CHILDHOOD INVASIVE BACTERIAL DISEASE IN KATHMANDU, NEPAL, 2005–2013

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Nepal, invasive bacterial disease, epidemiology

ABSTRACT

Background

Invasive bacterial disease (IBD; including pneumonia, meningitis, sepsis) is a major cause of morbidity and mortality in children in low-income countries.

Methods

We analyzed data from a surveillance study of suspected community-acquired IBD in children <15 years of age in Kathmandu, Nepal from 2005–2013 prior to introduction of pneumococcal conjugate vaccines (PCV). We detailed the serotype-specific distribution of invasive pneumococcal disease (IPD), and incorporated antigen and PCR testing of CSF from children with meningitis.

Results

Enhanced surveillance of IBD was undertaken during 2005–2006 and 2010–2013. During enhanced surveillance, a total of 7956 children were recruited of whom 7754 had blood and/or CSF culture results available for analysis, and 342 (4%) had a pathogen isolated. From 2007–2009 all 376 positive culture results were available, with 259 pathogens isolated (and 117 contaminants). *Salmonella enterica* serovar Typhi was the most prevalent pathogen isolated (167 cases, 28% of pathogens), followed by *Streptococcus pneumoniae* (98 cases, 16% pathogens). Approximately 73% and 78% of pneumococcal serotypes were contained in 10-valent and 13-valent PCV, respectively. Most cases of invasive pneumococcal disease (IPD) were among children ≥5 years of age from 2008 onwards. Antigen and PCR testing of CSF for pneumococci, *Haemophilus influenzae* type b and meningococci increased the number of these pathogens identified from 33 (culture) to 68 (culture/antigen/PCR testing).

Conclusions

S. enterica serovar Typhi and *S. pneumoniae* accounted for 44% of pathogens isolated. Most pneumococcal isolates were of serotypes contained in PCVs. Antigen and PCR testing of CSF improves sensitivity for IBD pathogens.

INTRODUCTION

Severe infections, including invasive bacterial disease (IBD), remain the major cause of mortality in children <5 years of age worldwide[1]. The highest burdens of microbiologically-confirmed IBD (bacterial pathogens cultured from normally sterile sites[2]) are in low- and middle-income countries in Africa and Asia where both the incidence of disease, and absolute numbers of children are high[1]. In these settings Enterobacteriaceae, *Streptococcus pneumoniae*, *Haemophilus influenzae* type b and *Staphylococcus aureus* predominate[3-5]. Implementation of conjugate vaccines against *H. influenzae* type b and serotypes of *S. pneumoniae*[5], socio-economic changes[6], and the changing epidemiology of antimicrobial resistance[7] may affect the pathogen-specific IBD burden.

Culture of pathogens from blood and/or cerebrospinal fluid (CSF) provides important data on the pathogen-specific aetiology of bacterial infection in children. However, culture techniques have low sensitivity for pathogens, and significantly underestimate bacterial disease in children[6]. This may be due to the paucity of bacteria in blood, since bacteria may be constrained to non-blood tissue (e.g. the lung mucosa) in many cases of IBD, and is compounded by the treatment of children with antibiotics prior to blood sampling, and inherent limitations of culture techniques. Alternative techniques for the etiologic diagnosis pneumonia and bacteraemia in children, including antigen testing and molecular detection tests from blood, generally lack specificity or sensitivity or both[8]. However, these may be useful in meningitis, where the blood brain barrier inhibits translocation of bacterial fragments into CSF during healthy carriage or non-meningitis infection[9,10].

Surveillance of IBD in children provides important insight into the epidemiology of infection in children, may enhance clinical care by enabling targeted antibiotic prescribing, and knowledge of

serotype-specific invasive pneumococcal disease (IPD) enables rational implementation of pneumococcal conjugate vaccines (PCVs)[11]. Here, we describe the burden of community-acquired IBD in neonates, infants and children admitted to Patan Hospital, Nepal, extending previous analyses[12,13] and providing a focused analysis of IPD, over a 9-year period of surveillance 2005–2013. At present, Patan Hospital is the only site for this surveillance in Nepal. Data from 2014 onwards are to be incorporated into a multifactorial assessment of PCV impact on childhood pneumonia and pneumococcal disease in Nepal.

MATERIALS AND METHODS

Study Setting

These sentinel surveillance data are from a single centre, Patan Hospital in Kathmandu valley in Nepal. Patan Hospital serves as the main hospital for the local community, and is one of the main teaching and referral hospitals, with one of two paediatric critical care units, in the Kathmandu Valley. Nepal is a low-income country, with a mortality rate in children under five years of age of 37 per 1000 live births in 2016[14]. Until 2015, the Nepal infant immunisation schedule included diphtheria-tetanus-pertussis, bacille Camille-Guérin (BCG), hepatitis B virus, *H. influenzae* type b (Hib, from 2009), oral polio virus and measles virus vaccines. In 2011, 87% of children less than two years of age had received all of these vaccinations[15]. HIV prevalence in young adults was <0.5% through the study period[16].

Clinical Data Collection

From March 2005–end 2006 enhanced surveillance was undertaken with the enrolment of all children admitted to Patan Hospital with suspected community-acquired IBD; from 2007–2009

data were available from children with bacterial pathogens isolated from blood and/or other normally sterile sites; with a further period of enhanced surveillance from 2010–2013 (Figure 1). During periods of enhanced surveillance, children <15 years of age with a clinical diagnosis of suspected pneumonia, sepsis, or meningitis made by the admitting pediatrician were recruited (see Table, Supplemental Digital Content 1)[17-19]. Neonates born at our institution who developed infection prior to discharge home, and children with onset of fever ≥48 hour following hospital admission were excluded. Data were prospectively recorded on a specific central database.

Laboratory Methods

Samples for blood culture were taken on admission for children with clinically suspected IBD. Blood was cultured using Bactec Peds Plus culture bottles (Becton Dickinson, USA) and incubated aerobically in 5% CO_2 at 35–37 ºC (from 2009 onwards a Bactec automated system was used). Subculture of turbid samples was onto sheep blood and chocolate agar (blood samples) or Columbia agar plates containing 5% sheep blood (CSF samples) with organisms identified using standard microbiologic and biochemical methods. Six monthly audits of blood culture techniques and volumes were undertaken.

CSF samples with ≥5 white cells per mm^3 were also tested for pneumococcal antigens using BinaxNOW test (Alere Diagnostics, USA), and latex agglutination for *S. pneumoniae*, *N. meningitidis* and *H. influenzae* type b (Wellcogen, ThermoFisher, USA). Except in 2007 (when culture negative samples were not stored) CSF was stored at -80 ºC at Patan Hospital before being sent to the WHO IBD Regional Surveillance Program (Department of Microbiology, Christian Medical College, Vellore, India) on dry ice for molecular testing of *S. pneumoniae*, *N. meningitidis* and *H. influenzae* type b. Molecular testing was done using real-time polymerase

chain reaction (PCR) according to US Center for Communicable Disease Control and Prevention methods. During 2008–2009 CSF samples were analysed for only *S. pneumoniae* and *H. influenzae* type b by PCR.

Serotyping of pneumococci was undertaken using the Quellung method using serotype-specific sera to pneumococcal polysaccharides (Statens Serum Institute, Denmark) by experienced microbiologists at Patan Hospital using established guidelines[20], with additional quality control checks using Quellung serotyping at an independent institute (Oxford Vaccine Group, UK).

Data analysis

Data were cleaned and cross-checked in detail using Stata v.14 (StataCorp, USA). Descriptive clinical analyses were undertaken in *R*, including the packages of *tidyverse*.

We classified serotypes of pneumococci by their presence in commercially available PCVs, including 10-valent PCV (PCV10, Synflorix, GSK: polysaccharides 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F) and 13-valent PCV (PCV13, Prevnar 13, Pfizer: additional polysaccharides 3, 6A, 19A). For analysis of the use of culture, antigen testing and PCR testing for CSF pathogens, data are presented as additional numbers of pathogens identified by antigen testing and PCR in that order.

Negative blood and CSF cultures were not systematically recorded during 2007–2009. Therefore only cultures from which a pathogen or contaminant was isolated are reported for this period.

Ethical approval

Approval was obtained from the Nepal Health Research Council and Oxford Tropical Research Network Review Board (026-04) for the collection and presentation of anonymised data without consent.

RESULTS

Recruitment

During periods of enhanced surveillance (2005–2006 and 2010–2013), 7956 children were admitted to Patan Hospital with suspected IBD and were therefore recruited to the surveillance study. Of these children, 7754 had blood and/or CSF cultures available for analysis. Additional positive culture data were available for 376 children between 2007–2009. Therefore, of a total of 8332 children, 8130 (98%) had blood cultures taken and available for analysis (see Figure, Supplemental Digital Content 2) during the whole period 2005–2013. Of these 8130 children, 3296 (41%) were female; 3316 (41%) children were <6 months of age, 943 (12%) children were 6 months to 1 year of age, 1177 (14%) children were 1 year to <2 years of age, 1337 (16%) children were 2 to <5 years of age, and 1357 (17%) children were ≥5 years of age (Table 1). Numbers of total admissions to Patan Hospital were available for the period 2010–2013: of 7985 children admitted during this period, 5691 (71%) were admitted with suspected IBD (Figure 1). During the period 2010–2013, 1646 (29%) of children were reported as having received antibiotics prior to admission to hospital (see Tables Supplemental Digital Content 3 and 4).

Organisms isolated

During 2005–2006, a pathogen was identified in 146/2063 (7.0%) cases on whom a blood and/or CSF culture was done; during 2010–2013, a pathogen was identified in 194/5691 (3.4%) cases on whom a blood and/or CSF culture was done (Table 2).

In total 599 pathogens were isolated from 2005–2013. The most frequently isolated pathogen was *S. enterica* serovar Typhi (167 total cases; 1.6% blood cultures done during enhanced surveillance, or 28% of pathogens), followed by *S. pneumoniae* (98 total cases, 0.7% blood cultures done during enhanced surveillance, or 16% of pathogens). Other prevalent pathogens cultured from blood and/or CSF were viridans streptococci (58 cases, treated as pathogens in this setting), *Enterobacter* spp. (59 cases), *Acinetobacter* spp. (39 cases, treated as pathogens in this setting) and *S. aureus* (37 cases). Coagulase-negative staphylococci were isolated in 115 neonates (where there was uncertainty as to whether this represented a skin contaminant) and 161 older infants and children (almost none of whom had central venous catheters, and these were considered contaminants). *Haemophilus influenzae* type b was isolated once from blood following introduction of the Hib vaccine to the infant immunisation schedule in 2009 (in 2012 from a child 12 years of age).

Childhood IPD at Patan Hospital 2005–2013

Number of isolates and age distribution

S. pneumoniae was the second most prevalent pathogen cultured from blood and/or CSF in the cohort. Demographics of children with IPD are shown in Table 1. Of 98 children with IPD, 39 (40%) were female. The median age of children with IPD was 3.4 years (interquartile range, IQR, 0.9–6.9). A total of 26 (26%) children with IPD were <1 year of age, 10 (10%) children were between 1 and <2 years of age, 31 (32%) children were between 2 and <5 years of age and

31 (32%) were ≥5 years of age. There was a non-significant decrease in the proportion of children <2 years of age with IPD between 2005 and 2013 (Figure 2A, Chi^2 test of trend, p=0.063). During the first period of enhanced surveillance (2005–2006), 24/2063 (1.2%) children on whom blood/CSF was cultured had IPD identified; in the second period of enhanced surveillance (2010–2013), 32/5691 (0.6%) children had IPD identified (Chi^2 test, p=0.006).

IPD serotype distribution

PCV coverage of IPD isolates is described in Figure 2B (detailed by serotype in Supplemental Digital Content 5, figure and age group in Supplemental Digital Content 6, figure). Of 98 isolates, 94 had Quellung serotyping available for analysis (the remaining 4 isolates, 2 from children <2 years of age and 2 from children 2 to 5 years of age, did not survive storage at -80 ºC). Of these isolates, 69 (73%) had polysaccharides covered by the 10-valent PCV and an additional 4 (4.3%) had isolates additionally covered by the 13-valent PCV.

Pneumococcal isolates from older children were more likely to be covered by the 10-valent PCV: 19/34 (56%) isolates from children <2 years of age; 23/29 (79%) isolates from children 2 to <5 years of age; and 27/31 (87%) isolates from children ≥5 years of age were contained in 10-valent PCV, respectively (p=0.012). There was no change in the proportion of isolates contained in 10-valent PCV between 2005 and 2013 (Chi^2 test of trend, p=0.258, Figure 2B and 2C).

The most prevalent individual serotypes were serotype 1 (40 isolates, 41%), serotype 5 (13 isolates, 13%) and serotype 14 (6 isolates, 6.1%). Isolation of serotype 1 pneumococci was associated with older age in comparison with isolation of other pneumococcal serotypes (median age 4.9, IQR 3.5–7.1, years vs median age 1.4, IQR 0.6–4.7, years; Kruskal-Wallis test, p<0.001; Figure 2D).

Culture, antigen testing and PCR for analysis of CSF

In total, 33 pathogens were cultured from CSF during the surveillance period. *S. pneumoniae* was the most prevalent organism isolated (18, 55% isolates). Of these, 15 (83%) isolates were from children <2 years of age. Pneumococcal serotypes isolated from CSF were diverse: 2 isolates each for serotypes 1, 12A and 23F; and 1 isolate each for serotypes 15A, 16, 18C, 19C, 2, 33, 9A/9V, 9N/9L, 9V and non-typeable. *H. influenzae* type b was the second-most prevalent pathogen cultured from CSF, with 7 isolates. Other pathogens cultured from CSF were *N. meningitidis* (3 isolates), other streptococci (1 beta-haemolytic of unknown group and 2 alpha-haemolytic viridans streptococci), and 1 isolate each for *Pseudomonas* spp. and *S. aureus* (Figure 3).

Antigen and PCR testing increased the number of pneumococcal meningitis cases identified from 18 cases to 40 cases, the number of *N. meningitidis* meningitis cases identified from 3 cases to 12 cases, and the number of *H. influenzae* type b meningitis cases identified from 7 to 11 cases (Figure 3).

DISCUSSION

IBD is a major cause of morbidity for children presenting to hospital in urban Nepal. *S. enterica* serovar Typhi (28% of all pathogens isolated) and *S. pneumoniae* (16% of all pathogens isolated) were the most prevalent pathogens identified in the cohort. The contaminant rate (which included coagulase-negative staphylococci, *Bacillus* spp., *Micrococcus* spp. and unknown contaminants) was similar to that reported in other studies in the region[21,22]. We did not classify viridans streptococci as contaminants in this setting, where malnutrition and poor dentition are prevalent.

Systematic reviews have described the prevalence of pathogens in children with suspected IBD in Asia[4], and in Africa[3]. In children in Asia, and in particular where HIV prevalence is low (such as Nepal), *S. enterica* serovar Typhi was the most prevalent pathogen, comprising 25% of pathogens cultured, and *S. pneumoniae* was the second-most prevalent pathogen, comprising 13% of pathogens cultured. Other important pathogens identified were *H. influenzae* type b (8% of pathogens, in data mainly collected prior to routine Hib vaccination), and *S. aureus* (4% of pathogens). The pathogen-specific prevalence of IBD in children in urban Nepal presented here therefore reflects wider regional data. In contrast, in Africa, particularly where HIV prevalence is high, *S. pneumoniae* (23% of pathogens), non-typhoidal salmonellae (19% of pathogens) and *S. aureus* (12% of pathogens) were prevalent in children with suspected IBD.

Culture-based methods for the identification of pathogens causing IBD are insensitive for bacterial disease due to low numbers of organisms, low volumes of blood taken for culture, and pre-treatment with antibiotics. In 2005–2006 some 32% of parents of febrile children admitted to Patan Hospital reported use of antibiotics prior to presentation[12], and a similar prevalence was noted in 2010–2013 (29%), particularly in children ≥5 years of age (42%, Supplemental Digital Content 3 (table)), and in whom a lumbar puncture was done (47%, Supplemental Digital Content 4 (table)). There is also circumstantial evidence of increasing antimicrobial prescription in the community in Kathmandu[23]. Pathogens also differ in their fastidiousness, with blood culture estimated to have approximately 60% sensitivity for *S. enterica* serovar Typhi in typhoid disease[24], and possibly <10% for *S. pneumoniae* during pneumococcal pneumonia (based on vaccine impact studies)[8,25,26], where bacteria are typically constrained to the lung.

Antigen testing and PCR of blood are more sensitive than culture-based methods for detection of pathogens. However, these methods are hampered by low specificity, particularly for organisms

such as *S. pneumoniae* that are carried in the nasopharynx of a high proportion of healthy children (with commensurate low level antigenaemia)[27]. They are also practically limited to a small number of pathogens on which commercial/standardised tests are readily available. We therefore undertook antigen and PCR testing for *S. pneumoniae*, *H. influenzae* type b and *N. meningitidis* only on CSF, to which the immunologic protection afforded to the brain prevents transfer of antigen or DNA in the absence of infection. This approach led to a large increase in the number of these pathogens identified in CSF, providing additional clarity to the causes of meningitis in children in urban Nepal, and supporting the continued use of antigen testing and/or PCR testing for IBD surveillance in this setting.

Diseases caused by *S. enterica* serovar Typhi, *S. pneumoniae,* and *N. meningitidis* are largely vaccine preventable. This surveillance period was undertaken prior to introduction of the 10-valent PCV to the routine infant immunisation schedule in 2015, and prior to a large randomised controlled trial of a typhoid conjugate vaccine from 2017 onwards[28]. We provide detailed data on the serotype-specific prevalence of IPD in the cohort, supporting the need for PCV in the child population of Nepal. In total, 73% of pneumococcal isolates are of serotypes covered by the 10-valent PCV, and an additional 4% by the 13-valent PCV. In the first three year of this surveillance study (2005–2007), the majority of IPD was identified in children <2 years of age, but IPD became increasingly rare in this age group over the course of the surveillance study. IPD in children <2 years of age was also associated with non-PCV serotypes of pneumococci, leading to (non-significant) changes in both the age and serotype distribution of IPD during the period of the surveillance study. This may be secondary to increasing use of antibiotics in the community for younger children, or may represent an independent epidemiologic phenomenon. This has important implications for the assessment of PCV introduction to the Nepali infant immunisation

schedule in 2015. For example, unless there is significant indirect immunity from vaccinating children <2 years of age on transmission of pneumococci to older children, the impact of 10-valent PCV on IPD and other pneumococcal diseases may be delayed for several years following implementation.

One characteristic and persistent feature of the serotype distribution of IPD in the cohort is the predominance of serotype 1 pneumococci, comprising 41% of all IPD in the cohort, and isolated predominantly from older children. The inclusion of children ≥5 years of age in this study (often excluded from childhood IBD surveillance)[1] highlights the importance of this serotype[11,29] and suggests that these pneumococci have acquired specific traits to enable them to persist, and cause disease, in a population of older children.

This study details the pathogen-specific prevalence of IBD in a large cohort of children in urban South Asia. Enhanced surveillance was undertaken prospectively, with detailed data collection of children with, and without, culture-confirmed IBD at Patan Hospital. However, the cohort recruited during the period 2007–2009 consists only of those patients with culture-confirmed IBD, limiting any formal time series analysis. In common with all infectious disease surveillance studies that use primarily culture-based methods, our data will significantly underestimate the true prevalence of IBD (and non-invasive bacterial disease) in the cohort. It is also possible that a small number of CSF samples were not subject to antigen or PCR testing due to small volumes or temporary unavailability of testing kits; and no PCR testing was undertaken for samples from 2007, thus underestimating the prevalence of bacterial meningitis in the cohort. We also did not survey severe viral infections, such as Japanese encephalitis. Antimicrobial susceptibility testing methods were not consistent through the surveillance period, and we have not presented these data here.

In summary, we instituted an IBD surveillance study in urban Nepal that details the pathogen-specific prevalence of IBD in children presenting to hospital. These data may also be of use in estimating the potential effects of routine vaccination against pneumococci, and potentially *S. enterica* serovar Typhi, in future.

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Table 1. Clinical characteristics of patients with suspected IBD, and with IPD at Patan Hospital during the surveillance period.

	Year of admission									
	2005	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**Total**
Suspected IBD	949	1117	–	–	–	1753	1509	1327	1301	7956
Cultures done and available	949	1114	152	76	148	1753	1509	1288	1141	8130
Female sex	390 (41%)	494 (44%)	72 (47%)	31 (41%)	60 (41%)	727 (41%)	579 (38%)	494 (38%)	449 (39%)	3296 (41%)
Age (years, median, IQR)	0.9 (0.2–2.8)	0.8 (0.1–2.6)	1.1 (0.1–4.9)	1.1 (0.1–4.4)	0.9 (0.1–4.6)	1.0 (0.1–3.6)	0.8 (0.1–3.0)	0.8 (0.1–2.8)	0.9 (0.1–2.9)	0.9 (0.1–3.0)
<1 month	143 (15%)	187 (16%)	37 (24%)	16 (21%)	32 (22%)	354 (20%)	301 (20%)	304 (24%)	260 (23%)	1634 (20%)
1 month to <6 months	247 (26%)	285 (26%)	25 (16%)	13 (17%)	24 (16%)	319 (18%)	307 (20%)	230 (18%)	232 (20%)	1682 (21%)
6 months to <1 year	105 (11%)	117 (11%)	11 (7%)	6 (8%)	19 (13%)	208 (12%)	202 (13%)	156 (12%)	119 (10%)	943 (12%)
1 year to <2 years	144 (15%)	167 (15%)	14 (9%)	8 (11%)	15 (10%)	267 (15%)	199 (13%)	194 (15%)	169 (15%)	1177 (14%)
2 years to <5 years	158 (17%)	222 (20%)	27 (18%)	16 (21%)	21 (14%)	267 (15%)	256 (17%)	180 (14%)	190 (17%)	1337 (16%)
5 years and older	152 (16%)	136 (12%)	38 (25%)	17 (22%)	37 (25%)	338 (19%)	244 (16%)	224 (17%)	171 (15%)	1357 (17%)
Total with IPD	16	8	15	16	11	8	7	10	7	98
Female sex	8	3	5	8	3	0	2	7	3	39 (40%)
Age (years, median, IQR)	1.2 (0.2–6.0)	3.1 (0.8–5.1)	3.5 (0.7–5.3)	3.1 (2.3–5.4)	3.6 (2.7–5.0)	4.6 (1.2–7.9)	4.9 (3.4–9.3)	1.7 (0.7–5.3)	4.7 (3.2–5.6)	3.4 (0.9–6.9)
<1 month	0	0	0	0	0	0	0	0	0	0
1 month to <6 months	5 (31%)	2 (25%)	3 (20%)	1 (6%)	0	0	0	2 (20%)	0	13 (13%)
6 months to <1 year	1 (6%)	1 (13%)	3 (20%)	1 (6%)	1 (9%)	2 (25%)	1 (14%)	3 (30%)	0	13 (13%)
1 year to <2 years	3 (19%)	1 (13%)	1 (7%)	2 (13%)	1 (9%)	1 (13%)	0	0	1 (14%)	10 (10%)
2 years to <5 years	2 (13%)	2 (25%)	3 (20%)	7 (44%)	6 (55%)	2 (25%)	3 (43%)	2 (20%)	4 (57%)	31 (32%)
5 years and older	5 (31%)	2 (25%)	5 (33%)	5 (31%)	3 (27%)	3 (38%)	3 (43%)	3 (30%)	2 (29%)	31 (32%)

Table 2. Bacterial and fungal organisms isolated from children at Patan Hospital during the surveillance period. Percentages given for pathogens per 100 patients on whom cultures (either blood or CSF) were available. Note that only data from positive cultures (either pathogens or contaminants) were available between 2007–2009, while all blood cultures were available for analysis between 2005–2007 and 2010–2013.

Year*	2005	2006	2007	2008	2009	2010	2011	2012	2013	Total
n (cultures done and available)	949	1114	152	76	148	1753	1509	1288	1141	–
Gram positive										
Streptococcus pneumoniae	16 (1.7%)	8 (0.7%)	15 (9.9%)	16 (21%)	11 (7.4%)	8 (0.4%)	7 (0.5%)	10 (0.8%)	7 (0.6%)	98
Viridans streptococci	4 (0.4%)	8 (0.7%)	10 (6.6%)	12 (16%)	9 (6.1%)	5 (0.3%)	3 (0.2%)	4 (0.3%)	3 (0.3%)	58
Staphylococcus aureus	4 (0.4%)	7 (0.6%)	8 (5.3%)	3 (3.9%)	3 (2.0%)	3 (0.2%)	3 (0.2%)	3 (0.2%)	3 (0.3%)	37
Beta-haemolytic streptococci (unknown group)	0	0	2 (1.3%)	1 (1.3%)	4 (2.7%)	1 (0.1%)	0	1 (0.1%)	0	9
Enterococcus spp.	4 (0.4%)	1 (0.1%)	0	0	0	1 (0.1%)	0	0	0	6
Group A beta-haemolytic streptococci	0	5 (0.4%)	0	0	0	0	0	0	0	5
Group B beta-haemolytic streptococci	1 (0.1%)	0	0	0	0	0	0	0	0	1
Gram negative										
Salmonella enterica serovar Typhi	27 (2.8%)	31 (2.8%)	28 (18%)	6 (7.9%)	10 (6.8%)	20 (1.1%)	9 (0.6%)	14 (1.1%)	22 (1.9%)	167
Enterobacter spp.	4 (0.4%)	1 (0.1%)	31 (20%)	4 (5.3%)	10 (6.8%)	1 (0.1%)	1 (0.1%)	1 (0.1%)	6 (0.5%)	59
Acinetobacter spp.	4 (0.4%)	5 (0.4%)	9 (5.9%)	1 (1.3%)	6 (4.1%)	7 (0.4%)	2 (0.1%)	3 (0.2%)	2 (0.2%)	39
Salmonella enterica serovar Paratyphi	4 (0.4%)	2 (0.2%)	3 (2.0%)	0	5 (3.4%)	6 (0.4%)	5 (0.3%)	2 (0.2%)	0	27
Escherichia coli	3 (0.3%)	1 (0.1%)	2 (1.3%)	3 (3.9%)	2 (1.4%)	2 (0.1%)	1 (0.1%)	2 (0.2%)	1 (0.1%)	17
Pseudomonas spp.	1 (0.1%)	0	3 (2.0%)	4 (5.3%)	7 (4.7%)	1 (0.1%)	1 (0.1%)	0	0	17
Salmonella spp.	1 (0.1%)	0	1 (0.7%)	4 (5.3%)	3 (2.0%)	3 (0.2%)	1 (0.1%)	1 (0.1%)	0	14
Haemophilus influenzae type b	1 (0.1%)	2 (0.2%)	3 (2.0%)	3 (3.9%)	3 (2.0%)	0	0	1 (0.1%)	0	13
Klebsiella spp.	0	0	0	2 (2.6%)	3 (2.0%)	2 (0.1%)	1 (0.1%)	3 (0.2%)	3 (0.3%)	14
Serratia spp.	0	2 (0.2%)	1 (0.7%)	2 (2.6%)	1 (0.7%)	0	0	0	0	6
Neisseria meningitidis	0	0	1 (0.7%)	0	1 (0.7%)	1 (0.1%)	0	1 (0.1%)	0	4
Neisseria spp.	0	0	0	1 (1.3%)	0	2 (0.1%)	0	0	0	3
Citrobacter spp.	0	0	0	0	1 (0.7%)	0	0	0	0	1
Haemophilus parainfluenzae	0	0	0	1 (1.3%)	0	0	0	0	0	1
Stenotrophomonas maltophilia	0	1 (0.1%)	0	0	0	0	0	0	0	1
Other										
Yeast	0	0	0	0	0	1 (0.1%)	1 (0.1%)	1 (0.1%)	1 (0.1%)	4
Total pathogens	74 (7.8%)	74 (6.6%)	117 (76%)	63 (83%)	79 (53%)	64 (3.7%)	35 (2.3%)	47 (3.6%)	48 (4.2%)	599
Negative	783 (83%)	984 (88%)	0	0	0	1549 (88%)	1374 (91%)	1169 (91%)	1043 (91%)	6902
Mixed Gram negative infection	1 (<0.1%)	5 (0.4%)	3 (2.0%)	0	1 (0.7%)	1 (0.1%)	0	0	0	11
Coagulase-negative staphylococci (neonates)	16 (1.7%)	7 (0.6%)	21 (14%)	2 (2.6%)	6 (4.1%)	16 (0.9%)	27 (1.8%)	8 (0.6%)	12 (1.1%)	115
Contaminants										
Bacillus spp.	3 (0.3%)	6 (0.5%)	0	1 (1.3%)	14 (9.5%)	41 (2.3%)	0	0	6 (0.5%)	71
Coagulase-negative staphylococci (non-neonates)	12 (1.3%)	9 (0.8%)	4 (2.6%)	9 (12%)	28 (19%)	61 (3.5%)	21 (1.4%)	5 (0.4%)	12 (1.1%)	161
Micrococcus spp.	2 (<0.1%)	4 (0.4%)	0	1 (1.3%)	12 (8.1%)	21 (1.2%)	6 (0.4%)	0	4 (0.4%)	50
Other or unknown contaminant	58 (6.1%)	26 (2.3%)	8 (5.3%)	0	8 (5.4%)	0	46 (3.0%)	59 (4.6%)	16 (1.4%)	219

FIGURE LEGENDS AND FIGURES

Figure 1. Recruitment of patients, and overview of laboratory investigations undertaken during the period of surveillance.

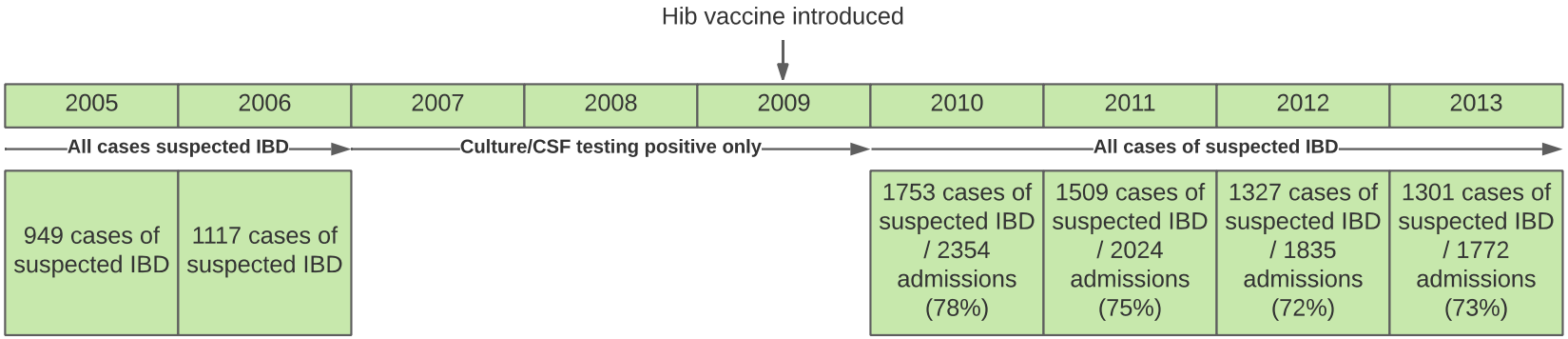

Hib vaccine introduced
2005
2006
2007
2008
2009
2010
2011
2012
2013
All cases suspected IBD
Culture/CSF testing positive only
All cases of suspected IBD
949 cases of suspected IBD
1117 cases of suspected IBD
1753 cases of suspected IBD / 2354 admissions (78%)
1509 cases of suspected IBD / 2024 admissions (75%)
1327 cases of suspected IBD / 1835 admissions (72%)
1301 cases of suspected IBD / 1772 admissions (73%)

Figure 2. IPD at Patan Hospital during the surveillance period. **A,** Distribution of IPD by year of admission and age group of child. **B,** Distribution of IPD isolates by year of admission and presence in pneumococcal conjugate vaccines (PCV). **C,** Distribution of IPD isolates by year of admission and presence in pneumococcal conjugate vaccines stratified by age group **D,** Age distribution of IPD associated with serotype 1 pneumococci.

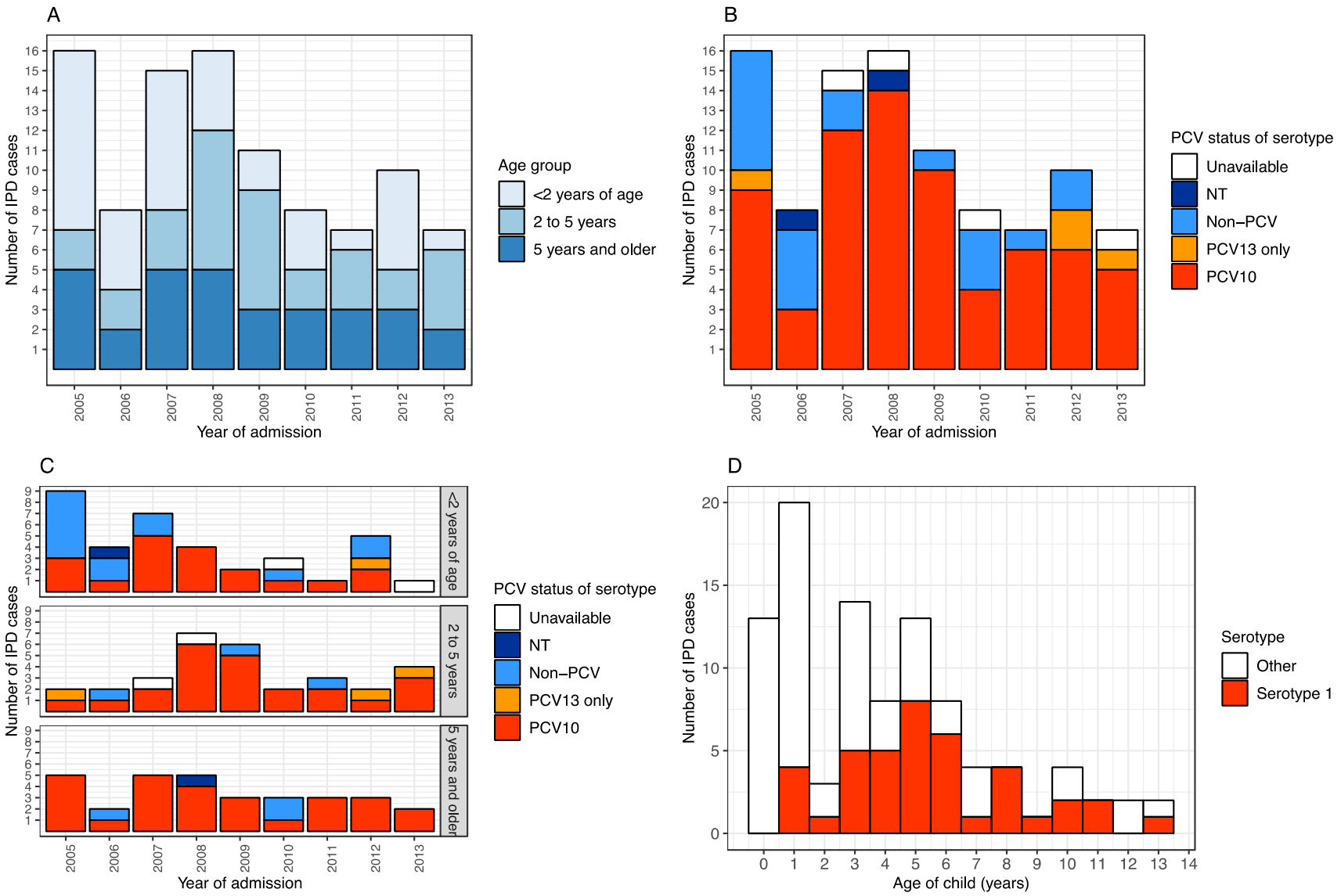
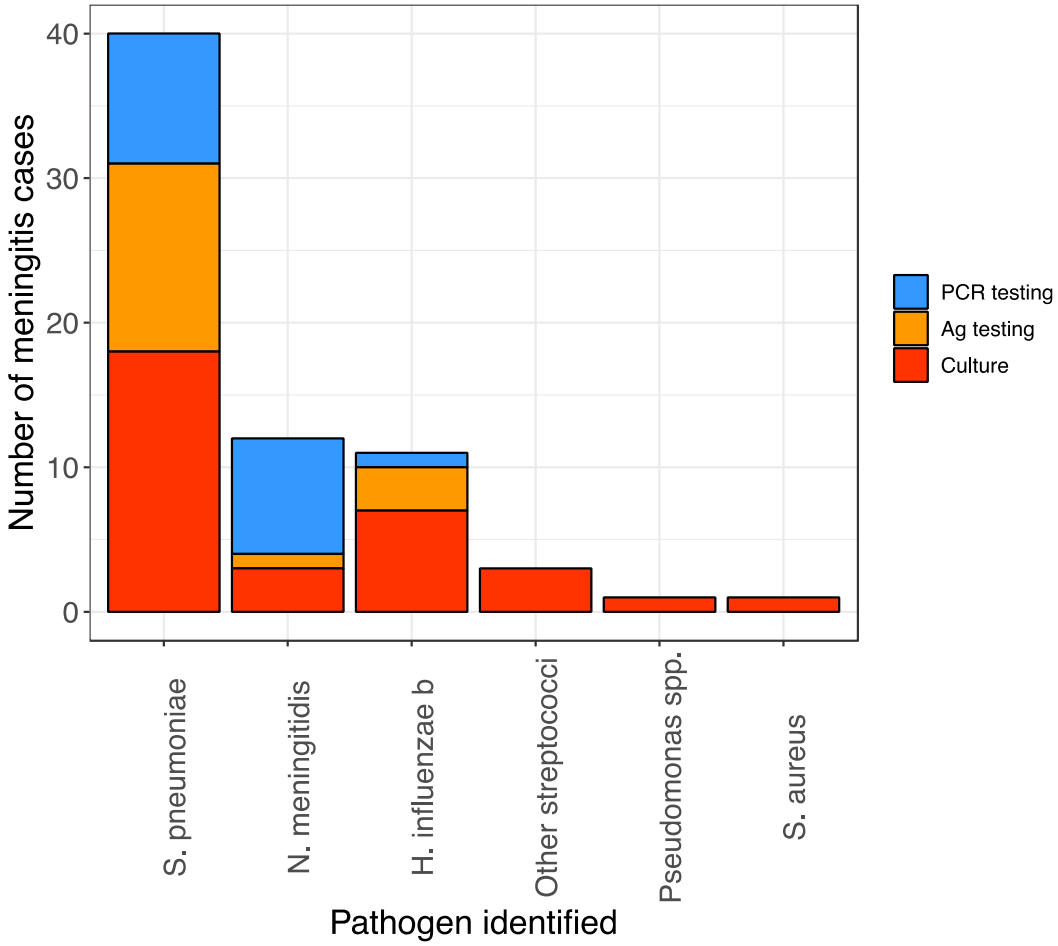

A
Number of IPD cases
Year of admission
Age group
<2 years of age
2 to 5 years
5 years and older
B
Number of IPD cases
Year of admission
PCV status of serotype
Unavailable
NT
Non–PCV
PCV13 only
PCV10
C
<2 years of age
2 to 5 years
5 years and older
Number of IPD cases
Year of admission
PCV status of serotype
Unavailable
NT
Non–PCV
PCV13 only
PCV10
D
Number of IPD cases
Age of child (years)
Serotype
Other
Serotype 1

Figure 3. Number of organisms identified in CSF using culture, additional organisms identified by antigen detection, and additional organisms identified by PCR alone in children with meningitis and CSF sampling undertaken at Patan Hospital 2005–2013.

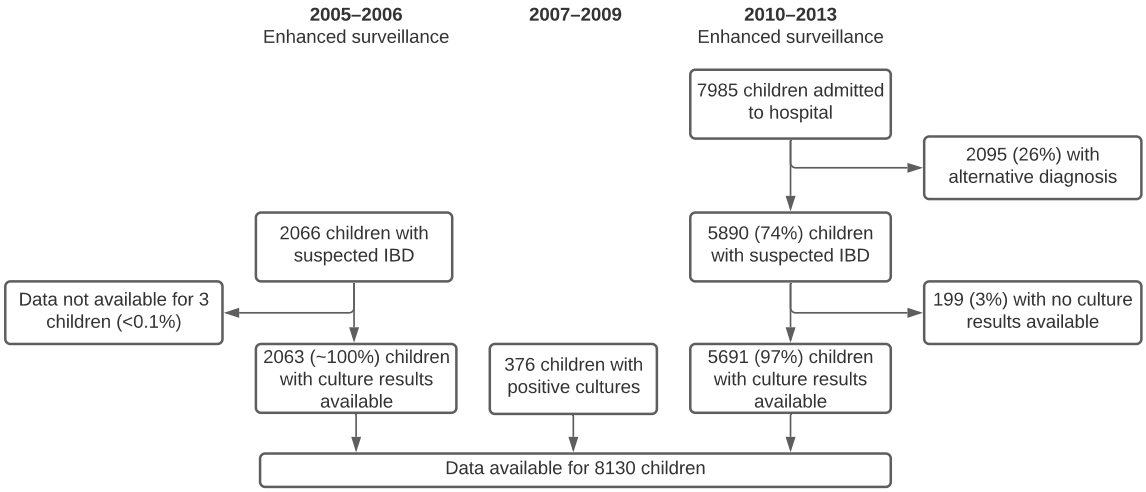


SUPPLEMENTAL DIGITAL CONTENT

Supplemental Digital Content 1. Clinical features of pneumonia, meningitis and sepsis as determined by the *WHO Integrated Management of Childhood Illness*[19] and *Pocket Book of Hospital Care for Children: Guidelines for the Management of Common Childhood Illness*[17]. Pneumonia and Severe Pneumonia have been recently combined into one category of Pneumonia[18].

Pneumonia	Severe pneumonia[a]	Pneumonia with danger signs	Meningitis	Sepsis
Cough or difficulty breathing with:	Cough or difficulty breathing with:	Not able to drink	History: fever with,	Fever with no focus of infection No specific features of meningitis
Fast breathing:	Chest indrawing	Persistent vomiting	Convulsions	
≥50 breaths/min in a child aged 2–11 months ≥40 breaths/min in a child aged 1–5 years		Convulsions	Vomiting	Confusion
		Lethargic or unconscious	Inability to drink	Signs of systemic upset
		Stridor when calm Severe malnutrition	Head or neck ache Recent head injury Examination: Altered consciousness Neck stiffness Bulging fontanelle Non-blanching rash Lethargy or irritability Evidence of head trauma Signs of raised ICP Decreased consciousness Unequal pupils Rigid posture or posturing Focal paralysis Irregular breathing	

Supplemental Digital Content 2. Recruitment to the cohort during the surveillance study period 2005–2013.



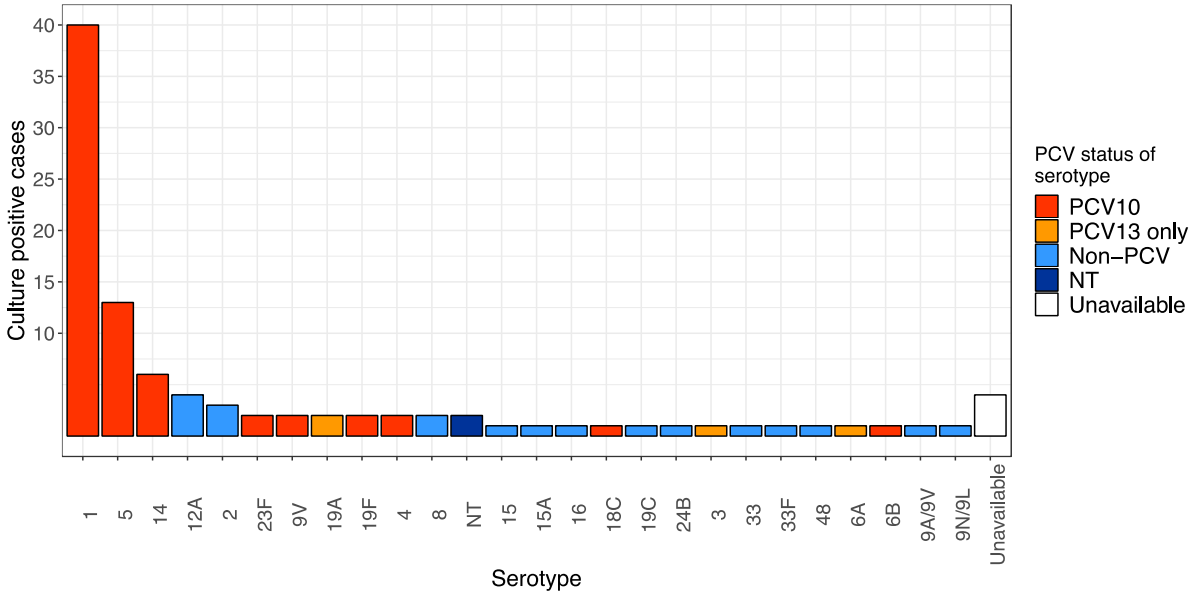
Supplemental Digital Content 3. Number of children with caregiver report of antibiotic administration (either over-the-counter or in a healthcare facility) prior to admission to Patan Hospital by year (for each year where detailed data exist), and by age group.

	2010	2011	2012	2013	Total
Age <2 years	290/1148 (25%)	323/1012 (32%)	183/884 (21%)	130/779 (17%)	926/3823 (24%)
Age 2–5 years	86/266 (32%)	100/256 (39%)	61/180 (34%)	63/192 (33%)	310/894 (35%)
Age ≥5 years	138/339 (41%)	116/241 (48%)	91/224 (41%)	65/170 (38%)	410/974 (42%)
Total	514/1753 (29%)	539/1509 (36%)	335/1288 (26%)	258/1141 (23%)	1646/5691 (29%)
Missing data	9	31	6	1	47

Supplemental Digital Content 4. Number of children with caregiver report of, or inpatient, administration of antibiotics prior to lumbar puncture at Patan Hospital by age group and year for children in whom a lumbar puncture was done.

	2010	2011	2012	2013	Total
Age <2 years	181/400 (45%)	142/242 (59%)	117/280 (42%)	59/241 (25%)	499/1163 (43%)
Age 2–5 years	12/22 (55%)	4/9 (44%)	11/15 (73%)	5/8 (63%)	32/54 (59%)
Age ≥5 years	32/46 (70%)	30/36 (83%)	20/24 (83%)	8/11 (73%)	90/117 (77%)
Total	225/468 (48%)	176/287 (61%)	148/319 (46%)	72/260 (28%)	621/1334 (47%)

Supplemental Digital Content 5. Serotype distribution of pneumococcal isolates from children with invasive pneumococcal disease at Patan Hospital 2005–2013. Isolates are colored by the presence of the serotype with the 10-valent (PCV10), 13-valent vaccines (PCV13), non-vaccine serotypes (non-PCV) or non-typeable pneumococci.



Supplemental Digital Content 6. Detailed age distribution of pneumococcal isolates from children with invasive pneumococcal disease at Patan Hospital 2005–2013. Isolates are colored by the presence of the serotype with the 10-valent (PCV10), 13-valent vaccines (PCV13), non-vaccine serotypes (non-PCV) or non-typeable pneumococci.