The principal aims of this study were to prospectively assess the burden of community-acquired invasive bacterial disease and pneumonia in children admitted to a hospital in urban Nepal.

2. Methods

2.1. Setting

Patan Hospital is a non-profit hospital with revenue generated from patient charges. It is one of two large hospitals in Kathmandu with pediatric referral and inpatient services. In 2001, the Kathmandu Valley had a total population of approximately 1 645 091 people, with about 129 784 of these being children under 5 years of age.[12] During the study period, pneumococcal conjugate vaccines were not available in the country. Vaccines against Hib and typhoid were available within the private market, but were not widely used.

2.2. Recruitment

Children recruited into the study were those aged ≤12 years who had been admitted to the pediatric ward at Patan Hospital with fever (>38 °C) or who were afebrile but had a possible clinical diagnosis of meningitis, pneumonia, or septicemia. Standard clinical criteria were used to identify children with these conditions (Table 1). At Patan Hospital the usual clinical practice involved blood culture for all such individuals. Written informed consent was obtained and then demographic and clinical data were collected by research officers and recorded on standard forms. Exclusion criteria were the absence of a blood culture, hospitalization for any illness in the 10 days prior to the admission, and transfer directly from the neonatal unit. The study was approved by the Nepal Health Research Council and Oxford Tropical Research Ethics Committee (OXTREC 026-04).

2.3. Laboratory methods

Blood cultures were taken from all enrolled children, as per the routine clinical practice within the hospital. Lumbar punctures were performed at the discretion of the attending clinician. All medical care was given by the hospital pediatric team according to usual clinical practice. Every effort was made to ensure the prompt transport of samples to the laboratory for incubation or, in the case of cerebrospinal fluid (CSF) samples, direct plating. The microbiology laboratory was within the hospital, close to the clinical areas, and samples were collected and processed at all times of the day.

Blood culture bottles were incubated at 35 °C and inspected twice daily for turbidity. All samples had sub-cultures irrespective of turbidity at 12–24 h and after 7 days of incubation. CSF samples were directly plated onto sheep blood and chocolate agar and incubated at 35 °C in a CO_2 enriched environment for 24 h. CSF samples with $>5 \times 10^6$ white cells/l were tested by the NOW® *Streptococcus pneumoniae* antigen test (Binax, Inc., Scarborough, ME, USA). Isolation and identification of microorganisms were performed using standard microbiological tests. Isolates were stored in Protect cryovials (Technical Service Consultants, Lancashire, UK) at −70 °C. Isolates of *S. pneumoniae* and Hib were sent to the South Asian Pneumococcal Alliance (SAPNA) central laboratory in Vellore, India for confirmation, serotype identification, and antibiotic susceptibility testing. Serotyping was performed within the Vellore laboratory using the Quellung method. The Patan microbiology laboratory participated in a regular quality assurance process directed by the SAPNA central laboratory in Vellore.

Urine samples taken at admission were frozen at −70 °C and subsequently tested for antibiotic activity as described previously.[13]

CSF samples were stored at −70 °C prior to shipping to the Health Protection Laboratory (Manchester, UK). Here, capsular transport (*ctrA*), capsulation (*bexA*), and pneumolysin (*ply*) gene targets specific for *N. meningitidis*, Hib, and *S. pneumoniae*, respectively, were tested for by PCR using previously described methods.[14]

Serum samples remaining after normal clinical care in 485 children were anonymized and stored at −70 °C. These were subsequently tested for the presence of anti-HIV antibody (Determine HIV-1/2, Inverness Medical, Cranfield, UK) and positive results were confirmed by Western blotting.

2.4. Radiography

Chest radiographs were taken according to usual clinical practice, which included all patients with pneumonia suspected on clinical grounds. Portable X-ray facilities were available for very sick children. Digital images of all radiographs were read by a radiologist in Oxford, UK using the standardized WHO criteria.[15] Radiographs were classified as showing: (1) primary endpoint consolidation (likely bacterial pneumonia) – dense fluffy consolidation of a portion of a lobe or entire lung, which may be associated

with a pleural effusion; (2) other consolidation or infiltrate – the presence of non-endpoint infiltrate in the absence of a pleural effusion; or (3) no consolidation/infiltration or pleural effusion. Radiograph quality was also assessed according to standardized WHO criteria to determine whether it was (1) sufficient to ascertain any of the outcomes described above ('acceptable'), (2) only sufficient to ascertain the presence or absence of primary endpoint consolidation ('suboptimal'), or (3) unreadable.

2.5. Nutritional status

Weight for age *Z*-scores were calculated for each subject (EpiInfo 2002; Centers for Disease Control and Prevention, Atlanta, GA, USA). Moderate or severe malnutrition was defined as a *Z*-score <-2 and severe malnutrition was defined as a *Z*-score <-3.

2.6. Statistical analysis

Dichotomous and ordinal variables were tested by the Chi-square test. The associations between bacteremia and malnutrition and between bacteremia and pre-hospital antibiotic use were estimated by Mantel–Haenszel odds ratios, stratified by age in four strata: <60 days, 60–364 days, 1–5 years and >5 years. Data were analyzed using Stata version 8.2 (StataCorp, College Station, TX, USA).

2.7. Role of the funding source

The study sponsor had no role in the study design, in the collection, analysis, and interpretation of data, in the writing of the report, or in the decision to submit the paper for publication.

3. Results

3.1. Recruitment and demographics

A total of 2039 children were enrolled in the study between April 1, 2005 and December 31, 2006. Information on total ward admissions was only available from April 24, 2005 to December 30, 2006 (88 of 91 weeks of the study). During that period 2335 children were eligible for recruitment, constituting 62% of total admissions; 85.8% of those eligible were enrolled in the study. Consequently 332 children were eligible but not enrolled. Of these, 19 died before consent could be taken, 67 refused consent, and 130 had no blood culture taken; for 116 the reason for non-recruitment was not recorded. Overall, 1166 of 2039 recruited children were male (57%). Amongst 2017 children with a recorded weight, 730 (36%) had moderate or severe malnutrition and 304 (15%) had severe malnutrition. There were 36 in-hospital deaths (2% of the 2039 children recruited) and 28 of these were children ≤1 year of age. Amongst these children, the clinical diagnosis was meningitis or encephalitis in three (8%), pneumonia in 17 (47%), septicemia in 11 (31%), and a variety of other diagnoses in five children (14%). Only one of the 485 serum samples tested (0.2%) was positive for HIV antibodies, and this was from a child aged <18 months.

3.2. Bacteremia

Blood cultures from 142 of the 2039 children (7%) grew organisms considered to be contaminants. These patients were excluded from further analysis of bacteremias. Of the remaining 1897 patients, bacteremia was identified in 142 (7.5%) (Table 2). Where recorded, blood cultures were collected into BACTEC™ PEDS PLUS™/F blood culture bottles (BD, Shannon Ireland) in 84% of children and locally prepared culture bottles in 16%. The locally prepared culture bottles performed at least as well as the BACTEC™ PEDS PLUS™/F blood culture bottles in terms of overall prevalence of identification of bacteremia, but were more likely to grow organisms considered to be contaminants (4.8% vs. 17%).

The prevalence of bacteremia increased with increasing age ($p < 0.0001$). The agents of enteric fever, Salmonella Typhi (53 isolates) and Salmonella Paratyphi (6 isolates), were the most common pathogens isolated in children ≥1 year old (59/142 (42%)). *S. pneumoniae* was the next most frequently cultured organism and was the single most frequently cultured organism in children <1 year of age; all except one episode in this age group was associated with meningitis. After adjusting for age group, there was no association between moderate or severe malnutrition and bacteremia (odds ratio (OR) 0.92, 95% confidence interval (CI) 0.64–1.33).

All S. Typhi and S. Paratyphi isolates were susceptible to ampicillin, chloramphenicol, and ciprofloxacin, but 39 (66%) were resistant to nalidixic acid. Only one *S. pneumoniae* isolate had reduced susceptibility to penicillin (minimum inhibitory concentration (MIC) 0.125 mg/ml).

Table 2
Positive blood cultures by age group. For each organism the total number of positive blood cultures is given for each age group together with the percentage that this represents from among all positive cultures within that age group

Pathogen	Age					Rank proportion
	0–59 days	60–364 days	≥1–5 years	>5 years	Total	
Total blood cultures (excluding contaminants)	504	491	653	249	1897	
Total positive blood cultures (% of total blood cultures)	22 (4.4)	31 (6.3)	59 (9.0)	30 (12.0)	142 (7.5)[a]	
Positive cultures by organism (% total positive blood cultures within each age group)						
Gram-positive						
Streptococcus pneumoniae	0	9 (29)	7 (12)	6 (20)	22	2
Staphylococcus aureus	3 (14)	3 (10)	2 (3)	3 (10)	11	3
Group A streptococci	3 (14)	1 (3)	1 (2)	0	5	–
Group B streptococci	1 (5)	0	0	0	1	–
Others[b]	6 (27)	2 (6)	2 (3)	2 (7)	12	–
Gram-negative						
Haemophilus influenzae	0	1 (3)	1 (2)	0	2	–
Salmonella Typhi/Paratyphi	2 (9)	4 (13)	37 (63)	16 (53)	59	1
Escherichia coli	1 (5)	3 (10)	0	0	4	–
Acinetobacter species	3 (14)	3 (10)	1 (2)	1 (3)	8	4
Other Gram-negative rods[c]	4 (18)	7 (23)	8 (14)	2 (7)	21	–

[a] Polymicrobial bacteremia in three patients, hence the number of isolates is greater than the number of patients with bacteremia.
[b] Species included Enterococcus (9), *Streptococcus parasanguis* (1), *Aerococcus viridans* (1), non-hemolytic Streptococcus (1).
[c] Species included Pseudomonas (3), Serratia (4), Enterobacter (8), non-typhoidal Salmonella (4), *Stenotrophomonas maltophilia* (1), Klebsiella (1).

Table 3
Odds ratios (with 95% confidence intervals) for bacteremia in children with evidence of pre-hospital antibiotic use, adjusted for age

	Reported antibiotic use ($n = 2013$)	Urinary antibiotic activity ($n = 457$)
Any bacteremia	1.07 (0.73–1.57)	0.91 (0.45–1.86)
Typhoidal salmonella bacteremia	2.82 (1.60–4.99)	1.73 (0.71–4.26)
Pneumococcal bacteremia	0.23 (0.07–0.80)	0.40 (0.05–3.00)

Some 32% of children had a history of antibiotic use within 48 h of admission, and this was more common in children ≥60 days old (41%) compared with those <60 days old (8%) ($p < 0.01$). Pre-hospital antibiotic use, as assessed by parental history or urine antibiotic activity on admission, was analyzed for its effect on the odds of detection of bacteremia (Table 3). There was no association between pre-hospital antibiotic use and all-cause bacteremia. However, pre-hospital antibiotic use, as assessed by parental history, was associated with a reduced odds of pneumococcal bacteremia and increased odds of Salmonella bacteremia. Analysis of 457 urine samples collected on admission and prior to in-hospital antibiotic treatment, demonstrated that in the 311 children with no history of antibiotic use, 98 (32%) had detectable urinary antibiotic activity.

3.3. Meningitis

CSF samples were obtained for microbiological culture from 700 patients with suspected meningitis or encephalitis. Sufficient CSF was available for additional pneumococcal antigen testing in 84% and for PCR in 74% in samples with a CSF white cell count >5 × 10^6/l. There was a discharge diagnosis of meningitis or encephalitis in 23 out of 379 children (6%) <60 days of age and 101 out of 321 children (32%) ≥60 days of age. In these 124 children a bacterial pathogen was identified in 19 (15%), with *S. pneumoniae* the most common organism (Table 4). Out of the 124 children, 70 had a CSF sample collected after the administration of antibiotics in hospital. For a history of administration of antibiotics prior to hospital admission there was no discernible effect on CSF culture

Table 4
CSF culture/pneumococcal antigen testing/PCR results according to CSF white cell count

Age	No. of cases						
	No. of CSF specimens	CSF white cell count distribution (cases with pathogen identified), ×10^6/l			Organism		
		<10	≥10–99	≥100	Sp[a]	Hib[b]	Nm[c]
0–59 days	379	304 (1[d])	56 (0)	19 (2)	1	1	1
60–364 days	100	67 (1[d])	17 (2)	16 (7)	8	1	1
≥1–5 years	149	109 (0)	16 (0)	24 (5)	2	1	2
>5 years	72	36 (0)	14 (1)	22 (2)	3	0	0
Total	700	516 (2)	103 (3)	81 (16)	14	3	4

CSF, cerebrospinal fluid; PCR, polymerase chain reaction; Sp, *Streptococcus pneumoniae*; Hib, *Haemophilus influenzae* type b; Nm, *Neisseria meningitidis*.
Total numbers of bacteria identified for each group are for a combination of CSF culture, BINAX antigen testing (for *S. pneumoniae*) and PCR for Sp, Hib and Nm.
Contribution of different methods of detection: [a]*S. pneumoniae*: CSF culture eight cases, BINAX additional four cases, PCR additional two cases; [b]*H. influenzae* type b: CSF culture three cases, PCR 0 cases; [c]*N. meningitidis*: CSF culture 0 cases, PCR additional four cases. [d]Two children had a pathogen identified by PCR only, but had <10 × 10^6/l white cells in the CSF and did not have a final diagnosis of meningitis or encephalitis: a 2-week infant with a diagnosis of pneumonia with CSF PCR positive for *S. pneumoniae* and an 11-month-old with meningococcal septicemia with CSF PCR positive for *N. meningitidis*.

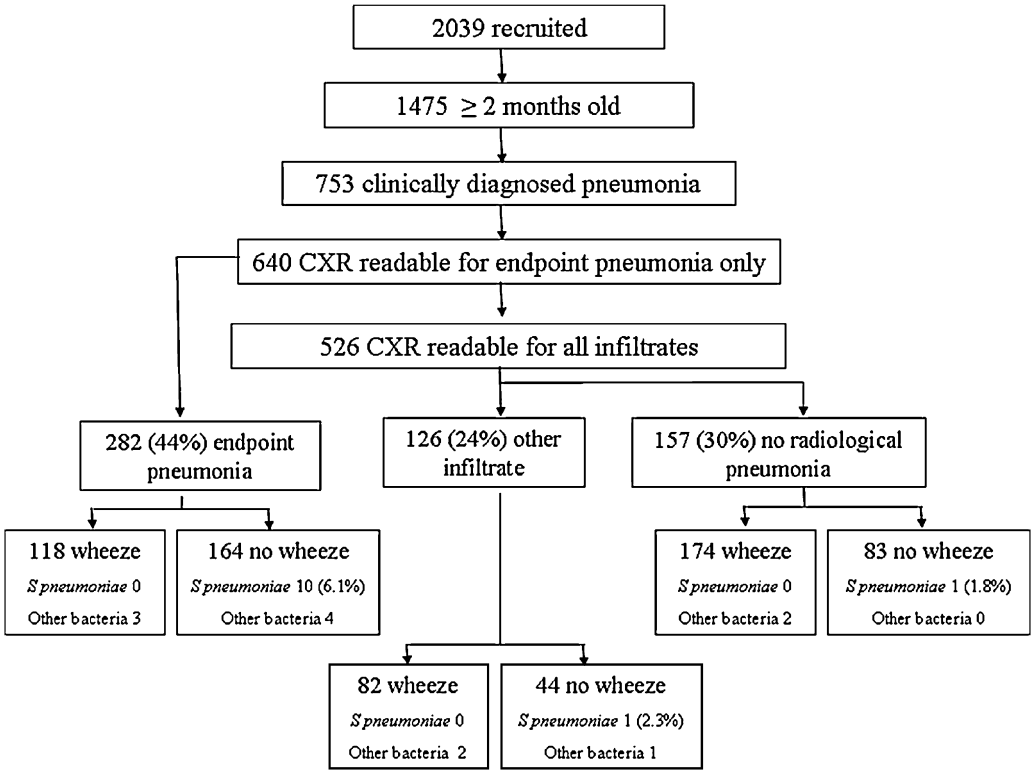

Figure 1. Flow diagram to illustrate the number of children with a clinical diagnosis of pneumonia and the associated blood culture results and chest X-ray (CXR) readings by standardized World Health Organization criteria. CXRs of both 'acceptable' and 'suboptimal' quality ($n = 640$) could be assessed for the presence of endpoint pneumonia, whereas only those with 'acceptable' quality could be assessed for less obvious infiltrates or for the absence of any infiltrate ($n = 526$). The percentages of children with 'endpoint pneumonia' are therefore given using a different denominator (640) to those children with 'other infiltrate' or 'no radiological pneumonia' (526).

Table 5
Serotype distribution of blood or CSF pneumococcal isolates from children with and without meningitis[a]

Pneumococcal serotype	Number of isolates of *Streptococcus pneumoniae*		
	Non-meningitis, blood culture	Meningitis, blood/CSF culture	Total
1	10	0	10
12A	1	3	4
5	2	0	2
9A	2	0	2
9V	1	1	2
2	0	1	1
8	1	0	1
15A	0	1	1
16	0	1	1
18C	0	1	1
19A	1	0	1
19C	0	1	1
23B	0	1	1
25F	1	0	1
33	0	1	1
NT	2	2	4
Vaccine coverage			
PCV7	1/21 (5%)	2/13 (15%)	3/34 (9%)
PCV10	13/21 (62%)	2/13 (15%)	15/34 (44%)
PCV13	14/21 (67%)	2/13 (15%)	16/34 (47%)

CSF, cerebrospinal fluid; NT, non-typeable; PCV, pneumococcal conjugate vaccine (7-, 10-, and 13-valent).
Serotypes are ordered by frequency of individual serotypes from combined meningitis and non-meningitis cases. The data include an additional 10 isolates from January 1, 2007 to September 30, 2007 in addition to the 24 from the study period described in the current paper. Four CSF isolates had a blood culture *S. pneumoniae* isolate with different serotype (2, 9A, 25F and 25F) from that of CSF (NT, 9 V, 12A and 19C, respectively).
[a]In the original study period 22 children had pneumococcal bacteremia (Table 2) and this group included eight children with meningitis. A total of 14 children had evidence of pneumococcal meningitis, from positive CSF culture ($n=8$), negative culture but positive Binax ($n=4$), or negative culture and Binax but positive PCR ($n=2$) (Table 4). Only two of the children with CSF culture-positive pneumococcal meningitis were not bacteremic and thus provided an additional two individuals with pneumococcal isolates for serotyping in addition to the children with bacteremia alone. This provided a total of 24 pneumococci from cases of invasive disease for serotyping. A further 10 isolates were available from continued surveillance in 2007, three from cases of meningitis and seven from non-meningitis bacteremia.

positivity (age-adjusted OR 1.18; 95% CI 0.25–5.72). However there was a reduction in CSF culture positivity for samples collected after the administration of antibiotics in hospital (age-adjusted OR 0.22; 95% CI 0.05–1.01).

3.4. Pneumonia

A clinical discharge diagnosis of pneumonia was made in 753 of the 1475 (51%) children ≥60 days old, the group most likely to have pneumonia due to *S. pneumoniae* or Hib, with 44% of these children having radiographically defined primary endpoint pneumonia. Digital copies of admission chest radiographs of sufficient quality were available for 640 (85%) of these children, as shown in Figure 1. In children with endpoint pneumonia (consolidation or pleural effusion) and no wheezing, 6% had pneumococcal bacteremia. No child with a clinical diagnosis of pneumonia and wheezing had pneumococcal bacteremia. There were no isolates of Hib from children with a primary clinical diagnosis of pneumonia.

Isolates of *S. pneumoniae* were obtained from 24 separate children during the study period, and a further 10 were collected during an additional 12 months of surveillance. Serotype data for these isolates are shown in Table 5, together with the proportion covered by various pneumococcal conjugate vaccine formulations.

4. Discussion

This study has demonstrated a significant burden of enteric fever, mainly due to Salmonella Typhi, and pneumonia, of which a large proportion is likely to be caused by *S. pneumoniae*, amongst children admitted to hospital in Kathmandu, Nepal. A subset of these data, involving 885 children, has been published separately in a supplement on pneumococcal disease.[16]

There are few systematic studies of bacteremia from resource-poor countries in Asia. Among children aged 1–15 years in Laos, Salmonella Typhi accounted for 60% of blood culture isolates, followed by *Staphylococcus aureus* (17%) and enteric Gram-negative bacilli (16%), with *S. pneumoniae* making up only 2% of isolates.[8] Studies from Africa are heavily influenced by high prevalences of HIV infection and are notable for a high incidence of non-typhoidal Salmonella bacteremia, accounting for between 7% and 30% of isolates in different studies.[7,17–20] This contrasts with the higher incidence of infection with typhoidal salmonellae in South Asia.[8]

The current study demonstrated a high prevalence of Salmonella Typhi bacteremia. However this is an underestimate of the true burden of enteric fever in Kathmandu, where this disease is predominantly managed in the outpatient or community setting. Population-based studies in Asia estimate that only 9% of enteric fever cases are hospitalized.[21] It is increasingly recognized that Salmonella Typhi is an important pathogen of young children.[21,22] In the current study, 73% of culture-confirmed cases of enteric fever were in children <5 years old and 28% in children <2 years old. Although improvements in sanitation and water supply would lead to a reduction in disease burden, such changes are unlikely to occur in the short term and immunization is a major prevention strategy. Currently available typhoid vaccines are only recommended for use in children ≥2 years old.[5,22] The development of improved typhoid vaccines for use in young children is a high priority for many countries in Asia.[23,24]

S. pneumoniae was the most common cause of bacteremia in children under 1 year of age, where it was almost always associated with meningitis, and overall the second most common cause of bacteremia. *S. pneumoniae* was also the most common pathogen identified causing meningitis. Pneumonia is one of the leading causes of childhood mortality in children ≤5 years of age,[2] and *S. pneumoniae* is one of the most important pathogens. In the present study, over 50% of children ≥2 months old had a discharge diagnosis of pneumonia; however it is more difficult to establish the etiology of bacterial pneumonia than of meningitis. A trial of a 9-valent pneumococcal conjugate vaccine in The Gambia indicated that 7.5 times the number of cases of radiographic pneumonia were prevented for every one culture-positive case.[25] In our study, 6% of children with endpoint pneumonia and no wheeze had a blood culture positive for *S. pneumoniae*. Using the same ratio of pneumonia cases prevented per culture-positive case would give 75 children in the current study who had pneumococcal pneumonia. This would represent 46% of all endpoint pneumonia without wheeze or 27% of all endpoint pneumonia. These figures are in keeping with the estimate of 20–35% from the vaccine probe studies.[26,27] Although the vaccine probe studies were conducted in children under the age of 5 years, in the present study the proportion of cases of radiographically confirmed pneumonia with a blood culture positive for *S. pneumoniae* was higher in those children >5 years of age compared to those under the age of 5 years, suggesting the continued importance of this organism in the older children.

Antibiotics are freely available within urban areas of Nepal and their use in children prior to admission to hospital might be expected to influence the detection of bacteremia. In the current study a history of pre-hospital antibiotic usage made it less likely

that *S. pneumoniae* would be isolated from blood culture. Whilst it cannot be excluded that this is due to the rapid deterioration of such children before there is time to administer antibiotics, this seems unlikely for a disease such as pneumonia. These data suggest that the estimates derived above are a minimal estimate of hospital disease burden.

The serotype distribution of pneumococcal isolates from the current study and the following 12 months (Table 5) indicates that the 10- and 13-valent pneumococcal conjugate vaccines would provide coverage of around half of all cases of invasive pneumococcal disease in children ≤12 years of age. Importantly the proportion of isolates covered is much greater for the pneumococci isolated from children with pneumonia (62–67%) as opposed to those with meningitis (15%). Although the latter is a more severe illness, the incidence of pneumonia, the majority of which is non-bacteremic, is far higher. More deaths occurred from pneumonia than from meningitis during the study period. Recent estimates of the global disease burden due to *S. pneumoniae* reinforce the importance of the disease burden due to pneumonia. For Southeast Asia it is estimated that the annual incidence of pneumonia is 2911 cases per 100 000 children under 5 years of age with 169 000 deaths per year in comparison with meningitis for which the estimates are 13 cases per 100 000 and 13 700 deaths per year.[28]

Although there has been controversy over the importance of Hib as a cause of invasive disease in Asia,[29] Hib has been identified as a cause of pneumonia, bacteremia, and meningitis in many regions of South Asia.[30,31] In the present study Hib constituted 2% of all bacteremias. This is a similar prevalence to that recorded in Laos,[8] but is in contrast to the documented prevalence in Africa where Hib constitutes 9–14% of bacteremias.[7,17,20] It is unclear whether this difference reflects actual differences in the incidence of Hib disease between the different populations.[32] The estimated incidence of Hib meningitis varies widely by global region, ranging from 16 to 46 cases per 100 000 children <5 years of age per year with an estimate of 27 per 100 000 for Southeast Asia.[33] There were only a small number of cases of Hib bacteremia and all were associated with a diagnosis of meningitis. This is consistent with several studies that have shown that Hib bacteremia with pneumonia occurs less frequently than cases of meningitis.[34–37]

It is unclear whether pre-hospital antibiotic usage affected the number of Hib isolates, as the number of cases was relatively low.[38] However, studies of Hib carriage in children in Kathmandu have indicated similar levels of nasopharyngeal carriage to those seen in areas where there is a high prevalence of disease.[39] Despite the severity of meningitis, the greatest burden of Hib disease is likely to be due to its role as an agent of pneumonia where it is more difficult to establish an etiological agent.

The information on Hib from the current study is similar to that at the other major pediatric unit in Kathmandu.[40] At Kanti Hospital, Hib was the second most common isolate cultured from CSF in children aged 2 months to 5 years after *S. pneumoniae*, and there were no episodes of Hib bacteremia in cases of meningitis. Although the incidence of Hib bacteremia associated with a primary diagnosis of pneumonia was too low to detect in the current study, the WHO has suggested using a ratio of 5:1 for estimating the burden of disease due to non-bacteremic Hib pneumonia from meningitis cases in children ≤2 years old.[41] In the current study this would give around 15 cases of Hib pneumonia from 55 children ≤2 years old with clinical and radiographic pneumonia and no wheeze. This figure of 27% of cases is within the range indicated by previous vaccine probe studies of 15–30% of radiographic pneumonia for children under 5 years of age.[26] If Hib were similarly prevalent in pneumonia in children aged 2–5 years, this would give 27 cases of Hib pneumonia in this age group.

Patan Hospital is a non-profit hospital with revenue generated from patient charges. Whilst this may bias the data in terms of the patient population that is seen, the hospital charges are small enough that they are not considered by local medical staff as a significant deterrent to presentation with an ill child. In terms of comparison to government hospitals, a study of pneumococcal and Hib disease undertaken at Kanti Hospital in Kathmandu as part of the SAPNA network had comparable mortality and pre-hospital antibiotic use reported.[40]

Vaccine preventable bacterial diseases are important causes of hospitalization in the children of Kathmandu. In this study meningitis and bacteremia due to Salmonella Typhi, *S. pneumoniae*, and Hib, together with estimates of pneumonia due to *S. pneumoniae* and Hib, indicate that over one in 10 of all children ≥2 months of age had diseases that in principal are preventable through immunization. These data estimate the incidence of disease severe enough to require hospitalization and substantially underestimate the true burden of these diseases in the community. Immunization programs directed against bacterial pathogens are likely to make a major impact on childhood illness in Nepal.

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