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RESEARCH ARTICLE

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Molecular detection of respiratory pathogens among children aged younger than 5 years hospitalized with febrile acute respiratory infections: A prospective hospital-based observational study in Niamey, Niger

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Abstract

Background and Aims: In Niger, acute respiratory infections (ARIs) are the second most common cause of death in children aged younger than 5 years. However, the etiology of ARI is poorly understood in the country. This study aims to describe viral and bacterial infections among children aged younger than 5 years hospitalized with febrile ARI at two hospitals in Niamey, Niger's capital city, and the reported clinical procedures.

Methods: We conducted a prospective study among children aged younger than 5 years hospitalized with febrile ARI at two national hospitals in Niamey between January and December 2015. Clinical presentation and procedures during admission were documented using a standardized case investigation form. Nasopharyngeal specimens collected from each patient were tested for a panel of respiratory viruses and bacteria using the Fast Track Diagnostic 21 Plus kit.

Results: We enrolled and tested 638 children aged younger than 5 years, of whom 411 (64.4%) were aged younger than 1 year, and 15 (2.4%) died during the study period. Overall, 496/638 (77.7%) specimens tested positive for at least one respiratory virus or bacterium; of these, 195 (39.3%) tested positive for respiratory viruses, 126 (25.4%) tested positive for respiratory bacteria, and 175 (35.3%) tested positive for both respiratory viruses and bacteria. The predominant viruses detected were respiratory syncytial virus (RSV) (149/638; 23.3%), human parainfluenza virus (HPIV) types 1 to 4 (78/638; 12.2%), human rhinovirus (HRV) (62/638; 9.4%), human adenovirus (HAV) (60/638; 9.4%), and influenza virus (INF) (52/638; 8.1%). *Streptococcus pneumoniae* (249/638; 39.0%) was the most frequently detected bacterium, followed by *Staphylococcus aureus* (112/638; 12.2%) and *Haemophilus influenzae* type B (16/638; 2.5%). Chest X-rays were performed at the discretion of the attending physician on 301 (47.2%) case patients. Of these patients, 231 (76.7%) had abnormal

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radiological findings. A total of 135/638 (21.2%) and 572/638 (89.7%) children received antibiotic treatment prior to admission and during admission, respectively.

Conclusion: A high proportion of respiratory viruses was detected among children aged younger than 5 years with febrile ARI, raising concerns about excessive use of antibiotics in Niger.

KEYWORDS

bacteria, children, febrile acute respiratory infection, Niger, viruses

1 | INTRODUCTION

Acute respiratory infections (ARIs) are responsible for elevated childhood morbidity and mortality globally,[1,2] accounting for approximately four million deaths among children aged younger than 5 years in 2010 and resulting in substantial burden of healthcare systems.[3,4] In developing countries, ARIs account for 19% of all deaths among children aged younger than 5 years and 8.2% of all disabilities and premature deaths.[5,6] Therefore, data on the epidemiology and seasonality of ARI are important to develop control and prevention strategies.[7]

In Niger, the estimated mortality rate among children aged younger than 5 years was 114 per 1000 in 2012 (UNICEF child mortality estimate), and more than 80% of these deaths were associated with malaria, respiratory infections, and diarrhea. According to the 2012 Niger health statistics yearbook, the estimated case fatality proportion associated with respiratory infections was 8.7%.

In the absence of laboratory diagnosis, it is difficult to clinically differentiate between ARI-related causative pathogens, due to similarities of symptoms.[8,9] The main causative agents of ARI are viruses and bacteria.[10] Major viruses include respiratory syncytial virus (RSV), human rhinovirus (HRV), influenza virus (INF) types A and B, human adenovirus (HAV), human parainfluenza virus (HPIV), human metapneumovirus (HMPV), and human coronavirus (HCOV), while major bacterial pathogens include *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Haemophilus influenzae*.[11-13]

In Niger, there is scarcity of data on ARIs, although a routine influenza surveillance system exists. INF has been detected in 11% of samples from children aged younger than 5 years hospitalized with severe acute respiratory illness (SARI).[14] Knowledge on the etiology of ARI in Niger could inform the selection of other important pathogens to be included in routine surveillance and improve the clinical management of patients.

This study aims to describe the viral and bacterial infections among children aged younger than 5 years hospitalized with febrile ARI at two national hospitals of Niamey, the capital city of Niger, and the reported clinical procedures.

2 | METHODS

2.1 | Study design and setting

We conducted a prospective study among children aged younger than 5 years hospitalized with febrile ARI between January and December 2015. Niger has four distinct seasons as categorized by the national directorate of meteorology: the cold season (mid-December to mid-February), the dry season (mid-February to May), the rainy season (June to September), and the hot season (October to mid-December).[15]

This study was part of the "TOTAL Niger Infection Respiratoire Aiguë" (TONIRA) Project, which was funded by the TOTAL Corporate Foundation in order to strengthen medical care of febrile ARI among children aged younger than 5 years. The study was conducted at the pediatric wards of two national tertiary hospitals situated in Niamey, namely, the Hôpital National de Niamey (HNN) and the Hôpital National Lamordé (HNL). These two hospitals provide general health care to the population of Niamey estimated at 1.1 million in 2015, as well as to patients referred from across the country. Laboratory detection of respiratory viruses and bacteria were conducted at the Centre de Recherche Médicale et Sanitaire (CERMES), Niamey, Niger (the National Reference Laboratory for influenza).

Demographic and clinical data, as well as the use of antibiotics before and during admission, were collected using standard data collection forms. Malaria testing was implemented on site using thick blood film microscopy. Chest X-rays were performed at the discretion of the attending physician, and typical abnormal radiological findings assessed included pulmonary opacities, acute bronchiolitis, and enlargement of intercostal spaces. Malnutrition was determined using the ratio of weight for height, and its level was classified as moderate or severe by the attending physician using national standards based on the 2010 Technical Guidelines for Integrated Disease Surveillance and Response in the African Region (Retrieved from https://www.afro.who.int/sites/default/files/2017-06/IDSR-Technical-Guidelines_Final_2010_0.pdf). Febrile ARI in conjunction with malaria, malnutrition, and diarrhea were assessed during admission using a standardized case investigation form. In-hospital outcome (ie., discharge, referral, or death) was recorded for all enrolled patients.

2.2 | Case definition

A febrile ARI case was defined as a hospitalized child aged younger than 5 years with onset of fever 38°C or higher and cough within 10 days prior to admission and at least one of the following signs: inability to drink or breastfeed, lethargy, vomiting, convulsions, nasal flaring, chest indrawing, stridor in a calm child, or tachypnea.

2.3 | Sampling and laboratory testing

Nasopharyngeal swabs were collected from all enrolled patients, placed in universal transport medium, stored at 4 to 8°C, and transported to CERMES within 72 hours of collection, for testing. Respiratory samples were tested for INF types A and B; HPIV types 1 to 4; HCoV NL63, 229E, OC43, and HKU1; HMPV A and B; RSV A and B; human parechovirus (HPV); human enterovirus (HEV); HAV; human bocavirus (HBoV); HRV; *S pneumoniae*; *H influenzae* type B (Hib); *S aureus*; *Mycoplasma pneumoniae*; and *Chlamydia pneumoniae* using the RT-qPCR Fast Track Diagnostic Respiratory pathogens 21 Plus kit from Bio-Mérieux (catalog no. FTD 2 +-96/12), following the manufacturer's instructions. An internal control provided as part of the kit was used to monitor the sample extraction and reverse transcription. RT-qPCR was performed using the FTD Respiratory pathogens 21 plus kit as follows: the reaction volume for each test was 25 μL, consisting of 10 μL of nucleic acid and 15 μL of buffer/enzyme mix from the AgPath-ID One-Step RT-PCR kit (Ambion, Life Technologies, USA, Catalog number 4387424). Amplification was performed using ABI 7500 Fast real-time PCR system thermocycler (Applied Biosystems, USA,) and the following cycling conditions were used: 50°C at 15 minutes for reverse transcription, 95°C at 10 minutes for enzyme activation, and 40 cycles of 95°C at 8 seconds and 60°C at 34 seconds for

TABLE 1 Detection of respiratory viruses and bacteria among children aged <5 years hospitalized with febrile acute respiratory infection in Niamey, Niger, 2015

		Detection n (%)			
Characteristics	**Population N (%)**	**Any virus or bacterium**	**Viruses only**	**Bacteria only**	**Viruses and bacteria**
Total	638	496 (77.7)	195 (30.6)	126 (19.7)	175 (27.4)
Age (in y)					
<1	411 (64.4)	327 (79.6)	141 (34.3)	70 (17.0)	116 (28.2)
1-4	227 (35.6)	169 (74.4)	54 (23.8)	56 (24.7)	59 (26.0)
Sex					
Female	291 (45.6)	231 (79.4)	86 (29.5)	61 (21.0)	84 (28.9)
Male	347 (54.4)	265 (76.4)	109 (31.4)	65 (18.7)	91 (26.2)
Season of the year[a]					
Cold	108 (84.3)	64 (59.3)	27 (25.0)	25 (23.1)	12 (11.1)
Dry	213 (33.4)	175 (82.2)	80 (37.6)	28 (13.1)	67 (31.5)
Rainy	191 (29.9)	160 (83.8)	40 (20.9)	57 (29.8)	63 (33.0)
Hot	126 (19.8)	97 (77.0)	48 (38.1)	16 (12.7)	33 (26.2)
Malaria					
No	538 (84.3)	415 (77.1)	159 (29.6)	110 (20.4)	146 (27.1)
Yes	100 (15.7)	81 (81.0)	36 (36.0)	16 (16.0)	29 (29.0)
Diarrhea					
No	524 (82.1)	407 (77.7)	164 (31.3)	100 (19.1)	143 (27.3)
Yes	114 (17.9)	89 (78.1)	31 (27.2)	26 (22.8)	32 (28.1)
Malnutrition					
No	524 (82.1)	406 (77.5)	154 (29.4)	102 (19.5)	150 (28.6)
Moderate	40 (6.3)	34 (85.0)	17 (42.5)	5 (12.5)	12 (30.0)
Severe	74 (11.6)	56 (75.7)	24 (32.4)	19 (25.7)	13 (17.6)
Antibiotics on admission					
No	66 (10.3)	50 (75.8)	20 (30.3)	9 (13.6)	21 (31.8)
Yes	572 (89.7)	446 (78.0)	175 (30.6)	117 (20.4)	154 (26.9)
Death					
No	623 (97.6)	482 (77.4)	188 (30.2)	122 (19.6)	172 (27.6)
Yes	15 (2.4)	14 (93.3)	7 (46.7)	4 (26.7)	3 (20.0)

[a]When the patient was hospitalized.

amplification. The fluorescence was assessed at the amplification step. The positive and negative virus plasmid controls provided in the kit were included in all runs to monitor assay performance.[16]

2.4 | Statistical analysis

Viral and/or bacterial detection was reported as percentage positive, overall and within selected categories (eg, age groups and seasons). Children aged younger than 5 years were stratified into two age groups: infants (aged younger than 1 year) and young children (aged 1-4 years), for comparability with other studies from Africa.[17-20] In addition, the majority of enrolled children were aged younger than 1 year hindering our ability to categorize age in smaller age groups. Stata version 14.2 (StataCorp, College Station, Texas, USA) was used for the analysis.

2.5 | Ethical approval

The protocol was approved by the National Ethical Consultative Committee (CCNE) of Niger and by the Clinical Research Committee (CoRC) of the Institut Pasteur, Paris. Proxy informed consent was obtained from parents or legal guardians of children. All children who did not meet the case definition or for whom verbal consent was not obtained were not included in the study.

3 | RESULTS

3.1 | Study population

Between January and December 2015, we enrolled and tested 638 children aged younger than 5 years. Of these, 411 (64.4%) were aged younger than 1 year, 347 (54.4%) were male, and 365 (57.3%) were admitted at the HNN. Of the comorbidities investigated, malaria, malnutrition, and diarrhea were diagnosed in 100 (15.7%), 114 (17.9%), and 114 (17.9%) case patients, respectively. Of the 114 malnourished children identified, 74 (64.9%) had severe malnutrition (Table 1). Chest X-rays were performed on 301 (47.2%) case patients. Of these patients, 231 (76.7%) had abnormal radiological findings, of which the most common were pulmonary opacity (106; 45.9%), extension of the intercostal space (99; 42.8%), and bronchiolitis (21; 9.1%). One hundred thirty-five (21.2%) children received antibiotic treatment within 3 days prior to admission, and 572 (89.7%) children were prescribed antibiotic treatment during admission. The in-hospital case fatality proportion was 2.4% (15/638) overall; 2.9% (12/411) and 1.3% (3/227) among children aged younger than 1 and 1 to 4 years, respectively.

3.2 | Detection of viral and bacterial agents

Overall, 496/638 (77.7%) specimens tested positive for at least one respiratory virus or bacterium (Table 1). Of the 496 positive specimens, 195 (39.3%) tested positive for respiratory viruses only, 126 (25.4%) tested positive for respiratory bacteria only, and 175 (35.3%) tested positive for both respiratory viruses and bacteria. Single virus or bacterium was detected in 229/496 (46.2%) positive specimens, of which 140 (61.1%) were single viral infections (Figure 1). The number of viral and/or bacterial detection among positive specimens ranged between 1 and 5.

Antibiotics were prescribed to 89.7% (175/195), 92.8% (117/126), and 88.0% (154/175) of patients with viral only, bacterial only, or viral and bacterial detection, respectively.

3.3 | Individual detection of respiratory viruses

Overall, respiratory viruses were detected in 370/638 (58.0%) specimens. RSV (149/638; 23.3%), HPIV types 1 to 4 (78/638; 12.2%), HRV (62/638; 9.4%), HAV (60/638; 9.4%), and INF (52/638; 8.1%) were the predominant viruses detected (Table 2). Of the HPIV-

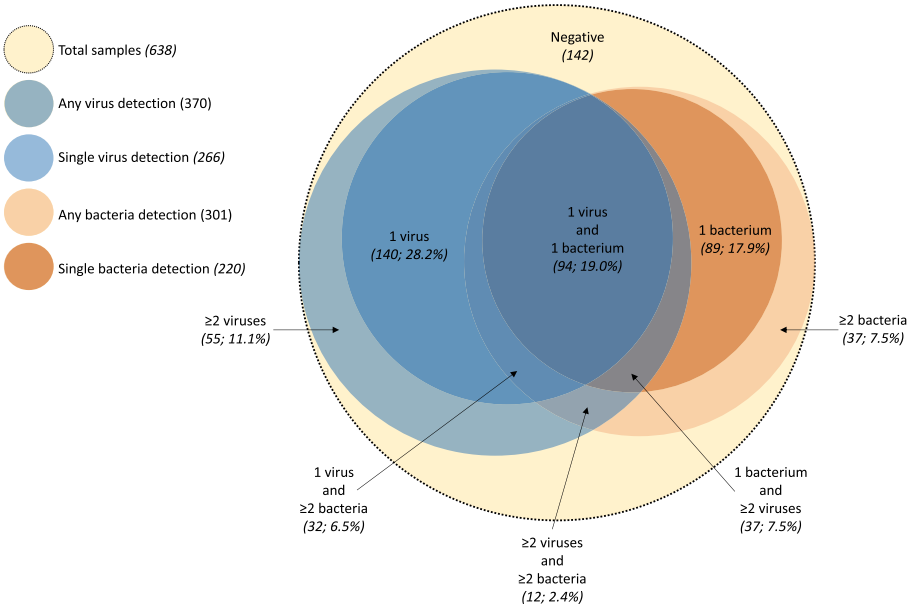

FIGURE 1 Detection (including codetection) of respiratory viruses and bacteria among children aged <5 years hospitalized with febrile acute respiratory infection in Niamey, Niger, 2015 (the size of the circles is proportional to the number of specimens within each category; percentages are expressed over total number of positive specimens: 496)

TABLE 2 Detection of individual respiratory viruses among children aged <5 years hospitalized with febrile acute respiratory infection in Niamey, Niger, 2015

	Detection n (%)										
Characteristics	**RSV**	**HPIV**	**HRV**	**HAV**	**INF**	**HCoV**	**HMPV**	**HBoV**	**HEV**	**HPV**	**Any virus**
Total (N = 638)	149 (23.3)	78 (12.2)	62 (9.7)	60 (9.4)	52 (8.1)	51 (8.0)	28 (4.4)	22 (3.4)	8 (1.2)	1 (0.2)	370 (58.0)
Age (in y)											
<1 (N = 411)	119 (28.9)	59 (14.4)	38 (9.2)	39 (9.5)	32 (7.8)	37 (9.0)	19 (4.6)	18 (4.4)	5 (1.2)	1 (0.2)	257 (62.5)
1-4 (N = 227)	30 (13.2)	19 (8.4)	24 (10.6)	21 (9.2)	20 (8.8)	14 (6.2)	9 (4.0)	4 (1.8)	3 (1.3)	0 (0.0)	113 (49.8)
Sex											
Female (N = 291)	65 (22.3)	39 (13.4)	25 (8.6)	25 (8.6)	26 (8.9)	26 (8.9)	14 (4.8)	9 (3.9)	2 (0.7)	0 (0.0)	170 (58.4)
Male (N = 347)	84 (24.2)	39 (11.2)	37 (10.7)	35 (10.1)	26 (7.5)	25 (7.2)	14 (4.0)	13 (3.7)	6 (1.7)	1 (0.3)	200 (57.6)
Season of the year[a]											
Cold (N = 108)	12 (11.1)	4 (3.7)	3 (2.8)	4 (3.7)	16 (14.8)	4 (3.7)	2 (1.8)	4 (3.7)	1 (0.9)	0 (0.0)	39 (36.1)
Dry (N = 213)	63 (29.6)	44 (20.7)	24 (11.3)	24 (11.3)	17 (8.0)	31 (14.5)	11 (5.1)	4 (1.9)	1 (0.5)	1 (0.5)	147 (69.0)
Rainy (N = 191)	18 (9.4)	19 (9.9)	24 (12.6)	22 (11.5)	13 (6.8)	16 (8.4)	4 (2.1)	12 (6.3)	6 (3.1)	0 (0.0)	103 (53.9)
Hot (N = 126)	56 (44.4)	11 (8.7)	11 (8.7)	10 (7.9)	6 (4.8)	0 (0.0)	11 (8.7)	2 (1.6)	0 (0.0)	0 (0.0)	81 (64.3)
Malaria											
No (N = 538)	116 (21.6)	63 (11.7)	47 (8.7)	49 (9.1)	46 (8.5)	48 (8.9)	23 (4.3)	20 (3.6	6 91.1)	1 (0.2)	305 (56.7)
Yes (N = 100)	33 (33.0)	15 (15.0)	15 (15.0)	11 (11.0)	6 (6.00)	3 (3.0)	5 (5.0)	2 (2.0)	2 (2.0)	0 (0.0)	65 (65.0)
Diarrhea											
No (N = 524)	126 (24.0)	67 (12.8)	50 (9.5)	48 (9.2)	42 (8.0)	37 (7.1)	24 (4.6)	18 (3.4)	8 (1.5)	0 (0.0)	307 (58.6)
Yes (N = 114)	23 (20.2)	11 (9.6)	12 (10.5)	12 (10.5)	10 (8.8)	14 (12.3)	4 (3.5)	4 (3.5)	0 (0.0)	1 (0.9)	63 (55.3)
Malnutrition											
No (N = 524)	124 (23.7)	65 (12.4)	48 (9.2)	42 (8.1)	42 (8.0)	41 (7.8)	25 (4.8)	20 (3.8)	6 (1.1)	1 (0.2)	304 (58.0)
Moderate (N = 40)	12 (30.0)	5 (12.5)	7 (17.5)	7 (17.5)	6 (15.0)	5 (12.5)	1 (2.5)	2 (5.0)	0 (0.0)	0 (0.0)	29 (72.5)
Severe (N = 74)	13 (17.6)	8 (10.8)	7 (9.5)	11 (14.9)	4 (5.4)	5 (6.8)	2 (2.7)	0 (0.0)	2 (2.7)	0 (0.0)	37 (50.0)
Antibiotics on admission											
No (N = 66)	17 (15.8)	9 (13.6)	10 (15.1)	6 (9.1)	6 (9.1)	8 (12.1)	5 (7.6)	2 (3.0)	0 (0.0)	0 (0.0)	41 (62.1)
Yes (N = 572)	132 (23.1)	69 (12.1)	59 (9.1)	54 (9.4)	46 (8.0)	43 (7.5)	23 (4.2)	20 (3.5)	8 (1.4)	1 (0.2)	329 (57.5)
Death	1.00	.704	.002	.644	1.000	.340	1.000	1.000	1.000	1.000	.602
No (N = 623)	146 (23.4)	76 (12.2)	56 (9.0)	58 (9.3)	51 (8.2)	49 (7.9)	28 (4.5)	22 (3.5)	8 (1.3)	1 (0.2)	360 (57.8)
Yes (N = 15)	3 (20.0)	2 (13.3)	6 (40.0)	2 (13.3)	1 (6.7)	2 (13.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	10 (66.7)
Bacteria											
Any (N = 301)	67 (22.3)	32 (10.6)	30 (10.0)	31 (10.3)	25 (8.3)	23 (7.6)	12 (4.0)	13 (4.3)	5 (1.7)	1 (0.3)	175 (58.1)
Spneu (N = 249)	50 (20.1)	28 (11.2)	24 (9.6)	28 (11.2)	20 (8.1)	18 (7.2)	11 (4.4)	10 (4.0)	3 (1.2)	1 (0.4)	142 (57.0)

(Continues)

TABLE 2 (Continued)

Characteristics	Detection n (%)										
	RSV	HPIV	HRV	HAV	INF	HCoV	HMPV	HBoV	HEV	HPV	Any virus
Saur (N = 112)	26 (23.2)	10 (8.9)	11 (9.8)	9 (8.0)	8 (7.1)	8 (7.1)	5 (4.5)	7 (6.2)	3 (2.7)	1 (0.9)	65 (58.0)
Hib (N = 16)	2 (12.5)	3 (18.7)	2 (12.5)	2 (12.5)	4 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	8 (50.0)
Mpneu (N = 4)	0 (0.0)	0 (0.0)	1 (25.0)	1 (25.0)	1 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (50.0)
Cpneu (N = 2)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100.0)

Abbreviations: Cpneu, *Chlamydia pneumonia*; HAV, human andenovirus; HBoV, human bocavirus; HCoV, human coronavirus; HEV, human enterovirus; Hib, *Haemophilus influenzae* type B; HMPV, human metapneumovirus; HPV, human parechovirus; HPVI, human parainfluenza virus; HRV, human rhinovirus; INF, influenza virus; Mpneu, *Mycoplasma pneumoniae*; RSV, respiratory syncytial virus; Saur, *Staphylococcus aureus*; Spneu, *Streptococcus pneumoniae*.

[a]When the patient was hospitalized.

positive specimens, HPIV-3 was the most commonly detected one (48/78; 61.5%), whereas INF type A predominated among the influenza-positive specimens (46/52; 88.5%). HCoVs were detected in 8.0% (51/638) of specimens, and OC43 was the mostly detected one (44/51; 86.3%). HMPV, HBoV, and HEV were detected individually in less than 5% of specimens. HPV was the least commonly detected respiratory virus (1/638; 0.2%).

Of the specimens that tested positive for at least one respiratory virus, two or more respiratory viruses were detected in 104/370 (28.1%) specimens, of which dual detection occurred in 72/104 (69.2%) specimens. RSV (95/149; 63.8%), HMPV (17/28; 60.7%), and INF (31/52; 59.6%) were mostly detected in specimens with single viral infection, whereas HPV (1/1; 100.0%), HEV (6/8; 75.0%), HAV (38/60; 63.3%), HBoV (13/22; 59.1%), and HRV (34/62; 54.8%) were mostly detected in specimens with two or more viral infections (Figure 2A). The number of viral detections among virus-positive specimens ranged between 1 and 4. Among patients with abnormal radiological findings, respiratory viruses were most commonly detected among patients with bronchiolitis (19/21; 90.5%). RSV and HPIV were mostly detected among children aged younger than 1 compared with 1 to 4 years (Table 2).

Regarding seasonality, INF was mostly detected during the cold season, HPIV and HCoV were mostly detected during the dry season, HRV was mostly detected during the rainy season, and RSV and HMPV were mostly detected during the hot season (Table 2 and Figure 3A).

3.4 | Individual detection of respiratory bacteria

Overall, respiratory bacteria were detected in 301/638 (47.2%) specimens. *S pneumoniae* (249/638; 39.0%) was the most frequently detected bacterium, followed by *S aureus* (112/638; 12.2%) and Hib (16/638; 2.5%) (Table 3). *M pneumoniae* and *C pneumoniae* were detected individually in less than 1% of specimens.

Of the specimens that tested positive for at least one respiratory bacteria, two or more respiratory bacteria were detected in 81/220 (26.9%) specimens, of which dual detection occurred in 80/81 (98.8%) of specimens. *S pneumoniae* (170/249; 68.3%) was mostly detected in specimens with a single bacterial infection, whereas Hib (13/16; 54.8%) and *S aureus* (68/112; 60.7%) were mostly detected in specimens with two or more bacterial infections (Figure 2B). The number of bacterial detections among bacteria-positive specimens ranged between 1 and 3. Among patients with abnormal radiological findings, respiratory bacteria were most commonly detected among patients with pulmonary opacity (87/106; 90.5%).

S pneumoniae and *S aureus* were mostly detected during the rainy season (June to September) (Table 3 and Figure 3B).

4 | DISCUSSION

We report the detection of respiratory viruses and bacteria among children aged younger than 5 years hospitalized with febrile ARI in

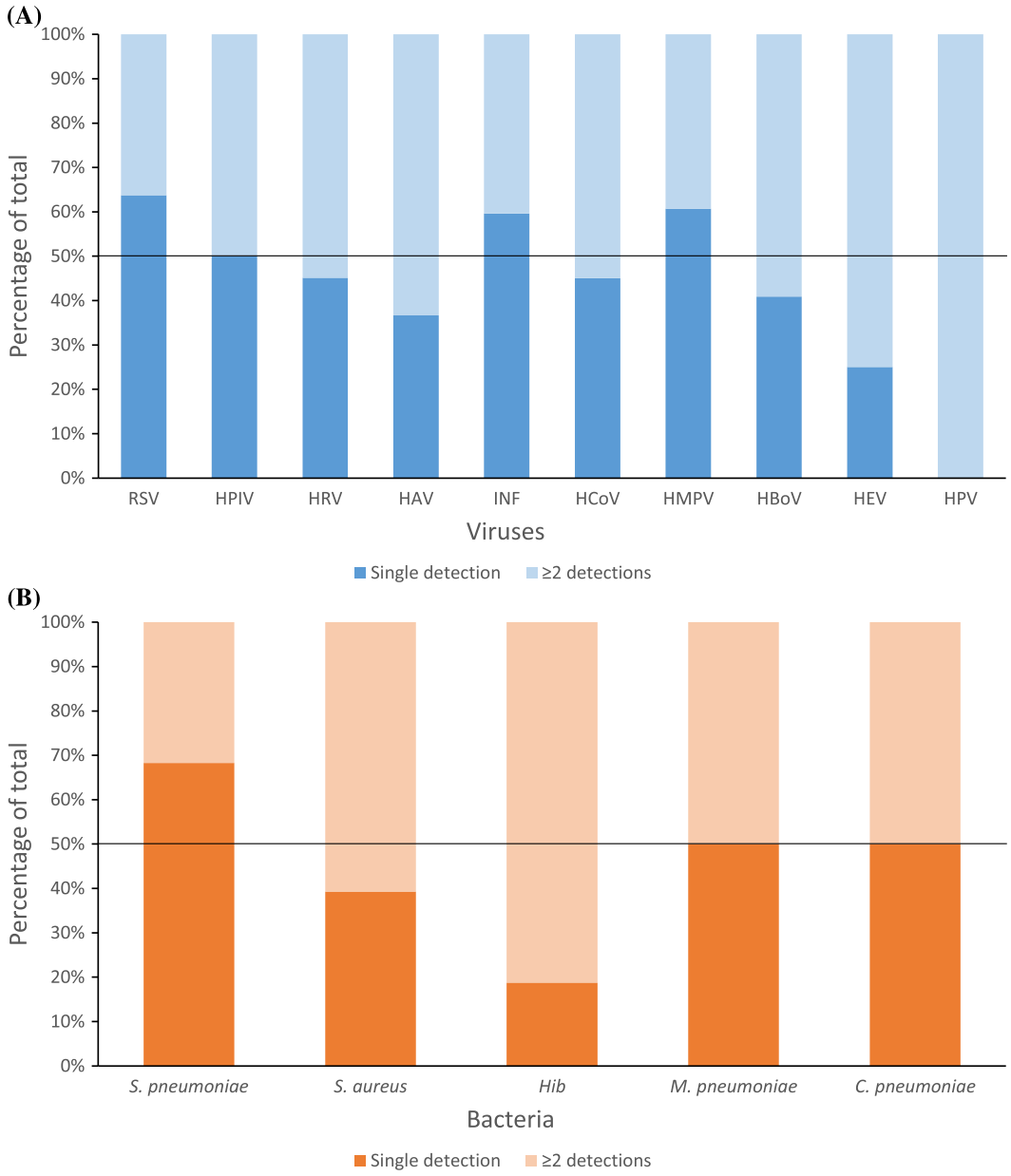

FIGURE 2 Proportion of single and multiple (A) viral and (B) bacterial detections among children aged <5 years hospitalized with febrile acute respiratory infection in Niamey, Niger, 2015. HAV, human adenovirus; HBoV, human bocavirus; HCoV, human coronavirus; HEV, human enterovirus; HPIV, human parainfluenza virus; HPV, human parechovirus; HRV, human rhinovirus; INF, influenza virus; RSV, respiratory syncytial virus

Niger between January and December 2015. At least one respiratory virus or bacteria was found in 77.7% of samples tested. This proportion was similar to that reported in a previous study on the etiology of ARI in children aged younger than 5 years in Niger (78%), although on a limited number of samples.[8] This finding was also consistent with those of other similar studies conducted in Africa (range 55%-70%)[21,22] and Asia (range 45%-65%).[23-25]

The positivity proportion of respiratory viruses varied considerably. RSV, HPIV, HRV, HAV, and INF were the most commonly detected viruses. As reported in other studies, RSV, INF, and HMPV were mostly detected as single viral infections.[26,27] RSV, HRV, and HPIV were also the most predominant viruses detected in children aged younger than 5 years in a previous study conducted in Niger.[8] RSV is considered to be a major cause of ARI in children aged younger than 5 years,[26,28-30] and it was the most commonly detected virus also in this study (149/638; 23.3%).

Among the cases in which HPIV and HCoV were detected, HPIV type 3 and HCoV type OC43 were the predominant types, and this is consistent with previous studies.[27,31] In this study, INFs were detected in 8.5% of the cases, which is in agreement with findings from the national influenza surveillance[14] and other related studies in Africa among patients hospitalized with SARI.[18,32,33] A higher influenza positivity proportion has been reported among outpatients with influenza-like illness.[13,28]

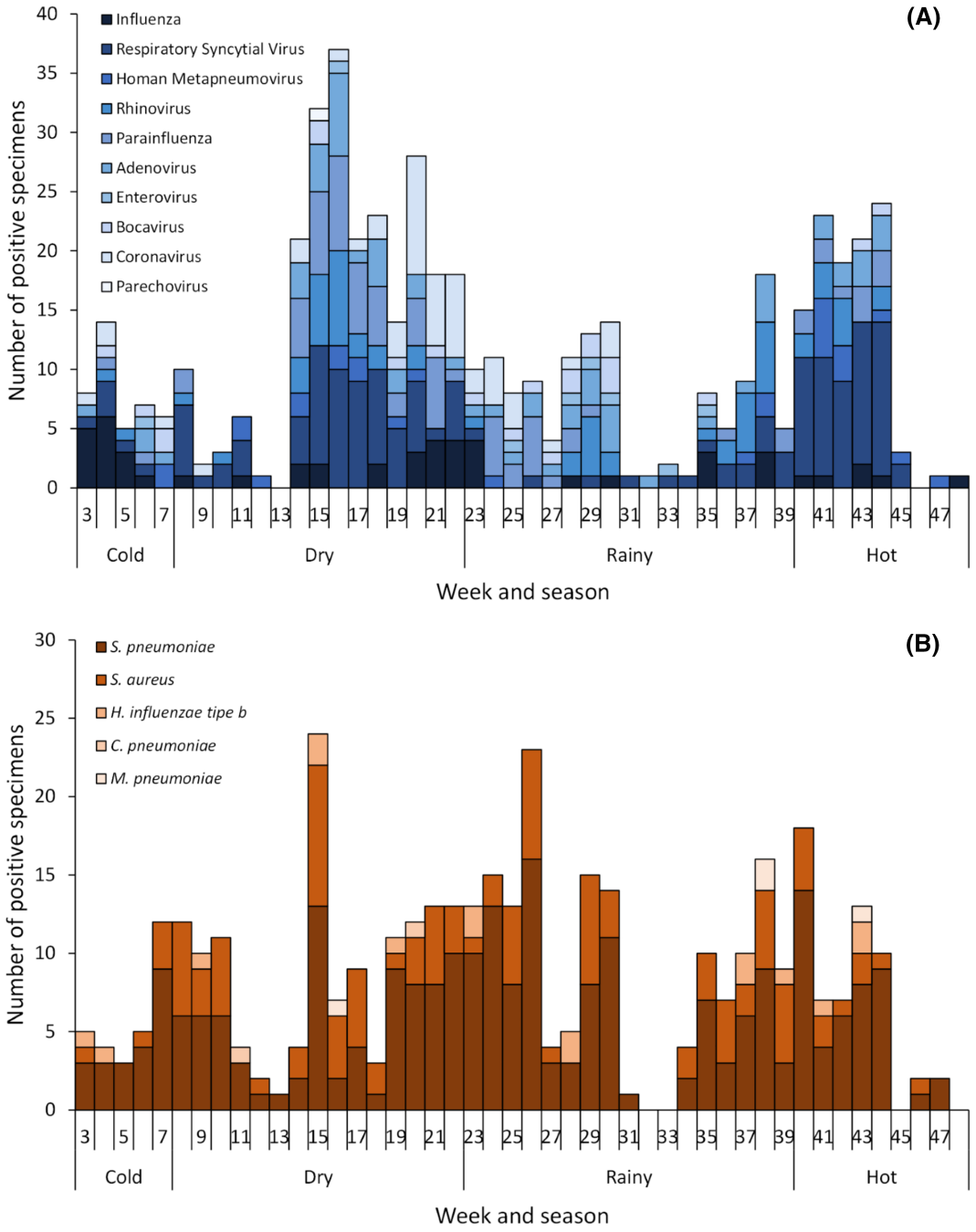

FIGURE 3 Weekly number of specimens positive for respiratory (A) viruses and (B) bacteria in Niamey, Niger, 2015

During the 1-year study period, respiratory viruses were detected throughout the four seasons.[15] However, RSV was mostly detected during the hot and dry seasons with higher temperature and lower relative humidity,[7] whereas INFs were mostly detected during the cold and rainy seasons, as reported from influenza sentinel surveillance in Niger.[14,34] If these seasonal patterns are confirmed in multiyear studies, they could be useful for epidemic preparedness and response plans.

High colonization rates of *S pneumoniae*, *S aureus*, and Hib have been reported in children, but only a fraction of colonization events have been reported to result in disease.[35,36] *S pneumoniae* was detected in 39.5% of specimens, and it was the bacteria predominantly detected in our samples. However, the colonization rate found in this study was lower than that reported in a study conducted in 2014 (54.5%), which could be attributed to the introduction of the 13-valent pneumococcal conjugate vaccination (PCV13) in Niger that same year.[37] The very low detection rate of Hib (2.5%) may also be attributed to the introduction of the Hib conjugate (tetanus toxoid) vaccine in the expanded immunization program in 2008 in Niger.[38] Uncommon respiratory bacterial pathogens including *M pneumoniae* and *C pneumoniae* were detected at a very low rate (less than 1%), and this correlates with the results reported in previous studies.[10,26,27]

TABLE 3 Detection of individual respiratory bacteria among children aged <5 years hospitalized with febrile acute respiratory infection in Niamey, Niger, 2015

Characteristics	Detection n (%)					
	Streptococcus pneumoniae	*Staphylococcus aureus*	*Haemophilus influenzae* type B	*Mycoplasma pneumoniae*	*Chlamydia pneumoniae*	Any bacterium
Total (N = 638)	249 (39.0)	112 (17.5)	16 (2.5)	4 (0.6)	2 (0.3)	301 (47.2)
Age (in y)						
<1 (N = 411)	152 (37.0)	68 (16.5)	8 (1.9)	3 (0.7)	1 (0.2)	186 (45.3)
1-4 (N = 227)	97 (42.7)	44 (19.4)	8 (3.5)	1 (0.4)	1 (0.4)	115 (50.7)
Sex						
Female (N = 291)	121 (41.6)	51 (17.5)	10 (3.4)	2 (0.7)	1 (0.3)	145 (49.8)
Male (N = 347)	128 (36.9)	61 (17.6)	6 (1.7)	2 (0.6)	1 (0.3)	156 (45.0)
Season of the year[a]						
Cold (N = 108)	31 (28.7)	11 (10.2)	3 (2.8)	0 (0.0)	0 (0.0)	37 (34.3)
Dry (N = 213)	69 (32.4)	43 (20.2)	3 (1.4)	1 (0.5)	2 (0.9)	95 (44.6)
Rainy (N = 191)	105 (55.0)	47 (24.6)	7 (3.7)	2 (1.0)	0 (0.0)	120 (62.8)
Hot (N = 126)	44 (34.9)	11 (8.7)	3 (2.4)	2 (0.8)	0 (0.0)	49 (38.9)
Malaria						
No (N = 538)	210 (39.0)	93 (17.3)	14 (2.6)	2 (0.4)	2 (0.4)	256 (47.6)
Yes (N = 100)	39 (39.0)	19 (19.0)	2 (2.0)	2 (2.0)	0 (0.0)	45 (45.0)
Diarrhea						
No (N = 524)	196 (37.4)	95 (18.1)	12 (2.3)	3 (0.6)	2 (0.4)	243 (46.4)
Yes (N = 114)	53 (46.5)	17 (14.9)	4 (3.5)	1 (0.9)	0 (0.0)	58 (50.9)
Malnutrition						
No (N = 524)	208 (39.7)	95 (18.1)	14 (2.7)	4 (0.8)	2 (0.4)	252 (48.1)
Moderate (N = 40)	14 (35.0)	6 (15.0)	0 (0.0)	0 (0.0)	0 (0.0)	17 (42.5)
Severe (N = 74)	27 (36.5)	11 (14.9(	2 (2.7)	0 (0.0)	0 (0.0)	32 (43.2)
Antibiotics on admission						
No (N = 66)	21 (31.8)	15 (22.7)	0 (0.0)	0 (0.0)	1 (1.5)	30 (45.4)
Yes (N = 572)	228 (39.9)	97 (17.0)	16 (2.8)	4 (0.7)	1 (0.2)	271 (47.4)
Death						
No (N = 623)	243 (39.0)	111 (17.8)	16 (2.6)	3 (0.5)	2 (0.3)	294 (47.2)
Yes (N = 15)	6 (40.0)	1 (6.7)	0 (0.0)	1 (6.7)	0 (0.0)	7 (46.7)
Viruses						
Any (N = 370)	142 (38.4)	65 (17.6)	8 (2.2)	2 (0.5)	2 (0.3)	175 (47.3)
RSV (N = 149)	50 (33.6)	26 (17.4)	2 (1.3)	0 (0.0)	1 (0.7)	67 (45.0)
HPIV (N = 78)	28 (35.9)	10 (12.8)	3 (3.8)	0 (0.0)	0 (0.0)	32 (41.0)
HRV (N = 62)	24 (38.7)	11 (17.7)	2 (3.2)	1 (1.6)	0 (0.0)	30 (48.4)
HAV (N = 60)	28 (46.7)	9 (15.0)	2 (3.3)	1 (1.7)	0 (0.0)	31 (51.7)
INF (N = 52)	20 (38.5)	8 (15.4)	4 (7.7)	1 (1.9)	0 (0.0)	25 (48.1)
HCoV (N = 51)	18 (35.3)	8 (15.7)	0 (0.0)	0 (0.0)	1 (2.0)	23 (45.1)
HMPV (N = 28)	11 (39.0)	5 (17.9)	0 (0.0)	0 (0.0)	0 (0.0)	12 (42.9)
HBoV (N = 22)	10 (45.4)	7 (31.8)	0 (0.0)	0 (0.0)	0 (0.0)	13 (59.1)
HPV (N=1)	1 (100.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)

Abbreviations: HAV, human andenovirus; HBoV, human bocavirus; HCoV, human coronavirus; HEV, human enterovirus; HMPV, human metapneumovirus; HPV, human parechovirus. HPVI, human parainfluenza virus; HRV, human rhinovirus; INF, influenza virus; RSV, respiratory syncytial virus.
[a]When the patient was hospitalized.

In our study, 21.2% of children had already received antibiotics before hospitalization, and nearly all children (89.7%) received antibiotics during admission. These findings are consistent with a study from Mali, which reported a rate of antibiotics uptake of 39.8% before admission and elevated antibiotics usage (more than 95%) on admission.[39] The national guidelines for ARI treatment based on the Integrated Management of Childhood Illness (Manuel sur la Prise en Charge Integrée des Maladies de l'Enfant (PCIME) (2001), retrieved from https://apps.who.int/iris/bitstream/handle/10665/67167/WHO_FCH_CAH_00.12_fre.pdf;jsessionid=65C124F019A55837AF0B229E9AB1B21A?sequence=1) include the systematic use of antibiotics for empiric treatment to prevent superinfection. However, considering the high number of patients from whom only respiratory viruses were detected, this practice raises concern about the potentially excessive use of antibiotics—and resistance development—in Niger.[28]

About 15% to 17% of patients with febrile ARI also had diarrhea, were malnourished, or were coinfected with malaria. This could be explained by the fact that these infections constitute some of the leading public health concerns in Niger and are responsible for elevated childhood mortality (Annuaire des statistiques sanitaires du Niger) (2016). Retrieved from http://www.stat-niger.org/statistique/file/Annuaires_Statistiques/snis/Annuaire_statistiques_2016.pdf).

Our study presents some limitations. First, the study spanned over a period of only 12 months, limiting our ability to fully assess the temporal circulation pattern of the investigated agents. Due to the small sample size, we may also have been underpowered to detect small variations in the temporal distribution of the investigated agents. Second, attribution of causality remains challenging due to the lack of controls in our study. The association of pathogen detection with illness could not be well assessed, although most of the viral and bacterial pathogens identified in this study have been described in previous case control studies as causative agents of ARI.[10,39] In addition, we did not collect blood samples to assess whether the detection of common bacterial colonizers of the nasopharynx, such as *S pneumoniae*, were associated with invasive disease. Third, we did not record the number of patients with febrile ARI that refused enrollment, hindering our ability to estimate underenrollment. Last, given that only patients with febrile ARI were enrolled, this could have resulted in an underestimation of the RSV burden since a large proportion of RSV-related illness is not associated with fever.[40]

5 | CONCLUSIONS

In this 1-year prospective study, both viral and bacterial pathogens were detected in high proportion among hospitalized children aged younger than 5 years with febrile ARI in Niamey, Niger. However, further investigations should be carried out to assess risk factors and association of pathogens with illness. Although national guidelines for ARI treatment recommend systematic empiric antibiotic therapy, our results suggest that antibiotic use might be unnecessary in most cases, given the predominance of viral infections as potential cause of febrile ARI. Furthermore, other predisposing diseases such as malaria, malnutrition, and diarrhea may be contributory factors to febrile ARI exacerbation among hospitalized children.

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Adamou Lagare had full access to the data and takes responsibility for the integrity of the data and the accuracy of the data analysis.

TRANSPARENCY STATEMENT

The lead author/manuscript guarantor (Adamou Lagare) affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

CONFLICTS OF INTERESTS

All authors declare that they have no commercial or other associations that may pose a conflict of interest.

DISCLAIMER

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the US Centers for Disease Control and Prevention, USA.

DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

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REFERENCES

1. Roth DE, Gaffey MF, Smith-Romero E, Fitzpatrick T, Morris SK. Acute respiratory infection case definitions for young children: a systematic review of community-based epidemiologic studies in South Asia. *Trop Med Int Health*. 2015;20(12):1607-1620.
2. Williams BG, Gouws E, Boschi-Pinto C, Bryce J, Dye C. Estimates of world-wide distribution of child deaths from acute respiratory infections. *Lancet Infect Dis*. 2002;2(1):25-32.
3. Nair H, Simoes EA, Rudan I, et al. Global and regional burden of hospital admissions for severe acute lower respiratory infections in young children in 2010: a systematic analysis. *Lancet*. 2013;381(9875):1380-1390.
4. Lozano R, Naghavi M, Foreman K, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*. 2012;380(9859):2095-2128.
5. Sentilhes AC, Choumlivong K, Celhay O, et al. Respiratory virus infections in hospitalized children and adults in Lao PDR. *Influenza Other Respi Viruses*. 2013;7(6):1070-1078.
6. Communiqué from the International Conference on Acute Respiratory Infections hiC, Australia. 7-10et al.: Acute respiratory infections: the forgotten pandemic. *Int J Tuberc Lung Dis* 1998. July 1997;2(1):2-4.
7. Shek LP, Lee BW. Epidemiology and seasonality of respiratory tract virus infections in the tropics. *Paediatr Respir Rev*. 2003;4(2):105-111.
8. Lagare A, Mainassara HB, Issaka B, Sidiki A, Tempia S. Viral and bacterial etiology of severe acute respiratory illness among children < 5 years of age without influenza in Niger. *BMC Infect Dis*. 2015;15(1):515.
9. Njouom R, Yekwa EL, Cappy P, Vabret A, Boisier P, Rousset D. Viral etiology of influenza-like illnesses in Cameroon, January-December 2009. *J Infect Dis*. 2012;206(Suppl 1):S29-S35.
10. Feikin DR, Njenga MK, Bigogo G, et al. Viral and bacterial causes of severe acute respiratory illness among children aged less than 5 years in a high malaria prevalence area of western Kenya, 2007-2010. *Pediatr Infect Dis J*. 2013;32(1):e14-e19.
11. Viegas M, Barrero PR, Maffey AF, Mistchenko AS. Respiratory viruses seasonality in children under five years of age in Buenos Aires, Argentina: a five-year analysis. *J Infect*. 2004;49(3):222-228.
12. Chang ML, Jordan-Villegas A, Evans A, et al. Respiratory viruses identified in an urban children's hospital emergency department during the 2009 influenza A(H1N1) pandemic. *Pediatr Emerg Care*. 2012;28(10):990-997.
13. Tsai HP, Kuo PH, Liu CC, Wang JR. Respiratory viral infections among pediatric inpatients and outpatients in Taiwan from 1997 to 1999. *J Clin Microbiol*. 2001;39(1):111-118.
14. Maïnassara HB, Lagare A, Tempia S, Sidiki A, Issaka B, Abdou Sidikou BO. OMO-B: Influenza sentinel surveillance among patients with influenza-like-illness and severe acute respiratory illness within the framework of the National Reference Laboratory, Niger, 2009-2013. *PLoS ONE*. 2015;10(7):0133178.
15. Halima B, Maïnassara AL, Tempia S, Sidiki A, Issaka B, Sidikou BA. Oukem-Boyer OOM: Influenza sentinel surveillance among patients with influenza-like-illness and severe acute respiratory illness within the framework of the National Reference Laboratory, Niger, 2009-2013. *PLoS ONE*. 2015;10(7):e0133178.
16. Martins Junior RB, Carney S, Goldemberg D, et al. Detection of respiratory viruses by real-time polymerase chain reaction in outpatients with acute respiratory infection. *Mem Inst Oswaldo Cruz*. 2014;109(6):716-721.
17. Kadjo HA, Ekaza E, Coulibaly D, et al. Sentinel surveillance for influenza and other respiratory viruses in Cote d'Ivoire, 2003-2010. *Influenza Other Respi Viruses*. 2013;7(3):296-303.
18. Kenmoe S, Tchendjou P, Vernet MA, et al. Viral etiology of severe acute respiratory infections in hospitalized children in Cameroon, 2011-2013. *Influenza Other Respi Viruses*. 2016;10(5):386-393.
19. Pretorius MA, Madhi SA, Cohen C, et al. Respiratory viral coinfections identified by a 10-plex real-time reverse-transcription polymerase chain reaction assay in patients hospitalized with severe acute respiratory illness—South Africa, 2009-2010. *J Infect Dis*. 2012;206(Suppl 1):S159-S165.
20. Subramoney K, Hellferscee O, Pretorius M, et al. Human bocavirus, coronavirus, and polyomavirus detected among patients hospitalised with severe acute respiratory illness in South Africa, 2012 to 2013. *Health Sci Rep*. 2018;1(8):e59.
21. Shafik CF, Mohareb EW, Yassin AS, et al. Viral etiologies of lower respiratory tract infections among Egyptian children under five years of age. *BMC Infect Dis*. 2012;12(1):350.
22. Niang MN, Diop OM, Sarr FD, et al. Viral etiology of respiratory infections in children under 5 years old living in tropical rural areas of Senegal: the EVIRA project. *J Med Virol*. 2010;82(5):866-872.
23. Tran DN, Trinh QD, Pham NT, et al. Clinical and epidemiological characteristics of acute respiratory virus infections in Vietnamese children. *Epidemiol Infect*. 2016;144(3):527-536.
24. Liu WK, Liu Q, de Chen H, et al. Epidemiology of acute respiratory infections in children in Guangzhou: a three-year study. *PLoS ONE*. 2014;9(5):e96674.
25. Bhuyan GS, Hossain MA, Sarker SK, et al. Bacterial and viral pathogen spectra of acute respiratory infections in under-5 children in hospital settings in Dhaka city. *PLoS ONE*. 2017;12(3):e0174488.
26. Bezerra PG, Britto MC, Correia JB, et al. Viral and atypical bacterial detection in acute respiratory infection in children under five years. *PLoS ONE*. 2011;6(4):e18928.
27. Hoffmann J, Rabezanahary H, Randriamarotia M, et al. Viral and atypical bacterial etiology of acute respiratory infections in children under 5 years old living in a rural tropical area of Madagascar. *PLoS ONE*. 2012;7(8):e43666.
28. Do AH, van Doorn HR, Nghiem MN, et al. Viral etiologies of acute respiratory infections among hospitalized Vietnamese children in Ho Chi Minh City, 2004-2008. *PLoS ONE*. 2011;6(3):e18176.
29. Chew FT, Doraisingham S, Ling AE, Kumarasinghe G, Lee BW. Seasonal trends of viral respiratory tract infections in the tropics. *Epidemiol Infect*. 1998;121(1):121-128.
30. Prill MM, Iwane MK, Little D, Gerber SI. Investigation of respiratory syncytial virus-associated deaths among US children aged <2 years, 2004-2007. *J Pediatric Infect Dis Soc*. 2016;5(3):333-336.
31. Linster M, Do LAH, Minh NNQ, et al. Clinical and molecular epidemiology of human parainfluenza viruses 1-4 in children from Viet Nam. *Sci Rep*. 2018;8(1):6833.

32. Ouedraogo S, Traore B, Nene Bi ZA, et al. Viral etiology of respiratory tract infections in children at the pediatric hospital in Ouagadougou (Burkina Faso). *PLoS ONE*. 2014;9(10):e110435.
33. Heraud JM, Njouom R, Rousset D, et al. Spatiotemporal circulation of influenza viruses in 5 African countries during 2008-2009: a collaborative study of the Institut Pasteur International Network. *J Infect Dis*. 2012;206(Suppl 1):S5-S13.
34. Jusot JF, Adamou L, Collard JM. Influenza transmission during a one-year period (2009-2010) in a Sahelian city: low temperature plays a major role. *Influenza Other Respi Viruses*. 2012;6(2):87-89.
35. Aydemir O, Aydemir Y, Ozdemir M. The role of multiplex PCR test in identification of bacterial pathogens in lower respiratory tract infections. *Pak J Med Sci*. 2014;30(5):1011-1016.
36. Maysaa G. Clinico-pathological study of atypical pathogens in community-acquired pneumonia: a prospective study. *J Infect Dev Ctries*. 2009;3(3):199-205.
37. Ousmane S, Diallo BA, Ouedraogo R, Sanda AA, Soussou AM, Collard JM. Serotype distribution and antimicrobial sensitivity profile of Streptococcus pneumoniae carried in healthy toddlers before PCV13 introduction in Niamey, Niger. *PLoS ONE*. 2017;12(1):e0169547.
38. Campagne G, Garba A, Schuchat A, et al. Response to conjugate Haemophilus influenzae B vaccine among infants in Niamey, Niger. *Am J Trop Med Hyg*. 1998;59(5):837-842.
39. Benet T, Sylla M, Messaoudi M, et al. Etiology and factors associated with pneumonia in children under 5 years of age in Mali: a prospective case-control study. *PLoS ONE*. 2015;10(12):e0145447.
40. Rha B, Dahl RM, Moyes J, et al. Performance of surveillance case definitions in detecting respiratory syncytial virus infection among young children hospitalized with severe respiratory illness-South Africa, 2009-2014. *J Pediatric Infect Dis Soc*. 2018;2(7):91-176.

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