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Pneumonia in children admitted to the national referral hospital in Bhutan: A prospective cohort study

Sophie Jullien[a,b,*], Dinesh Pradhan[c], Tashi Tshering[c], Ragunath Sharma[b], Kumbu Dema[b], Selene Garcia-Garcia[d], Jose Luis Ribó[e], Carmen Muñoz-Almagro[d,f,g], Quique Bassat[a,d,f,h,i]

[a] ISGlobal, Hospital Clínic – Universitat de Barcelona, carrer Rosselló, 132, 08036 Barcelona, Spain
[b] Jigme Dorji Wangchuck National Referral Hospital (JDWNRH), Gongphel Lam, Thimphu, Bhutan
[c] Khesar Gyalpo University of Medical Sciences of Bhutan (KGUMSB), PO box 446, Old Medical Block, JDWNRH, Menkhang Lam, Thimphu, Bhutan
[d] Hospital Sant Joan de Déu (University of Barcelona), Pg. Sant Joan de Déu, 2, 08950 Esplugues de Llobregat, Barcelona, Spain
[e] Hospital Universitari General de Catalunya, carrer Pedro i Pons 1, 08195 Sant Cugat del Vallés, Barcelona, Spain
[f] Consorcio de Investigación Biomédica en Red de Epidemiología y Salud Pública (CIBERESP), Av. Monforte de Lemos, 3-5, 28029 Madrid, Spain
[g] Department of Medicine, Universitat Internacional de Catalunya, Carrer de Josep Trueta, 08195 Sant Cugat del Vallés, Barcelona, Spain
[h] Centro de Investigação em Saúde de Manhiça (CISM), Rua 12, Cambeve, Vila de Manhiça, CP 1929, Maputo, Mozambique
[i] Catalan Institution for Research and Advanced Studies (ICREA), Pg. Lluís Companys 23, 08010 Barcelona, Spain

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ABSTRACT

Objectives: The study aim was to describe the etiological profile and clinical characteristics of pneumonia among children hospitalized in Thimphu, Bhutan.
Methods: This prospective study enrolled children aged 2–59 months admitted to the Jigme Dorji Wangchuck National Referral Hospital with World Health Organization (WHO)-defined clinical pneumonia. Demographic and clinico-radiological data were collected through questionnaires, physical examination, and chest radiography. Blood samples and nasopharyngeal washing were collected for microbiological analysis including culture and molecular methods.
Results: From July 2017 to June 2018, 189 children were enrolled, of which 53.4% were infants. Pneumonia-related admissions were less frequent over the winter. Chest radiographies were obtained in 149 children; endpoints included pneumonia in 39 cases (26.2%), other infiltrates in 31 (20.8%), and were normal in 79 children (53.0%). Non-contaminated bacterial growth was detected in 8/152 (5.3%) blood cultures, with only two cases of *Streptococcus pneumoniae*. Viral detection in upper respiratory secretions was common, with at least one virus detected in 103/115 (89.6%). The three most-commonly isolated viruses were respiratory syncytial virus (52/115; 45.2%), rhinovirus (42/115; 36.5%), and human parainfluenza virus (19/115; 16.5%). A third of patients with viral infections showed mixed infections. Case fatality rate was 3.2% (6/189).
Conclusion: Respiratory viral infections predominated among this cohort of WHO-defined clinical pneumonia cases, whereas bacterial aetiologies were uncommon, highlighting the epidemiologic transition that Bhutan seems to have reached.

Abbreviations: BiPAP, bilevel positive airway pressure; CPAP, continuous positive airway pressure; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; GCS, Glasgow coma scale; Hb, haemoglobin; IQR, interquartile range; JDWNRH, Jigme Dorji Wangchuck National Referral Hospital; LMICs, low- and middle-income countries; NPW, nasopharyngeal washing; PCV, pneumococcal conjugate vaccine; PICU, Paediatric Intensive Care Unit; RR, respiratory rate; RSV, respiratory syncytial virus; RT-PCR, real-time polymerase chain reaction; SD, standard deviation; WAZ, weight-for-age *Z*-score; WBC, white blood cells; WHO, World Health Organization.

* Corresponding author at: ISGlobal, Hospital Clínic – Universitat de Barcelona, carrer Rosselló, 132, 08036 Barcelona, Spain.
E-mail addresses: sophie.jullien@isglobal.org (S. Jullien), dinesh.bhutan@gmail.com (D. Pradhan), ttshering@mrrh.gov.bt (T. Tshering), ragunathsharma959@yahoo.com (R. Sharma), kdematnor1979@gmail.com (K. Dema), selenegarciagg@gmail.com (S. Garcia-Garcia), joseluisribo@gmail.com (J.L. Ribó), cma@sjdhospitalbarcelona.org (C. Muñoz-Almagro), quique.bassat@isglobal.org (Q. Bassat).

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Background

Pneumonia is the single largest cause of mortality in children aged under five years, causing an estimated 15.5% of all deaths in children under five years of age, and over 800,000 paediatric deaths annually (Liu et al., 2016; UN IGME, 2018). Most of these lives could be saved through more effective and equitable health system interventions, combining prevention, early and accurate diagnosis, and treatment (Walker et al., 2013; Rambaud-Althaus et al., 2015). The main pneumonia burden remains disproportionately concentrated in low- and middle-income countries (LMICs) in Southeast Asia and sub-Saharan Africa (Walker et al., 2013). Pneumonia deaths are decreasing, but more slowly than for other major causes of mortality, and too slowly to achieve the Sustainable Development Goal ambition of "ending preventable child deaths" by 2030 (United Nations, 2018).

Risk factors and causative pathogens of childhood pneumonia differ across the world. Obtaining reliable local data, including the burden of the disease, epidemiological trends, and the determination of the main pathogens involved, is imperative to help develop targeted interventions. Therefore, adequate surveillance systems are required to monitor the effectiveness of national strategies implemented towards the reduction of the disease burden. However, the lack of local data and weak surveillance systems in many LMICs hamper an adequate knowledge of the epidemiology and aetiology of childhood pneumonia in those settings where reliable data are most needed.

One country that exemplifies the dearth of data regarding childhood pneumonia is the Kingdom of Bhutan (Jullien et al., 2020), a small country locked in the Himalayas, with an estimated population of 779,666 in 2017 (Department of Information Technology, 2016; Ministry of Health, 2018). In this predominantly mountainous country, elevation rises from around 100 m in the southern foothills to over 7500 m in the northern Himalayan range, with the capital, Thimphu, standing at 2334 m (Central Intelligence Agency, 2019). The climate varies with the altitude, from tropical in the southern plains to alpine with very cold winters in the North. In Thimphu, the temperature ranges from −3 °C in winter to 22 °C in summer on average, coinciding with the monsoon that brings precipitations of around 350 mm in July (Climate-data org, 2019). Bhutan is classified as a lower-middle income country as of 2020 (The World Bank, 2020). Essential health services in both modern and traditional medicines are free for Bhutanese citizens, based on a primary healthcare approach (World Health Organization, 2017).

We conducted this prospective hospital-based observational study to describe the epidemiology, aetiology, and clinical and radiological presentation of World Health Organization (WHO)-defined pneumonia among children aged between 2 and 59 months admitted to the Jigme Dorji Wangchuck National Referral Hospital in Thimphu.

Methods

Study design and participants

This was a prospective hospital-based study conducted for 12 consecutive months at the Jigme Dorji Wangchuck National Referral Hospital (JDWNRH) in Thimphu. The hospital has 38 paediatric beds, including five in the paediatric intensive care unit (PICU).

All children aged 2–59 months hospitalized with WHO-defined pneumonia (irrespective of severity) were eligible for recruitment (World Health Organization, 2014) (see Box 1). Children admitted in the preceding seven days or with evidence of a foreign body in the respiratory tract were excluded. Potential participants were identified during day and night by the study co-investigators with the collaboration of paediatricians, paediatric residents, and nurses from the outpatient department, the emergency room, the PICU, and the paediatric ward. If an eligible participant was missed during the night, the child was assessed and recruited the following morning. All eligible children were recruited provided parent(s) or guardian(s) consented to study participation.

Box 1. WHO definitions of pneumonia and severe pneumonia used as inclusion criteria (World Health Organization, 2014).

Pneumonia:
- History of cough or reported breathing difficulty, AND
- Increased respiratory rate (RR) OR chest indrawing.

Severe pneumonia:
- History of cough or reported breathing difficulty

AND at least one of the following:
- Oxygen saturation <90% or central cyanosis,
- Severe respiratory distress (e.g. grunting, very severe chest indrawing),
- Signs of pneumonia with a general danger sign: inability to breastfeed or drink, lethargy or reduced level of consciousness, convulsions.

Increased RR is defined according to age as follows:
- RR ≥ 50 breaths per minute in children aged two months or more and less than 12 months.
- RR ≥ 40 breaths per minute in children aged 12 months or more and less than 60 months.

Data collection

On study admission, a study identification number was assigned and a comprehensive physical examination was performed, including anthropometric measurements, vital signs, axillary temperature, and peripheral oxygen saturation in room air. Demographic and clinical data were collected from the medical records and through family interviews. Sample collection upon enrolment, or as soon as possible after enrolment, included blood samples and nasopharyngeal washing (NPW). All the nurses in the PICU and paediatric ward were trained at the beginning of the study by the lead investigator on how to collect these samples. When a child was identified for recruitment but blood had already been collected, no further blood sampling was conducted. However, if another blood analysis was clinically indicated, additional blood was obtained for the specific purpose of the study. Fluid from pleural effusion was collected when clinically indicated. All recruited patients underwent a postero-anterior chest radiography upon admission. Additional information of potential diagnostic interest, such as computed tomography scans, ultrasound, or cerebrospinal fluid investigation available throughout admission, was also collected. Children were clinically managed and discharged as per existing hospital protocols and discretion of the treating paediatricians, and were followed-up by one study investigator in terms of outcome determination. All data were collected using digitalized and standardized forms (see Supplementary material for clinical definitions and details of variables measured).

Chest radiograph interpretation

The WHO protocol used in clinical trials of pneumococcal conjugate vaccines (PCV) was followed to interpret chest radiographs (Cherian et al., 2005). In brief, readers first judged the

quality of the film (uninterpretable or interpretable, the latter stratified as suboptimal or adequate) and then classified findings for all interpretable radiographs. Significant pathology was defined as the presence of consolidation, other infiltrates, and/or pleural effusion. Endpoint radiologically confirmed pneumonia was defined as consolidation, pleural effusion, or both on any hemithorax. Initially, two paediatricians independently interpreted the radiographs. Discordant results were read by a third reader, trained in WHO criteria for interpretation of chest radiographs. An additional external quality control measure was included in the study protocol, whereby a paediatric radiologist would read a random sample of 10% of the chest radiographs. However, as substantial discordance was observed between the two primary readers, all chest radiographs were again independently interpreted by the paediatric radiologist using the WHO criteria. This last reading was accepted as final interpretation for analysis.

Biological sample testing and laboratory methods

Blood was collected under aseptic conditions following the hospital's validated standardized procedures. Blood for haematology, biochemistry, and culture was processed following standard procedures. Blood was cultured using an automated blood culture system (BacT/ALERT®). Bacterial isolates were identified by colony morphology, growth requirements, and basic biochemical tests. Antibiotic susceptibility was determined using disk diffusion in accordance with the guidelines of the Clinical Laboratory Standard Institute (CLSI, 2015).

Additionally, real-time polymerase chain reaction (RT-PCR) for LytA gene of *Streptococcus pneumoniae* in dried-spot collected blood, and host-response biomarkers in additional blood (2 mL, EDTA tube) were investigated (findings reported elsewhere) (Brotons et al., 2017). The blood samples were centrifuged at $3000 \times g$ for three minutes, and the serum was separated and stored at $-80\,^{\circ}C$.

NPW samples were homogenized and aliquots frozen at $-80\,^{\circ}C$ and subsequently shipped to Barcelona, Spain, where they were subjected to molecular screening (multiplex RT-PCR QIAStat respiratory panel, Qiagen, for 17 viral targets and four bacterial targets). NPW were also subjected to detection of pneumococcus and capsular typing (findings reported elsewhere).

Rapid influenza diagnostic tests (Alere BinaxNOW®) were performed as per discretion of the treating clinicians and nurses, independently of the current study. Investigations for active tuberculosis included Mantoux test and gastric aspirates for microscopy and GeneXpert®.

Data management and statistical analysis

The lead investigator entered data into a computerized password-protected database (ODK®) with study identification number. Errors in data entry were limited by pre-defined ranges for every value. Stata 15.1 was used for data analyses (StataCorp, 2017). Mean with standard deviation (SD) and median with interquartile range (IQR) were used to summarize normally and non-normally distributed variables respectively.

Results

Study profile and demographic characteristics

Between 1st July 2017 and 30th June 2018, 1591 children were admitted to the paediatric department of JDWNRH. Among them, 286 (18.0%) were children aged 2–59 months with respiratory symptoms, of which 189 (66.1%) were recruited (Figure 1).

The baseline characteristics of the 189 children are presented in Table 1. Median age was 10.8 months; over half of the children were infants. Most children were adequately immunized according to age. There was no known case of HIV infection. Children were mainly from the district of Thimphu, although the study included patients from 16 out of the 20 districts in Bhutan. On average, families reported that it had taken around 15 min to reach the closest healthcare facility. Twenty-seven children (14.3%) were referred from another health centre. Summer, fall, and spring each comprised around 30% of the recruited cases, while winter had the lowest number of pneumonia admissions (10.1%). October was the month with the highest number of cases (37; 19.6%) (Figure 2).

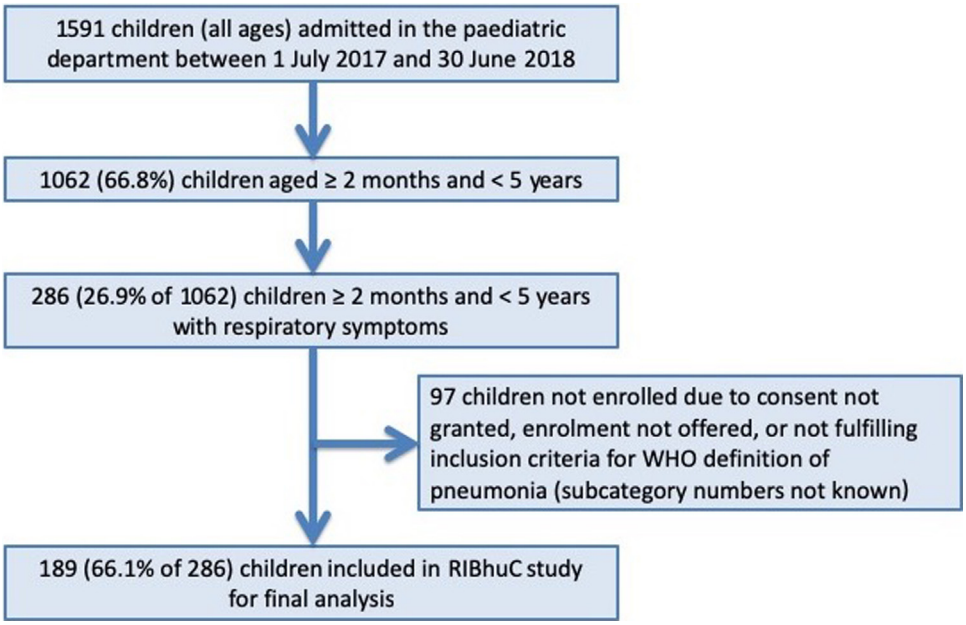

Figure 1. Study profile.

Clinical characteristics

Clinical characteristics upon admission are presented in Tables 2 and 3. Wasting (WAZ ≤ -2 SD) was detected in 17 children (9.0%). On admission, 77 children (41.2%) presented with fever, half of the children were breathing fast according to age, and three-quarters were hypoxemic. Median basal oxygen saturation was 85% (IQR 80–90) among the 173 children with available measurement in room air without oxygen therapy. On auscultation, typical lung consolidation-related sign (crackles) was most common (57.5%), followed by rhonchi (45.2%) and wheezing (25.0%).

On admission, 35.8% of the children were anaemic, 36.9% had leucocytosis, and 25.3% had neutrophilia. Two common inflammatory markers were tested at JDWNRH: C-reactive protein (CRP) with a mean of 2.06 mg/dL (SD 2.09), and erythrocyte sedimentation rate (ESR) with a mean of 24.89 mm (SD 28.02). Twenty-five

Table 1
Baseline characteristics of recruited children

Patients characteristics		*n*/*N*	%
Sex	Female	80/189	42.3
	Male	109/189	57.7
Age group	2 to <6 months	46/189	24.3
	6 to <12 months	55/189	29.1
	12 to <24 months	38/189	20.1
	24 to <36 months	20/189	10.6
	36 to <48 months	15/189	7.9
	48 to <60 months	15/189	7.9
Immunization	Fully immunized according to age	143/189	75.7
	Partially immunized according to age	43/189	22.7
	Not immunized	0/189	0
	Unknown	3/189	1.6
Preterm birth (<37 weeks of gestation)	No	174/189	92.1
	Yes	13/189	6.9
	Unknown	2/189	1.0
Co-morbidities	Known case of HIV infection	0/189	0
	Suspected case of tuberculosis	4/189	2.1
	Known underlying chronic respiratory disease	1/189[a]	0.5
Previous admission due to pneumonia	Yes	43/189	22.7
	No	143/189	75.7
	Unknown	3/189	1.6
Education	Both parents are illiterate	26/189	13.8
	Only one parent has basic (primary) education	26/189	13.8
	Both parents have basic (primary) education	78/189	41.3
	At least one parent has university education	48/189	25.4
	Unknown	11/189	5.8
Employment	Both parents are unemployed	2/189	1.1
	Only one parent is employed	105/189	55.6
	Both parents are employed	67/189	35.4
	Unknown	15/189	7.9
Number of people living in the household	$\leq$5 people living in household	117/189	61.9
	>5 people living in household	62/189	32.8
	Unknown	10/189	5.3
Exposure factors in the household	Smokers	21/189	11.1
	Non-smokers	158/189	83.6
	Smokers, unknown	10/189	5.3
	People chewing betel nut (*doma*)	115/189	60.8
	No people chewing betel nut	64/189	33.9
	People chewing betel nut, unknown	10/189	5.3
Type of heater used in the household (>1 option possible for each household)	Electrical	138/189	73.0
	Wood-burning stove (*bukhari*)	21/189	11.1
	Open fire	4/189	2.1
	Kerosene	14/189	7.4
Residency of the family	Thimphu	133/189	70.4
	Paro	15/189	7.9
	Chukha	5/189	2.7
	Wangdue	5/189	2.7
	Others	31/189	16.3
Closest health facility	JDWNRH	85/189	45.0
	Other hospital	57/189	30.2
	Basic health unit	39/189	20.6
	Unknown	8/189	4.2
Time to access healthcare facility	$\leq$15 min	107/189	56.6
	>15 but $\leq$30 min	58/189	30.7
	>30 but $\leq$60 min	6/189	3.2
	>60 min	5/189	2.7
	Unknown	13/189	6.9
Transport to access healthcare facility	Taxi	68/189	36.0
	Car	65/189	34.4
	Walk	42/189	22.2
	Public transport	1/189	0.5
	Unknown	13/189	6.9

[a] One patient was diagnosed with asthma.

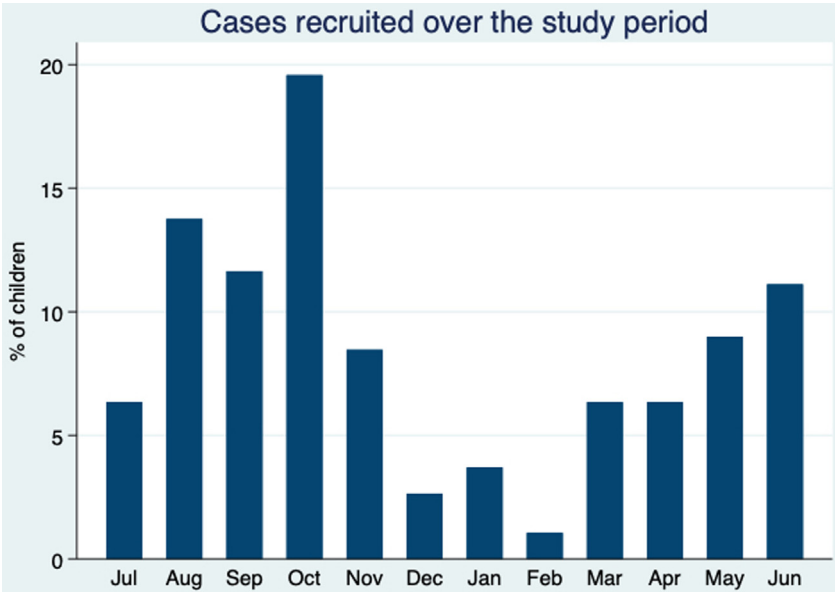

Figure 2. Proportion of pneumonia cases distributed per month.

children (13.2%) had CRP levels above the threshold (>4 mg/dL) commonly considered suggestive of high risk of bacterial infection, whereas 25 children had high ESR (≥50 mm) (Sanders et al., 2008; Bruel et al., 2011). Only four children presented with both high CRP and ESR.

Chest radiography was performed in 178/189 children (94.2%). Images were available for interpretation by the study investigators in 150 of them (84.3%). In 28 cases, children were discharged before investigators could interpret the radiography findings and the radiograph was missing. One film was judged uninterpretable. Among the final 149 readable chest radiographs, 79 (53.0%) were normal, 39 (26.2%) were classified as primary endpoint pneumonia, and 31 (20.8%) as other infiltrates.

Microbiological findings

While HIV infection was not suspected in any child by the treating physicians, active tuberculosis was suspected in 10 children (5.3%) but was not confirmed by the laboratory tests in any of them.

Blood culture was performed in 148/189 children (78.3%), of which 45 (30.4%) had received antibiotics prior to sample collection (Table 4). Thoracocentesis was performed in one child with pleural effusion. Six different pathogens were isolated among the eight non-contaminated positive blood cultures: *S. pneumoniae* (two cases), *Pseudomonas* sp. (two cases), *Escherichia coli*, *Acinetobacter* sp., *Salmonella typhi*, and *Serratia rubidaea* (one case each). Drug sensitivity results are shown in Supplementary Table 2. *S. pneumoniae* was isolated in the only sample of pleural fluid that was collected, which corresponds to the same child with positive blood culture, subsequently also confirmed by RT-PCR in blood.

NPW was collected in 129/189 children (68.3%). The NPW sample was too scarce or of bad quality to run the test in 14 children (10.9%). Among the remaining 115 children, 52 (45.2%) had received antibiotics prior to sample collection. *Bordetella pertussis* was detected in three (2.6%) children, and *Mycoplasma pneumoniae* in one (0.9%) child; *Chlamydophila pneumoniae* and *Legionella pneumophila* were not detected among respiratory samples.

At least one virus was identified in 103/115 NPW samples (89.6%) (Table 4). Viral co-infection was detected in 35/103 children (34.0%): 22 presented double infection, 10 presented triple infection, and three children were infected with four viruses. The most commonly isolated virus was respiratory syncytial virus (RSV) (52; 45.2%), followed by rhinovirus (42; 36.5%), human parainfluenza virus (19; 16.5%), and influenza virus (16; 13.9%). Coronavirus were detected in two children (1.7%). Routine rapid flu test was performed under the Influenza national surveillance programme in 32/189 children (16.9%), being positive for influenza A in seven cases, for influenza B in one case, and for co-infection of influenza A and B in one case. Analysis by RT-PCR confirmed the detection of influenza virus in 4/9 children with positive rapid flu test, and detected 10 additional cases with influenza virus.

Among children with at least one virus detected, 4/86 (4.6%) had a positive blood culture for bacteria and 24/89 (27.0%) had radiological endpoint pneumonia. Among children with no virus detected, 3/9 (23.3%) had a positive blood culture and 4/11 (36.4%) had radiological endpoint pneumonia (Supplementary Table 3). No children with influenza had a positive blood culture. However, 6/15 (40.0%) children with influenza identified in their nasopharynx had radiological endpoint pneumonia.

Lumbar puncture was not indicated in any of the children.

Evolution during admission

Children were hospitalized for a median of four days (IQR 2–6) (Table 5). Thirty children required PICU admission, with a median stay of 72 h (IQR 24–96). Three-quarters of the children were put on oxygen therapy, of which half for at least three days. Most children (72.0%) received antibiotics during admission. Antibiotics were stopped in the first two days of admission in 10 children (7.4%) and advised to be continued after discharge in 90 (66.2%). Main diagnoses given by the treating physician at discharge are shown in Supplementary Table 4. Half of the children were discharged with a diagnosis of pneumonia or bronchopneumonia. In terms of the seasonal variability of the most common clinical syndromes given by the treating physician at discharge, bronchopneumonia was mainly in fall (50.0%), bronchiolitis in spring (43.6%), and pneumonia did not show a clear seasonal pattern (Supplementary Figure 1).

Six children had a fatal outcome (case fatality rate 3.2%); all had been referred from other centres in critical condition.

Table 2
Clinical characteristics of recruited children at time of admission

		n/N	%
History of the current episode			
Duration of illness	<24 h	4/188	2.1
	≥24 to <72 h	41/188	21.8
	≥72 h to <7 days	93/188	49.5
	≥7 days	50/188	26.6
Reported fever prior to admission	No fever	29/184	15.8
	Median duration of fever, in hours (IQR)	72 (24–120)	NA
Danger sign (as per WHO definition)	Any danger sign	37/189	19.6
	Unable to drink or breastfeed	34/189	18.0
	Lethargy or reduced level of consciousness (GCS <15)	8/189	4.2
	Convulsion during the present episode[a]	2/189	1.1
Medical treatment sought prior to admission	Yes	102/186	54.8
Child started on antibiotics prior to admission	Yes	43/187	23.0
Antibiotics received prior to admission (more than one per child possible)	Amoxicillin or ampicillin	35/43	81.4
	Gentamycin	12/43	27.9
	Ceftriaxone	4/43	9.3
	Cefotaxime	2/43	4.7
	Erythromycin	2/43	4.7
	Cloxacillin	1/43	2.3
	Azithromycin	1/43	2.3
Severity of clinical pneumonia			
WHO definition on admission	WHO severe pneumonia	150/189	79.4
	WHO non-severe pneumonia[b]	19/189	10.0
	Do not meet WHO definition[c]	20/189	10.6
Severity during hospitalization	Severe pneumonia[c]	164/189	86.8
	Non-severe pneumonia[c]	25/189	13.2
Clinical examination at time of admission			
Nutritional status	No wasting (WAZ > −2 SD)	170/187	90.9
	Moderate wasting (WAZ ≤ −2 SD and > −3 SD)	10/187	5.4
	Severe wasting (WAZ ≤ -3 SD)	7/187	3.7
Vital signs	Increased respiratory rate according to age[d]	92/184	50.0
	Hypoxemia (SpO2 < 90%)	140/187	74.9
	Fever (≥37.5 °C)	77/187	41.2
	High fever (>39 °C)	9/187	4.8
Inspection	Central cyanosis	13/188	6.9
	Rhinorrhoea	63/188	33.5
	Lower chest wall indrawing	102/188	54.3
	Severe chest indrawing (supraclavicular and/or suprasternal)	22/187	11.8
	Nasal flaring	39/188	20.7
	Head nodding	2/187	1.1
	Grunting	10/188	5.3
	Deep breathing	0/188	0
	Digital clubbing	0/188	0
Auscultation	Crackles	108/188	57.5
	Rhonchi	85/188	45.2
	Wheezing	47/188	25.0
	Prolonged expiration	30/188	16.0
	Reduced air entry	17/188	9.0
	Inspiratory stridor	6/188	3.2
	Tubaric murmur	1/188	0.5
	Heart murmur	8/188	4.3
Other signs	Time for capillary refill > 2 s	7/188	3.7
	Weak peripheral pulses	7/188	3.7
	Weak central pulses	4/188	2.1
	Clinical shock	7/188	3.7
	Hepatomegaly	17/188	9.0
	Splenomegaly	2/188	1.1
	Glasgow coma score <15	8/188	4.3
	Prostration	2/188	1.1

Abbreviations: GCS: Glasgow coma scale; NA: not applicable; WAZ: weight-for-age *Z*-score.
[a] Two children presented with convulsions. One was diagnosed as febrile convulsion, while the other child was a severe case of pneumonia which led to a fatal outcome.
[b] Some children who presented with non-severe pneumonia developed hypoxemia during their hospitalization, which is a sign of severity as per the WHO definition.
[c] Twenty children (10.6%) did not strictly meet the WHO definition of pneumonia at the time of admission but were admitted to the paediatric ward with suspected pneumonia or bronchiolitis as per the clinical discretion of the treating paediatricians. Four of them developed hypoxemia during hospitalization requiring oxygen therapy and were therefore classified as severe pneumonia. None of the remaining 16 children were admitted to PICU or presented other signs of severity, and were classified as non-severe pneumonia (Supplementary Table 1).
[d] Increased respiratory rate (RR) according to age is defined as RR ≥ 50 bpm in children aged 2–12 months and RR ≥ 40 bpm in children aged ≥12 months.

NPW was not collected in three children due to the severity of their illness upon arrival. Of the other three children, one child presented a triple co-infection by *B. pertussis*, parainfluenza virus, and influenza virus. Four fatal cases were diagnosed as suffering of pneumonia, and two of bronchiolitis. Two deaths occurred within the first 24 h of admission to our centre. A summary of the main characteristics of these six children is presented in Supplementary Table 5.

Table 3
Laboratory findings on admission, blood sample

		n/N	%
Haematology			
Anaemia	Yes (Hb < 11 g/dL)	67/187	35.8
	Mild (Hb ≥ 10 and <11 g/dL)	31/187	16.6
	Moderate (Hb ≥ 7 and <10 g/dL)	35/187	18.7
	Severe (Hb < 7 g/dL)	1/187	0.5
Abnormal count of WBC (10^9/L)	Leucopenia (<5.0)	7/187	3.7
	Leucocytosis[a]	69/187	36.9
	Neutrophilia (≥70% of WBC)	47/186	25.3
	Neutropenia (<1.5)	3/186	1.6
Abnormal count of platelets (10^9/L)	Thrombocytosis (>450)	47/183	25.7
	Thrombocytopenia (<150)	2/183	1.1
Biochemistry			
Urea (mg/dL)	Urea > 40	4/116	3.4
Creatinine (mg/dL)	Creatinine > 1.2	4/117	3.4
Sodium (mEq/L)	Hyponatremia (<135)	15/119	12.6
	Hypernatremia (>145)	11/119	9.2
Potassium (mEq/L)	Hypokalemia (<3.5)	5/119	4.2
	Hyperkalemia (>5.5)	4/119	3.4
Inflammatory markers			
CRP	High CRP (>4 mg/dL)	25/178	14.0
ESR	High ESR (≥50 mm)	25/168	14.9

Abbreviations: CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; Hb: haemoglobin; WBC: white blood cells.
[a] Leucocytosis was defined as white blood cells greater than 15×10^9 cells/L for children aged between 2 and 11 months and greater than 13×10^9 cells/L for children aged between 12 and 59 months.

Table 4
Microbiological findings

	n/N	%
Invasive bacterial disease[a]		
Non-contaminated positive blood culture[b]	8/148[c]	5.4
S. pneumoniae isolated by blood culture	2/148	1.4
S. pneumoniae isolated by RT-PCR in dried blood spot sample (Ct LytA)	1/148	0.7
Non-contaminated positive pleural culture	1/1	100
S. pneumoniae isolated by pleural fluid culture	1/1	100
Viral detection		
Rapid flu test in pharyngeal swab	9/32[d]	28.0
At least one virus detected in NPW	103/115	89.6
Among children with positive virus findings in NPW		
Single viral infection in NPW	68/103	66.0
Mixed viral infection in NPW	35/103	34.0
RSV	52/115	45.2
Rhinovirus	42/115	36.5
Parainfluenza virus[e]	19/115	16.5
Influenza virus	16/115[f]	13.9
Adenovirus	8/115	7.0
Bocavirus	6/115	5.2
Human Metapneumovirus	4/115	3.5
Coronavirus (Cor229E, CorHKU1, CorNL63, CorOC43)	2/115	1.7

Abbreviations: NPW: nasopharyngeal washing; PCR: polymerase chain reaction; RSV: respiratory syncytial virus; RT-PCR: real-time polymerase chain reaction.
[a] Vials for blood culture were out of stock at the hospital for few weeks during the study period, leading to blood culture not being performed in 12 participants, although molecular screening in bloodspots in filter paper was conducted for all 10 of these children.
[b] Coagulase-negative staphylococci, and *Bacillus* spp were considered contaminants, as per our protocol.
[c] Bacterial growth was detected in 22 blood cultures, but it was attributed to contamination in 14 cases.
[d] Seven children had positive rapid flu test for influenza A, one child for influenza B, and one child for influenza A and B. Out of the seven children with rapid flu test positive for influenza A, detection of influenza A by RT-PCR in NPW was also positive in four cases, but negative in one case, and "failed/inhibited" in the remaining two cases. For the child with rapid flu test positive for influenza B and for the child with rapid flu test positive for both influenza A and B, RT-PCR in NPW was negative for both influenza A and B in both children.
[e] Parainfluenza viruses 1, 2, 3, and 4 were detected in 2 (1.7%), 1 (0.9%), 14 (12.2%), and 3 (2.6%) children respectively.
[f] Fourteen were influenza A, and two were influenza B.

Discussion

This is the first published series of comprehensive epidemiological, clinical, and microbiological data describing Bhutanese children under five years of age hospitalized with WHO-defined clinical pneumonia. Mortality related to pneumonia was 3.2%, similar to other studies from LMICs (Jroundi et al., 2014; Lazzerini et al., 2016; Bénet et al., 2017; Chen et al., 2018; O'Brien et al., 2019). Nevertheless, this remains high for Bhutan in spite of the country offering free and easily accessible healthcare services. The six children who died were referred from other health centres and reached the study hospital in critical condition.

The high proportion of infants in our study highlights that infants are particularly vulnerable and prone to hospitalization due to severe pneumonia (Fancourt et al., 2017; Chen et al., 2018; Jakhar et al., 2018). There was no child known or suspected to be infected with HIV, which is consistent with the very low number of under-five year old children infected with HIV in Bhutan (UNAIDS, 2018).

Table 5
Evolution during admission

		n/N	%
Evolution and outcome			
Hospital stay	<24 h	9/189	4.8
	≥24 to <72 h	67/189	35.4
	≥72 h to <7 days	82/189	43.4
	≥7 days	31/189	16.4
	Admission to paediatric intensive care unit	30/189	15.9
	Admission to high dependency unit	41/189	21.7
Management	Invasive mechanical ventilation	7/189	3.7
	Non-invasive mechanical ventilation	13/189[a]	6.9
	Oxygen therapy	142/189	75.1
	Antibiotics during admission	136/189	72.0
Outcome	Alive at discharge	183/189	96.8
	Death	6/189	3.2
	Transferred	1/189	0.5
	Absconded	0/189	0
	Withdrawn from the study	0/189	0

[a] Twelve children required continuous positive airway pressure (CPAP). One child was put on bilevel positive airway pressure (BiPAP) and was changed to CPAP after improvement. One child only required high flow nasal cannula oxygen.

Winter, which is the coldest season in Bhutan, surprisingly showed the lowest number of cases (10.1%); this finding differs from what is commonly seen in other settings, whereby hospitalization of childhood pneumonia tends to peak during the coldest season (Murdoch et al., 2014; Ben-shimol et al., 2015). However, this finding is consistent with those reported by the national sentinel surveillance programme for severe acute respiratory infections, and with the proportion of all-cause paediatric admissions, lower during winter (Royal Centre for Disease Control, 2018). This could be partially explained by the fact that winter coincides with the school break in Bhutan, with less contact among children; and families moving from the capital to the villages with lower population density.

Hypoxemia is a well-established predictor of severity in children with pneumonia (Duke et al., 2001; Lozano, 2001). A high proportion of children in this study (74.9%) presented with hypoxemia, which is much higher than reported in other settings (Subhi et al., 2009; O'Brien et al., 2019). We defined hypoxemia as SpO2 < 90%, which is considered appropriate for altitudes under 2500 m, as is the case with Thimphu (2334 m). This characteristic might therefore not be generalizable to Bhutanese children who live at different altitudes than that of Thimphu.

While bacterial aetiology was infrequent, viruses were identified in a considerable proportion of children. These microbiological findings coincide more with the etiological profile of pneumonia in children from high-income countries, highlighting the advanced stage of the epidemiologic transition that Bhutan seems to have reached (Omran, 2005; Prayle et al., 2011). The findings from the PERCH study, conducted in seven LMICs with routine use of PCV, are similar (O'Brien et al., 2019). Even in the absence of a deployed PCV in Bhutan (PCV was introduced only in January 2019), the burden of pneumococcal invasive disease appears to be low in children.

The low proportion of confirmed bacterial cases could be explained by several reasons. First, vaccination coverage was high, which is representative of the rest of the country, although the PCV was not in routine use during the recruitment period (WHO, 2016). Second, almost one-third of the children had received antibiotics prior to collection of blood sample, which reduces the yield of blood culture by around 45% (Berkley et al., 2005; Rhodes et al., 2010; Driscoll et al., 2017; O'Brien et al., 2019). Small blood volume is another factor known to compromise the sensitivity of blood culture (Berkley et al., 2005; Bouza et al., 2007; Driscoll et al., 2017). Blood collection is challenging in children, especially in infants. Blood volumes collected for each child were not recorded in this study but, in practice, around 1 mL was dedicated for blood culture in most cases, despite the 2–3 mL recommended in the protocol. Nevertheless, these findings confirm the low yield of blood culture in hospitalized children with pneumonia and question both the need of blood culture for uncomplicated cases of pneumonia and using blood culture as the preferred screening tool for invasive bacterial disease in children with pneumonia. Molecular methods have been found to be more sensitive than blood culture to detect pneumococcal invasive disease (Muñoz-almagro et al., 2011; Selva et al., 2013; O'Brien et al., 2019). This was not the case in this study.

B. pertussis was isolated in respiratory samples of three children. This is similar to the detection rate of around 1% of hospitalized pneumonia cases in similar studies (Jroundi et al., 2014; Barger-kamate et al., 2016). One of these three children, aged five months, had a fatal outcome. This underlines the high fatality ratio of pertussis-infected pneumonia, especially in infants who are unvaccinated, and suggests the need of intervention such as maternal vaccination to reduce morbi-mortality associated with pertussis in vulnerable populations.

Viral detection was common. The use of PCR techniques has increased the ability to detect respiratory viruses (Ruuskanen et al., 2011). However, evidence of the detection of viruses in asymptomatic individuals has raised concern about the clinical significance of these positive findings. Attribution of causality is not straightforward, as viruses can commonly be found both in symptomatic but also asymptomatic individuals (Jartti et al., 2008; Ruuskanen et al., 2011; Rudan et al., 2013; O'Brien et al., 2019). While the causative role of RSV, influenza, adenovirus, human metapneumovirus, and bocavirus in childhood pneumonia is well-established, the pathogenic role of other viruses such as rhinovirus is still questioned (Fry et al., 2007; Caracciolo et al., 2008; Ruuskanen et al., 2011; Shi et al., 2017; Jayaweera et al., 2018; O'Brien et al., 2019). Using molecular methods, rhinovirus has been shown to be the most frequent respiratory pathogen isolated in children, and its detection in asymptomatic children is significantly higher than other respiratory viruses (Kusel et al., 2006; Jartti et al., 2008; Ruuskanen et al., 2011). Nevertheless, clinical relevance of rhinovirus has been proven by the association of this virus with respiratory symptoms in children, mainly wheezing (Kusel et al., 2006; Khetsuriani et al., 2007). In our series, 27.5% of the children with rhinovirus presented with wheezing. Infection with coronavirus (Cor229E, CorHKU1, CorNL63, CorOC43) was low in the present study. Similarly, the new coronavirus (SARS-CoV-2) seems to cause a low infection rate in children (World Health Organization, 2020). The reason why coronavirus infection rate in children is low is unknown.

In addition, the interpretation of positive viral findings is challenging due to the identification of multiple co-existing viral infections (Jartti et al., 2008; Ruuskanen et al., 2011). Co-infections were common in the present study, which is consistent with the existing literature (Ruuskanen et al., 2011; Jroundi et al., 2014; Jiang et al., 2017). Considering radiological pneumonia endpoint as a proxy for bacterial pneumonia, 27.0% of children with positive NPW findings had a viral-bacterial co-infection, and 40.0% of children with influenza detected in NPW had an influenza-bacterial co-infection. The contribution of viral-bacterial co-infections is well-acknowledged in the aetiology of childhood pneumonia, particularly the interaction between influenza virus and *S. pneumoniae* (O'Brien et al., 2000; Kwofie et al., 2012; Brealey et al., 2015). The combined effect of bacteria and viruses was shown to increase the severity of the disease, and bidirectional interactions have been described: respiratory viruses leading to bacterial superinfection, and bacteria pathogens promoting respiratory viral superinfections (Brealey et al., 2015). However, there is still a lack of robustness supporting these findings.

This study has several limitations. Most children in the present study lived in Thimphu, and the microbiological findings may not be generalized to the rest of the country. Bhutan is very diverse: comprised of cities, such as Thimphu, and isolated households in very remote areas, leading to different lifestyles and environmental exposures; and also diverse in terms of altitude, with different climates and precipitations.

Conclusions

The burden of pneumonia requiring hospitalization was highest among infants. Respiratory viruses were detected in a considerable number of children, although a clear pathogenic role cannot be established. Together with the relatively low proportion of children presenting a likely bacterial pneumonia – around a quarter as per positive blood culture and radiological findings – these findings emphasize the advanced stage of the epidemiologic transition that Bhutan seems to have reached. This study is the first step to better understand the aetiology and clinicopathological characteristics of pneumonia in Bhutanese children. Henceforth, the development of targeted pneumonia interventions and hypothesis-driven research is encouraged to reduce the morbidity and mortality associated with this disease. Fostering a robust pneumonia aetiology surveillance in children under five years of age appears important and would allow the assessment of the impact of the recently introduced PCV in reducing the burden of pneumonia.

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Ethical approval

The study protocol was approved by the Research Ethics Board of Health, Ministry of Health, in Thimphu in March 2017 (protocol number PO/2016/086), and by the research ethics committee from the Hospital Clínic in Barcelona (HCB/2017/0741).

Conflict of interest

No conflict of interest to declare.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at https://doi.org/10.1016/j.ijid.2020.04.017.

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