Virology: Research & Reviews

Research Article ISSN: 2514-4138

Children under five years of age in senegal: A group highly exposed to respiratory viruses infections

Amary Fall[1], Assane Dieng[2], Serigne Fallou Wade[3], Abdoulaye Diop[2], Jean Baptisse Niokhor Diouf[4], Djibril Boiro[5], Younoussa Keita[6], Assane Sylla[5], Ousmane Ndiaye[5], Cheikh Saad Bouh Boye[2], Mbayame Niang[1*] and Ndongo Dia[1*]

[1]Pole de virologie, Institute Pasteur, Dakar, Senegal

[2]Bacteriology and Virology Laboratory, Dantec teaching Hospital, Dakar, Senegal

[3]Li Ka Shing Knowledge Institute, St. Michael's Hospital 209 Victoria St. Toronto, Canada

[4]Pediatric unit Roi BAUDOUIN hospital, Dakar, Senegal

[5]Pediatric Unit, Abass NDAO teaching Hospital, Dakar, Senegal

[6]Pediatric unit, Aristide Le Dantec Hospital, Senegal

Abstract

Background: Acute respiratory infections (ARI) continue to be the leading cause of pediatric morbidity and mortality worldwide.

Objectives: The present study is a prospective analysis of the prevalence and diversity of respiratory viruses associated with acute RTIs in children under 5 years of age in Senegal, and their association with disease severity.

Patients/Methods: An active surveillance of ARI was conducted from March 2014 to December 2015 in three pediatric referral Healthcare departments of Dakar. 288 children with ARI were enrolled and respiratory specimens collected. A two-step multiplex real-time RT-PCR for the simultaneous testing of 16 different respiratory viruses was performed.

Results: 93 children required hospitalization. Viral etiologies were identified in 224 patients while 64 were negative for all tested viruses. Single viral infections accounted for 30.5% and co-infections for 46.9%. A total of 439 respiratory viruses were identified in all children. Among these, 154 (35.3 %) were detected in hospitalized children. Adenoviruses with 44.4 %, influenza viruses 36.5%, rhinoviruses (HRV) 28.5%, enteroviruses 19.8% and respiratory syncytial virus (RSV) with 10.1% were the most detected. RSV infections were significantly more frequent in the first 6 months of life (p-value = 0.00213). RSV and HRV are mostly associated with bronchiolitis and bronchitis. Influenza detection is also the most related with pneumonia disease (47.6 %).

Conclusion: This study investigated the role of 16 different respiratory viruses in children with ARI in this Senegal. Data clearly suggest that respiratory viruses are major contributors to childhood acute respiratory infections in Senegal.

Abbreviations: ARI: Acute respiratory infection; RTI: respiratory tract infection; RT-PCR: Reverse Transcription Polymerase Chain Reaction; RSV: respiratory syncytial virus; HRV: human rhinoviruses; ILI: Influenza-like Illness; HAdV: human adenovirus; HEV: human enterovirus; HMPV: human metapneumovirus; HCoV: human coronaviruses; PIV: parainfluenza virus; LRTI: lower respiratory tract infections; URTI: upper respiratory tract infections; CRP: C-reactive protein; CBC: complete blood count; WHO: World Health Organization; MoH: Ministry of Health; IPD: Institut Pasteur de Dakar.

Background

Acute respiratory infections (ARI) continue to be the leading cause of pediatric morbidity and mortality worldwide [1,2]. In 2010, Nair and colleagues reported that 11.9 million and 3.0 million episodes of severe and very severe ARI respectively, contributed to childhood hospitalization [3]. Furthermore, 0.935 million deaths in 2013 were attributed to ARI [4]. In developing countries where pneumonia is responsible over 10–25 % of all deaths among children under 5 years of age the situation is more alarming [5]. Within the etiology of ARIs, viruses play an important role and are the main cause of ARIs in children under the age of 5 [6]. Unfortunately, in some cases, they also become an important cause of death [7]. In Senegal, recent data collected in the framework of the sentinel surveillance of respiratory viruses have shown a variety of respiratory viruses circulating in the community [8]. The most common viruses associated with influenza-like illness (ILI) in patients were influenza viruses, adenoviruses (ADVs), human rhinoviruses (HRVs), respiratory syncytial virus (RSV), enteroviruses (EVs), parainfluenza viruses (PIVs), human metapneumovirus (hMPV), and human coronaviruses (HCoVs) 229E, OC43, NL63, and HKU1. Studies focused on HRV and RSV, have revealed a high activity of both viruses in children under 5 in Senegal, especially during the rainy season [9,10]. Indeed, previous studies have well established that RSV is the leading cause of lower respiratory tract infections (LRTIs)

Correspondence to: Dr Ndongo Dia, Pediatric unit, Aristide Le Dantec Hospital, Senegal, Tel: +221 839 92 25; Fax: + 221 33 839 92 24; E-mail: ndia@pasteur.sn

Dr Mbayame Niang, Pediatric unit, Aristide Le Dantec Hospital, Senegal, Tel: +221 839 92 22 ; E-mail: niang@pasteur.sn

Key words: *epidemiology, adenoviruses, respiratory syncytial virus, influenza viruses, rhinoviruses, prevalence*

Received: July 16, 2017; **Accepted:** August 04, 2017; **Published:** August 04, 2017

in young children with 50% of children infected during their first year of life, and 100% having experienced at least one infection by 3 years of age [11]. Human rhinoviruses (HRV) were known as a frequent cause of upper respiratory tract infections (URTI). However, recently, HRV were found also to be associated with lower respiratory infections (LRTI), such as bronchiolitis [12], pneumonia, exacerbation of asthma or cystic fibrosis, [13,14] chronic obstructive pulmonary disease [15], and also hospitalization of children [16].

The present study is a prospective exploration of the prevalence and diversity of respiratory viruses associated with acute RTIs in children under 5 years of age, and their association with disease severity (clinical diagnostic).

Materials and methods

Samples and data collection

This prospective study was conducted from March 2014 to December 2015, and consisted of an active surveillance of ARI in three pediatric referral Healthcare departments of Dakar (Hôpital Aristide Ledantec, Hôpital Roi Baudoin and Hôpital Abass Ndao). A clinical team at each hospital was trained on the case definition identification and surveillance procedures; patients'carers/tutors were asked for verbal consent to participate. The clinical team enrolled any patients presented at the hospital who met an ARI case definition. ARI was defined as any patient with sudden onset of one or more of the following symptoms and signs: rhinitis, cough, sore throat, wheeze, dyspnea associated or not with an axillary temperature of more than 38.5°C. A respiratory specimen (nasal and / or throat swab) was collected from each child up to 5 years of age and placed in 2-mL cryovials containing viral transport medium (Universal Transport Medium; COPAN Diagnostics Inc., Murrieta, CA) and immediately transported at 4°C to the laboratory. At the laboratory, the specimens were processed immediately for viral diagnostic. Aliquots of each sample were also stored at −80°C for additional analysis.

Data regarding hospitalized patients were collected from medical records, using a standardized report form that includes basic epidemiologic data and a description of the symptoms prior to presentation. For hospitalized children, an input record was routinely performed including a complete blood count (CBC), a *C-reactive protein* CRP and radiography in the case of pulmonary signs. Case reports were entered into an Epi Info database (Centers for Disease Control and Prevention, Atlanta, GA) and merged with laboratory data.

PCR assay for respiratory viruses diagnostic

Total Viral nucleic acid (RNA/DNA) was obtained from 140 µl of clinical specimen using a Viral Nucleic Acid Extraction kit (QIAGEN, Valencia, CA, USA) according to the manufacturer's recommendation. RNA are eluted with 60µl nuclease-free water and stored at −80°C until use. A two-step multiplex real-time RT-PCR was performed with a Bio-Rad CFX-96 thermocycler (Bio-Rad Laboratories) as previously described [8] for the simultaneous testing for Influenza viruses (fluA and fluB), Human respiratory syncytial virus (RSVA and RSVB), Human adenoviruses (HAdV), Human metapneumovirus (HMPV), Human coronaviruses (229E, NL63, OC43), Human parainfluenza viruses (PIV1, -2, -3 and -4), Human rhinoviruses (HRV), Human enteroviruses (HEV) and Human bocaviruses (HBoV).

Statistical analyses

The Fisher test or Chi-2 test was used for comparison of all patients according to viral results and clinical data in inpatients and outpatient's groups. The logistic-regression model was used to examine association of virus detection with sex, age group and clinical data. A P value < 0.05 was considered statistically significant. The R software (R.3.0.1 version) was used to perform the statistical analyses.

Ethical management

This protocol was approved as less than minimal risk research by the Ministry of Health in its guidelines for influenza surveillance policy. The protocol and oral consent were determined as routine surveillance activity by the Senegalese National Ethics committee and the steering committee for 4S network [8], an entity representing MoH, IPD, WHO and Clinicians in compliance with all applicable National regulations governing the protection of human subjects. Data were collected anonymously in an objective of surveillance and applicable to a molecular epidemiology studies on the detected pathogen. The information provided to participants was an informal description of the study. Respiratory specimens were collected, only after informed consent was granted, verbally, to local health care workers by the parents or tutors of children. Oral consent was documented in the patient form with two questions about received information and about oral consent. For the surveillance activities, written consent is judged not necessary by the Senegalese national ethics committee, which has also previously approved the work of the National Influenza Center. Collections of non-sensitive data or an observation from normal care in which participants remain anonymous do not require ethics committee review. The patients included in this study were consulted the health care centers due to influenza-like symptoms; parents accept the sampling and the tests for respiratory viruses largely because they are free and safe.

Results

Demographic and clinical characteristics of children

From May 2014 to December 2015, nasopharyngeal swabs were collected from 288 children with ARI whose age varied from 0 up to 5 years (Table 1): 164 (56.9%) were from Roi Baudouin Hospital, 74 (25.7%) from Abass Ndao Hospital and 50 (17.4%) were from Aristide Ledantec Hospital. Of these children, 93 (32.3 %) required hospitalization.

The ages of the children ranged from 3 days to 5 years, with a mean age of 25.5 months and a median age of 18 months. The male to female ratio was 1.54 (173 [60.8%] males and 112 [39.2%] females). Sex information was not available in 3 cases. The most common age group was infants with age > 24 months, 42% (121/288). Children of age group 12-24 months represented 23.3% (67/288), while children of age groups 0-6 and 6-12 months represented 16.3% (47/288) and 18.4 % (53/288), respectively.

Regarding clinical symptoms the majority of children presented at admission suffered from fever (74.6 %; 215/288), cough (62.1%; 179/288), and rhinorrhea (39.9%; 115/288). Dyspnea (21.2%; 61/288), sore throat (9.4%; 27/288) and tachypnea (9%; 26/288) were also reported. We also noted that dyspnea (73.8%, 45/61), tachypnea (84.6%; 22/26), pulmonary condensation (90%, 9/10) and weight loss (60%, 6/10) were closely linked to hospitalization.

Clinically, rhinitis (33%; 95/288), bronchitis (39.2%; 113/288), bronchiolitis (17.4%; 50/288), tonsillitis (14.6%; 42/288) and pneumonia (7.3%; 21/288) were the most common diagnostic. Otitis (4.2%; 12/288) and pharyngitis (3.1%; 9/288) were also found. Pneumonia (19/21; $p < 0.0001$), bronchiolitis (22/50; $p < 0.001$), acute bronchitis (26/113; p =

Table 1. Demographic characteristics, symptoms and diagnosis.

	Inpatients(N=93)	Outpatients(N=195)	Total (N=288)	p Value
Gender				
Male	55(59.1)	118(61.5)	173(60.8)	-
Female	38(40.9)	74(38.5)	112(39.2)	0.697
Group Age (months)				
[0-6]	20(21.5)	27(13.9)	47(19.4)	0.3090
]6-12]	17(18.3)	36(18.6)	53(18.5)	0.2800
]12-24]	23(24.8)	44(22.2)	67(23.0)	0.3732
24+	33(35.5)	88(45.4)	121(42.2)	0.0578
Clinical signs				
Cough	64(68.8)	115(59.0)	179(62.2)	0.1199
Dyspnea	45(48.4)	16(8.2)	61(21.2)	**4.338e-14**
Sore throat	9(9.7)	18(9.2)	27(9.4)	0.903
Fever	73(78.5)	142(72.8)	215(74.7)	0.3152
Tachypnea	22(23.7)	4(2.1)	26(9.0)	**1.107e-08**
Weight loss	6(6.5)	4(2.1)	10(3.5)	0.0817
Otorrhoea	3(3.2)	2(1.0)	5(1.7)	0.3328
Pulmonary condensation	9(9.7)	1(0.5)	10(3.5)	**0.0002**
Rhinorrhea	39(41.9)	76(39.0)	115(39.9)	0.6997
Diagnosis				
Pneumonia	19(20.4)	2(0.7)	21(7.3)	**1.373e-08**
Acute bronchitis	26(28.0)	87(29.5)	113(39.2)	**0.0069**
Bronchiolitis	22(23.7)	28(9.5)	50(17.4)	0.0665
Acute otitis	4(4.3)	8(2.7)	12(4.2)	0.937
Rhinitis	18(19.4)	77(29.1)	95(33.0)	**0.0007**
Tonsillitis	12(12.9)	30(10.2)	42(14.6)	0.7213
Pharyngitis	4(4.3)	5(1.7)	9(3.1)	0.4766
Acute sinusitis	1(1.1)	0(0.0)	1(0.3)	0.3229
Meningitis	2(2.2)	0(0.0)	2(0.7)	0.1035
Tracheitis	2(2.2)	1(0.3)	3(1.0)	0.2447

0.006) and tonsillitis (12/42; $p < 0.001$) were significantly associated with hospitalization. All cases of tracheitis (3), meningitis (2) and acute sinusitis (1) were also hospitalized.

Patients and viral detection

Viral etiologies were identified in the vast majority (77.8%, 224/288) of patients while 64 (22.2 %) were negative for all viruses tested. Single viral infections accounted for 30.5% (88/288) of cases and co-infections with multiple viruses were found in 46.9% (135/288), mainly with the association of HAdV or influenza viruses (Table 2). In 56 samples (19.4 %) more than two viruses were detected.

A total of 439 respiratory viruses were identified in all children. Among these, 254 (57.8%) were detected in hospitalized children. Adenoviruses (44.4%; 128/288), influenza viruses (37.5%; 108/288) and rhinoviruses (28.5%; 82/288) were the most frequently detected among the children (Table 3). We also detected human enteroviruses (HEV) in 57 children (19.8%) and human respiratory syncytial virus (RSV) in 29 children (10.1%). Other viruses have been detected below 5% rate: human bocavirus (4.5%; 13/288), parainfluenza viruses (3.1%; 9/288), human coronaviruses (2.4%; 7/288) and human metapneumoviruses (2.1%; 6/288). Regarding mono-infection criterion, rates are proportional to the virus whole detection rates. Indeed, adenoviruses are the most frequently detected in single infection with 31 cases (24.2%; 31/128) followed by influenza viruses (24.8%; 26/105), HRV (19.5%; 16/82) and HEV with 9 cases (15.8%; 9/57). RSV and HBoV are detected respectively three and two times in single infection. HRV mono-infections are associated to hospitalization in 92.3 % (12/13), a significantly higher percentage than other viruses ($P < 0.01$), followed by influenza virus (57.1%; 8/14). RSV infections occurred at a younger age than other viral infections, and were significantly more frequent in the first 6 months of life (Fisher exact test p-value = 0.0213). In contrast, none of the other viruses exhibited significant age group distribution differences.

Taking into account the clinical diagnosis and detected viruses, RSV and HRV are the most associated viruses with bronchiolitis disease (24.1 % and 15.8% respectively), HMPV detection was mostly associated with acute bronchitis (50 %) and HCoV with rhinitis (Table 3). These different associations were not statistically significant ($P > 0.05$). Any significant association ($P > 0.05$) was also observed between a virus detection and a clinical symptom.

No significance difference was observed in the distribution of the viral detection rate of the viruses according to the age group. However, we noticed a detection ratio of 1.8 viruses per patient in children less than 6 months, which represented a high ratio as compared to other groups.

Discussion

This is the first study in Senegal that investigated the role of 16 different respiratory viruses in children with ARI using sensitive molecular methods over a 2-year period. Our data support the conclusion that respiratory viruses are significantly involved in childhood acute respiratory infections in Senegal. Interestingly, this study in line with previous reports suggests that acute respiratory infections are a major public health issue in children worldwide [2]. Therefore, these findings could be very useful for public health policies in our country and in the sub-Saharan region.

Indeed, we showed that 224 samples from the children with ARI out of 288 contained at least one of the targeted respiratory viruses. All targeted viruses have been detected in at least one patient.

The frequency of virus detection (77.8%) among the children with ARI in our study is high compared with those found in several other studies from other countries. For example Kaplan, *et al.* [17], in a similar study conducted in Jordan detected at least one respiratory virus in 75.5% of the children, Kenmoe, *et al.* [18], in Cameroon detected at least one respiratory virus in 65.4% of the children, Amer, *et al.* [19] in Saudi Arabia had a detection rate of 60.3%, Lee, *et al.* [20] in Taiwan 60.2%, Wertheim, *et al.* [21] 58.6% (in patients from Thailand, Vietnam and Indonesia), Lu, *et al.* [22] in China 55.8%, Giamberardin, *et al.* [23] in Brazil 55% or Khamis, *et al.* [24] in Oman with 50%. Even lower detection rates were obtained in studies performed in Europe or the USA: Moe, *et al.* [25] in Norway (43%), Pierangeli, *et al.* [26] in Italy (42.7%) or Wansaula, *et al.* [27] in USA (43.3%). However, higher rates than ours were observed in a study conducted by Do, *et al.* [28] in children less than 2 years old in Vietnam (91%) and another one in Cyprus (85.8%) by Richter, *et al.* [29]. However, it should be noted that the technical approaches used could at least partly explain the discrepancies in viral detection rates: primarily in their sensitivity differences and secondly in the number of targeted viruses. Alternatively differences in rates of detection could be due to true geographical differences in overall burden, differences in study populations (outpatients or hospitalized patients), ARI case definition differences and to the studies sample collection periods.

Of the 439 viruses detected in the children, adenovirus was the most common viral pathogen with 44.4% of the total number of detected viruses. This result is consistent with the data collected since

Table 2. Viral co-detections in children less than 5 years old with acute respiratory infections in Senegal.

Virus types	HAdV	PIV	FLU	HRV	HMPV	HBoV	HCoV	RSV	HEV
HAdV	128(100)								
PIV	4(3.1)	9(100)							
FLU	59(46.1)	5(55.6)	108(100)						
HRV	44(34.4)	4(44.4)	31(28.7)	82(100)					
HMPV	3(2.3)	0(0.0)	3(2.8)	0(0.0)	6(100)				
HBoV	6(4.7)	0(0.0)	6(5.6)	7(8.5)	1(16.7)	13(100)			
HCoV	5(3.9)	0(0.0)	2(1.9)	4(4.9)	0(0.0)	0(0.0)	7(100)		
RSV	15(11.7)	0(0.0)	14(13)	16(19.5)	1(16.7)	1(7.7)	0(0.0)	29(100)	
HEV	26(20.3)	3(33.3)	24(22.2)	21(25.6)	2(33.3)	5(38.5)	1(14.3)	14(48.3)	57(100)
Single detection	31(24.2)	0(0)	26(24.1)	16(19.5)	1(16.7)	2(15.4)	0(0)	3(10.3)	9(15.8)

Table 3. Clinical characteristic of acute respiratory infection and viral detection in children less than 5 years old in Senegal.

	HAdV	PIV	FLU	HRV	HMPV	HBoV	HCoV	RSV	HEV
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Detection	128(44.4)	9(3.1)	105(36.5)	82(28.5)	6(2.1)	13(4.5)	7(2.4)	29(10.1)	57(19.8)
Hospitalized	43(33.6)	4(44.4)	38(35.2)	30(36.6)	2(33.3)	3(23.1)	2(28.6)	12(41.4)	20(35.1)
Gender									
Male	76(53.4)	6(66.7)	67(62.0)	55(67.1)	3(50.0)	9(69.2)	5(71.4)	19(65.5)	35(61.4)
Female	52(40.6)	3(33.3)	41(38.0)	27(32.9)	3(50.0)	4(30.8)	2(28.6)	10(34.5)	22(38.6)
Diagnosis									
Pneumonia	14(10.3)	1(11.1)	10(9.3)	6(7.3)	(0.0)	2(15.4)	(0.3)	3(10.3)	4(7.0)
Acute bronchitis	48(37.5)	6(66.7)	37(34.3)	29(35.4)	3(50.0)	6(46.2)	3(42.5)	8(27.6)	24(42.1)
Bronchiolitis	21(16.4)	(0.0)	11(10.2)	14(17.1)	(0.0)	1(7.7)	1(14.4)	7(24.1)	6(10.5)
Acute otitis	6(4.7)	1(11.1)	8(7.4)	5(6.1)	(0.0)	(0.0)	1(14.7)	2(6.9)	1(1.8)
Rhinitis	43(33.6)	3(33.3)	42(38.9)	28(34.1)	1(16.6)	1(7.7)	4(57.6)	10(34.5)	23(40.4)
Tonsillitis	19(14.8)	(0.0)	21(19.4)	13(15.9)	(0.0)	2(15.4)	1(14.8)	1(3.4)	8(14.0)
sign clinical									
Cough	84(65.6)	7(77.8)	63(58.3)	50(61.0)	5(83.3)	6(46.2)	3(42.6)	18(62.1)	34(59.6)
Dyspnea	30(23.4)	2(22.2)	22(20.4)	20(24.4)	1(16.6)	2(15.4)	1(14.4)	7(24.1)	10(17.5)
Sore throat	12(3.4)	1(11.1)	11(10.2)	14(17.1)	(0.0)	1(7.7)	2(28.4)	1(3.4)	6(10.5)
Weight loss	7(5.5)	2(22.2)	4(3.7)	2(2.4)	(0.0)	1(7.7)	(0.5)	(0.0)	5(8.8)
Fever	93(72.7)	7(77.8)	80(74.1)	59(72.0)	5(83.3)	11(84.6)	3(42.7)	24(82.8)	42(73.7)
Tachypnea	15(11.7)	1(11.1)	10(9.3)	5(6.1)	(0.0)	(0.0)	(0.7)	4(13.8)	5(8.8)
Otorrhoea	2(1.6)	(0.0)	4(3.7)	1(1.2)	(0.0)	(0.0)	(0.6)	1(3.4)	1(1.8)
Condensation pulmonary	7(5.5)	1(11.1)	5(4.6)	3(3.7)	(0.0)	(0.0)	(0.5)	3(10.3)	3(5.3)
Rhinorrhea	53(41.4)	5(55.6)	45(41.7)	42(51.2)	3(50.0)	6(46.2)	5(71.4)	20(69.0)	28(49.1)
Age groups/Viral detection									
[0-6] months	22(46.8)	1(2.1)	17(36.2)	20(42.6)	1(2.1)	1(2.1)	2(4.3)	10(21.3)	10(21.3)
]6-12] months	24(45.3)	2(3.8)	14(26.4)	15(28.3)	2(3.8)	(0.0)	1(1.9)	7(13.2)	13(24.5)
]12-24] months	30(44.8)	1(1.5)	27(40.3)	14(20.9)	1(1.5)	6(9.0)	1(1.5)	3(4.5)	13(19.4)
<24 months	52(43.0)	5(4.1)	47(38.8)	33(27.3)	2(1.7)	6(5.0)	3(2.5)	9(7.4)	21(17.4)
p-Value	0.9759	0.7553	0.2218	0.1241	0.3935	0.1808	0.8985	**0.04677**	0.7103

2012 from our sentinel surveillance of other respiratory viruses in people with influenza-like illnesses in Senegal [8]. Indeed we observed a strong activity of adenoviruses in Senegal throughout the year (data not shown). Adenoviruses were observed in the same proportions (27.3%) in a similar study in Cameroon [18]. The detection rates found in Senegal and Cameroon are considerably higher than those found in other geographical areas: Nascimento-Carvalho, *et al.* [30] in Brazil with 3%, Moe, *et al.* [25] (2016) in Norway (1.7%), Wansaula, *et al.* [27] in USA (1%) or Lu, *et al.* [22] in China. These results suggest a strong association of adenoviruses in human respiratory tract in African populations even if disease causation has not convincingly been proven. In fact, the presence of a virus in the nasopharynx of a child does not necessarily mean that it is the etiological agent of the ILI or ARI. Therefore, it may only represent a coincidental upper airway infection, an asymptomatic carrier state, or prolonged shedding of a pathogen that caused a previous infection. For adenoviruses, additional investigations (epidemiology, circulating genotypes…) will help to assess the real importance of this virus in respiratory infection diseases.

Influenza viruses were detected in 36.5% of patients, which highlights the importance of these viruses in the genesis of local respiratory infections among children in Senegal. This rate is relatively high compared to those obtained in several other studies [4,18,27,31,32] even if others studies had concluded that influenza viruses are more likely to be detected in under 5-year-old patients, with an incidence of 9.4–9.6% in the last 2 years [33-35]. Moreover, Van Woensel, *et al.* [36] argued that infants and young children have a 12-fold increased risk of admission to hospital for respiratory tract infection caused by influenza virus compared with children aged 5–17 years. In any case, our results clearly support that children under 5 years of age are a highrisk group of complications regarding influenza virus's infection and the prospect

of implementing seasonal influenza vaccination in this age group in Senegal becomes a real public health necessity.

Picornaviruses (Rhinovirus and Enterovirus) accounted for about 48.3% of viral infections in our population. This high circulation of picornaviruses in children was clearly reflected in the data collected after many years of surveillance of these viruses in people in Senegal [9]. Interestingly, in the present pediatric study about 59.7% of picornaviruses infection cases are related to low respiratory tract infections (LRTIs) although these viruses are usually related to mild URTI. These results underline a need for picornaviruses investigation during viral diagnosis of respiratory tract diseases in light of recently published data regarding the association of rhinoviruses especially with severe respiratory tract infections in children [37-39], and in asthma exacerbations [16,40-42].

Regarding RSV, our results are lower than the rates reported in several other similar studies. Among many examples we can cite the studies carried out in other low and middle-income countries including Bangladesh [43], Kenya [44], Mozambique, South Africa [45] El Salvador [46], Costa Rica [47], in Jordan [17], in Vietnam [29] and Thailand [48]. Otherwise, the proportion in Senegal (10.1%) was similar to that reported in Cameroon [18], in Mozambique [49] or Nepal [50]. However, as shown in our study, it is globally well documented that RSV represents a substantial burden of acute respiratory tract illness particularly in the early years of life (especially in the first six months), leading to severe morbidity and hospitalization in very young children [43,51,52]. Therefore, in a World Health Organization (WHO) vaccine perspective against this virus, the importance of long-term molecular epidemiological surveys for early detection of prevalent strains and newly emerging genotypes cannot be over emphasized to foster better understanding of RSV infections in Senegal. In future study, for a better assessment of the RSV burden among children in Senegal focusing on pediatric hospitalized cases, data on disease outcome, atypical clinical signs, duration of symptoms, duration of hospitalization and treatment should be collected.

The remaining viral etiologies were identified below 5%: bocaviruses 4.5%, parainfluenza with 3.1%, human coronaviruses 2.4% and human metapneumovirus with 2.1%. These low circulation rates are in line with those reported in many other similar studies [18,23,24,26,29].

Mixed infections were observed in approximately 46.9% of all samples, which is much higher than those previously reported rates ranging from 4.8% in Italy [27] to 29.5% in Cameroon [18]. EV, RSV, HBoV, and HCoV were found most frequently in co-infections. Even though this study did not allow for investigating a possible association between multiple infections and disease severity, literary data show that such a potential association is still controversial.

Our study did have several limitations. The first weakness is the small number of samples collected in this study. An increase number of samples, along with the participation of more hospital sites will be critical to complete the picture for both the targeted viruses and the clinical diagnoses in children with ARI in Senegal. It will also be interesting to extend the recruitment period of the study as it would make possible to assess the seasonality of the infections. The lack of matched control groups is another limitation of the study especially to appreciate the asymptomatic viral carriage which will help to investigate a clinical causal link. In future work, it will be critical to compare the bacterial and viral etiologies, and also to study the clinical value of virus/bacteria co-infections in children with ARI. The role of bacteria as a cause of LRTI or as cause of super infection is well known, especially in RSV and influenza virus infection [53]. Bacteria investigation is also important for antibiotic intervention strategies. However, bacteria diagnostic is not easy as lower airway secretions (like sputum) considered as the optimal specimen type for detecting bacterial (co-) infection, are often difficult to obtain from young children, and can only be readily obtained from intubated children.

Conclusion

In summary, our study provides novel insights into the epidemiology and clinical impact of respiratory viruses among children under 5 years old, the most vulnerable age group, in a low-income West African country, Senegal. It highlights a high diversity and prevalence of respiratory viruses circulating in this age group. It arises from these results a need for implementation of surveillance programs for respiratory viral infections in order to collect exhaustive data for crucial analyses (seasonality, targeted group ages, causal roles of viral pathogens and the risk factors that may correlate with more severe disease …) seeking to guide preventive public health measures.

Authors' contributions

The work presented here was carried out in collaboration between all authors. *ND, MN, CSB, and ON* defined the research, analyzed and interpreted the data, and revised the manuscript; *AF and AD1* performed and coordinated technical work; *ND*, *AF*, *SFW* and *MNN* wrote the draft and revisions of the paper; *AD2, JBND, DB, YK and AS* contributed in the samples and data collection. All authors contributed to, seen and approved the manuscript.

Acknowledgments

This study would not have been possible without the excellent support from all the health-care workers of Abass Ndao, Aristide Ledantec and Roi Baudoin pediatric healthcare. We convey our special thanks to Kathleen Victoir from the International Network of Pasteur Institutes for her unwavering support to the Senegalese National Influenza Center. We acknowledge the Senegalese ministry of health for their help in implementing the 4S Network.

Funding information

This work was supported by the Department of Health and Human Service (DHSS) via the International Network of Pasteur Institutes and Pasteur Institute of Dakar.

Competing interests

The authors have no competing interests.

References

1. Scheltinga SA, Templeton KE, Beersma MF, Claas EC (2005) Diagnosis of human metapneumovirus and rhinovirus in patients with respiratory tract infections by an internally controlled multiplex real-time RNA PCR. *J Clin Virol* 33: 306-311.

2. Grassi T, Mancini F, Ciervo A, Vescio MF, Ghazal A, et al. (2014) Chlamydophila pneumoniae, Mycoplasma pneumoniae, and influenza in children with respiratory infections in Alexandria, Egypt. 2014. *J Infect Dev Ctries* 8: 379-383.

3. Nair GB, Niederman MS (2013) Year in review 2012: Critical Care--respiratory infections. *Crit Care* 17: 251. [Crossref]

4. Liu T, Li Z, Zhang S, Song S, Julong W, et al. (2015) Viral Etiology of acute respiratory tract infections in hospitalized children and adults in Shandong Province, China. *Virol J* 12: 168.

5. Williams BG1, Gouws E, Boschi-Pinto C, Bryce J, Dye C (2002) Estimates of world-wide distribution of child deaths from acute respiratory infections. *Lancet Infect Dis* 2: 25-32. [Crossref]

6. Luksic I, Kearns PK, Scott F, Rudan I, Campbell H, et al. (2013) Viral etiology of hospitalized acute lower respiratory infections in children under 5 years of age – a systematic review and meta-analysis. 2013. *Croat Med J* 54: 122–134.

7. Karaivanova GM (1995) Viral respiratory infections and their role as publichealth problem in tropical countries (review). *Afr J Med Med* Sci 24: 1-7.

8. Dia N, Diene Sarr F, Thiam D, Faye Sarr T, Espié E, et al. (2014) Influenza-like illnesses in Senegal: not only focus on influenza viruses. *PLoS One* 9: e93227.

9. Fall A, Dia N, Kébé O, Sarr FD, Kiori DE, et al. (2016) Enteroviruses and Rhinoviruses: Molecular Epidemiology of the Most Influenza-Like Illness Associated Viruses in Senegal. *Am J Trop Med Hyg* 95: 339-347.

10. Fall A, Dia N, Cisse el HA, Kiori DE, Sarr FD, et al. (2016) Epidemiology and Molecular Characterization of Human Respiratory Syncytial Virus in Senegal after Four Consecutive Years of Surveillance, 2012-2015. *PLoS One* 11: e0157163.

11. Mejias A, Chavez-Bueno S, Jafri HS, Ramilo O (2005) Respiratory syncytial virus infections: old challenges and new opportunities. 2005. *Pediatr Infect Dis J* 24: S189-196.

12. McIntyre CL, Knowles NJ, Simmonds P (2013) Proposals for the classification of human rhinovirus species A, B and C into genotypically assigned types. *J Gen Virol* 94:1791-1806.

13. Jackson DJ, Gangnon RE, Evans MD, Roberg KA, Anderson EL, et al. (2008) Wheezing rhinovirus illnesses in early life predict asthma development in high-risk children. *Am J Respir Crit Care Med* 178: 667-672.

14. de Almeida MB, Zerbinati RM, Tateno AF, Oliveira CM, Romão RM, et al. (2010) Rhinovirus C and respiratory exacerbations in children with cystic fibrosis. *Emerg Infect Dis* 16: 996-999.

15. Seemungal TA, Harper-Owen R, Bhowmik A, Jeffries DJ, Wedzicha JA (2000) Detection of rhinovirus in induced sputum at exacerbation of chronic obstructive pulmonary disease. *Eur Respir J* 16: 677-683. [Crossref]

16. Piotrowska Z, Vázquez M, Shapiro ED, Weibel C, Ferguson D, et al. (2009) Rhinoviruses are a major cause of wheezing and hospitalization in children less than 2 years of age. *Pediatr Infect Dis J* 1: 25-29.

17. Kaplan NM, Dove W, Abd-Eldayem SA, Abu-Zeid AF, Shamoon HE, et al. (2008) Molecular epidemiology and disease severity of respiratory syncytial virus in relation to other potential pathogens in children hospitalized with acute respiratory infection in Jordan. *J Med Virol* 80: 168-174.

18. Kenmoe S, Tchendjou P, Vernet MA, Moyo-Tetang S, Mossus T, et al. (2016) Viral etiology of severe acute respiratory infections in hospitalized children in Cameroon, 2011-2013. 2016. *Influenza Other Respir Viruses* 10: 386-393.

19. Amer HM, Alshaman MS, Farrag MA, Hamad ME, Alsaadi MM, et al. (2016) Epidemiology of 11 respiratory RNA viruses in a cohort of hospitalized children in Riyadh, Saudi Arabia. *J Med Virol* 88: 1086-1091.

20. Lee CY, Chang YF, Lee CL, Wu MC, Ho CL, et al. (2015) Molecular viral epidemiology and clinical characterization of acute febrile respiratory infections in hospitalized children in Taiwan. *J Med Virol* 87: 1860-1866.

21. Wertheim HF, Nadjm B, Thomas S, Agustiningsih A, Malik S, et al. (2015) Viral and atypical bacterial aetiologies of infection in hospitalised patients admitted with clinical suspicion of influenza in Thailand, Vietnam and Indonesia. *Influenza Other Respir Viruses* 6: 315–322.

22. Lu Y, Wang S, Zhang L, Xu C, Bian C, et al. (2013) Epidemiology of human respiratory viruses in children with acute respiratory tract infections in Jinan, China. *Clin Dev Immunol* 2013: 210490.

23. Giamberardin HI, Homsani S, Bricks LF, Pacheco AP, Guedes M, et al. (2016) Clinical and epidemiological features of respiratory virus infections in preschool children over two consecutive influenza seasons in southern Brazil. *J Med Virol* 8: 1325-1333.

24. Khamis FA, Al-Kobaisi MF, Al-Areimi WS, Al-Kindi H, Al-Zakwani I (2012) Epidemiology of respiratory virus infections among infants and young children admitted to hospital in Oman. *J Med Virol* 84: 1323-1329.

25. Moe N, Pedersen B, Nordbø SA, Skanke LH, Krokstad S, et al. (2016) Respiratory Virus Detection and Clinical Diagnosis in Children Attending Day Care. *PLoS One* 11: e0159196.

26. Pierangeli A, Gentile M, Di Marco P, Pagnotti P, Scagnolari C, et al. (2007) Detection and typing by molecular techniques of respiratory viruses in children hospitalized for acute respiratory infection in Rome, Italy. *J Med Virol* 79: 463-468.

27. Wansaula Z, Olsen SJ, Casal MG, Golenko C, Erhart LM, et al. (2016) Surveillance for severe acute respiratory infections in Southern Arizona, 2010-2014. *Influenza Other Respir Viruses* 10: 161-169.

28. Do LA, Bryant JE, Tran AT, Nguyen BH, Tran TT, et al. (2016) Respiratory Syncytial Virus and Other Viral Infections among Children under Two Years Old in Southern Vietnam 2009-2010: Clinical Characteristics and Disease Severity. *PLoS One* 11: e0160606.

29. Richter J, Panayiotou C, Tryfonos C, Koptides D, Koliou M, et al. (2016) Aetiology of Acute Respiratory Tract Infections in Hospitalised Children in Cyprus. *PLoS One* 11: e0147041.

30. Nascimento-Carvalho CM, Ribeiro CT, Cardoso MRA (2008) The role of respiratory viral infections among children hospitalized for community-acquired pneumonia in a developing country. *Pediatr Infect Dis* J 27: 939–941.

31. Khadadah M, Essa S, Higazi Z, Behbehani N, Al-Nakib W (2010) Respiratory syncytial virus and human rhinoviruses are the major causes of severe lower respiratory tract infections in Kuwait. *J Med Virol* 82: 1462-1467.

32. Wang H, Zheng Y, Deng J, Wang W, Liu P, et al. (2016) Prevalence of respiratory viruses among children hospitalized from respiratory infections in Shenzhen, China. *Virol J* 13: 39.

33. Watanabe AS, Carraro E, Moreira L, Camargo C, Sinohara J, et al. (2009) Respiratory virus infections among hospitalized patients with suspected influenza A H1N1 2009 virus during the first pandemic wave in Brazil. *Braz J Infect Dis* 15: 220-224.

34. Turner P, Turner C, Watthanaworawit W, Carrara V, Cicelia N, et al. (2013) Respiratory virus surveillance in hospitalised pneumonia patients on the Thailand-Myanmar border. *BMC Infect Dis* 13: 434.

35. Kwon JM, Shim JW, Kim DS, Jung HL, Park MS, et al. (2014) Prevalence of respiratory viral infection in children hospitalized for acute lower respiratory tract diseases, and association of rhinovirus and influenza virus with asthma exacerbations. *Korean J Pediatr* 57: 29-34.

36. van Woensel JB, van Aalderen WM, Kimpen JL (2003) Viral lower respiratory tract infection in infants and young children. *BMJ* 327: 36-40.

37. Iwane MK, Prill MM, Lu X, Miller EK, Edwards KM, et al. (2011) Human rhinovirus species associated with hospitalizations for acute respiratory illness in young US children. *J Infect Dis* 204: 1702–1710.

38. Fuji N, Suzuki A, Lupisan S, Sombrero L, Galang H, et al. (2011) Detection of human rhinovirus C viral genome in blood among children with severe respiratory infections in the Philippines. *PLoS One* 6: e27247.

39. Lauinger IL, Bible JM, Halligan EP, Bangalore H, Tosas O, et al. (2013) Patient characteristics and severity of human rhinovirus infections in children. *J Clin Virol* 58: 216-220. [Crossref]

40. Kotaniemi-Syrjänen A, Vainionpää R, Reijonen TM, Waris M, Korhonen K, et al. (2003) Rhinovirus-induced wheezing in infancy--the first sign of childhood asthma? *J Allergy Clin Immunol* 111: 66-71. [Crossref]

41. Miller EK, Edwards KM, Weinberg GA, Iwane MK, Griffin MR, et al. (2009) A novel group of rhinoviruses is associated with asthma hospitalizations. *J Allergy Clin Immunol* 123: 98-104. [Crossref]

42. Bizzintino J, Lee WM, Laing IA, Vang F, Pappas T, et al. (2011) Association between human rhinovirus C and severity of acute asthma in children. *Eur Respir J* 37: 1037-1042. [Crossref]

43. Homaira N, Luby SP, Hossain K, Islam K, Ahmed M, Rahman M, et al (2016) Respiratory Viruses Associated Hospitalization among Children Aged <5 Years in Bangladesh: 2010-2014. *PLoS One* 11: e0147982.

44. Feikin DR, Ope MO, Aura B, Fuller JA, Gikunju S, et al. (2012) The population-based burden of influenza-associated hospitalization in rural western Kenya, 2007–2009. *Bull World Health Organ* 90: 256–63A.

45. Robertson SE, Roca A, Alonso P, Simoes EA, Kartasasmita CB, et al. (2004) Respiratory syncytial virus infection: denominator-based studies in Indonesia, Mozambique, Nigeria and South Africa. *Bull World Health Organ* 82: 914–922.

46. Clara W, Armero J, Rodriguez D, de Lozano C, Bonilla L, et al. (2012) Estimated incidence of influenza-virus-associated severe pneumonia in children in El Salvador, 2008–2010. *Bull World Health Organ* 90: 756–763.

47. Saborio GG, Clara A, Garcia A, Quesada F, Palekar R, et al. (2014) Influenza-associated Hospitalizations and Deaths, Costa Rica, 2009–2012. *Emerg Infect Dis* 20: 878-881.

48. Fry AM, Chittaganpitch M, Baggett HC, Peret TC, Dare RK, et al. (2010) The burden of hospitalized lower respiratory tract infection due to respiratory syncytial virus in rural Thailand. PLoS One 5: e15098.

49. O'Callaghan-Gordo C, Bassat Q, Morais L, Díez-Padrisa N, Machevo S, et al. (2011) Etiology and epidemiology of viral pneumonia among hospitalized children in rural Mozambique: a malaria endemic area with high prevalence of human immunodeficiency virus. *Pediatr Infect Dis J* 30: 39-44.

50. Mathisen M, Basnet S, Sharma A, Shrestha PS, Sharma BN, et al. (2011) RNA viruses in young Nepalese children hospitalized with severe pneumonia. *Pediatr Infect Dis J* 30: 1032-1036. [Crossref]

51. Venter M, Madhi SA, Tiemessen CT, Schoub BD (2001) Genetic diversity and molecular epidemiology of respiratory syncytial virus over four consecutive seasons in South Africa: identification of new subgroup A and B genotypes. *J Gen Virol* 82: 2117–2124.

52. McLellan JS, Chen M, Kim A, Yang Y, Graham BS, et al. (2010) Structural basis of respiratory syncytial virus neutralization by motavizumab. *Nat Struct Mol Biol* 17: 248-250. [Crossref]

53. Navarini AA, Recher M, Lang KS, Georgiev P, Meury S, et al. (2006) Increased susceptibility to bacterial superinfection as a consequence of innate antiviral responses. *Proc Natl Acad Sci U S A* 103: 15535-15539.