Journal of Medical Virology

WILEY

JOURNAL OF MEDICAL VIROLOGY

RESEARCH ARTICLE OPEN ACCESS

Whole Genome Sequencing and Genetic Diversity of Respiratory Viruses Detected in Children With Acute Respiratory Infections: A One-Year Cross-Sectional Study in Senegal

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Received: 28 October 2024 | **Revised:** 26 January 2025 | **Accepted:** 28 March 2025

Keywords: acute respiratory infections | children | NGS | Senegal | virus | whole genome

ABSTRACT

Acute respiratory infections (ARI) are a health priority, especially in countries with limited resources. They are a major cause of morbidity and mortality, especially among children and the elderly. In Senegal, the endemic circulation of respiratory viruses other than influenza has been demonstrated. However, there is a paucity of data exploring the genetic diversity of these viruses based on whole-genome sequencing. In this study, we present data on the genetic diversity of respiratory viruses in children under 15 years old in Senegal, including an overview of the different pathogens detected. Between November 2022 and November 2023, we collected nasopharyngeal swabs from children seen in curative consultations for symptoms of acute respiratory infections. Of the 156 children included, 73.7% tested positive for at least one pathogen. The most frequently detected virus was rhinovirus (50.0%), followed by influenza B (41.6%) and human parainfluenza virus type 3 (7.6%). Combinations of rhinovirus/influenza B, human parainfluenza virus type 2/human parainfluenza virus type 4, and rhinovirus/influenza B/adenovirus were the most frequently identified. A statistically significant association was detected between some of the viruses detected. A high genetic diversity of respiratory viruses circulating in children was revealed. The strains were phylogenetically close to various strains circulating worldwide, suggesting a global circulation of respiratory viruses. Our study provides the first complete genome sequences of human parainfluenza viruses type 2, 3, 4 and human bocavirus from Senegal and thus contributes to the enrichment of international databases on sequences from Senegal and underlines the importance of sequencing in the dynamics of pathogen circulation.

1 | Introduction

Acute respiratory infections (ARI) represent a major public health problem, being the leading cause of morbidity and mortality attributed to infectious diseases worldwide. According to global estimates of the main causes of death, in 2019, endemic respiratory diseases caused 2.5 million deaths worldwide [1]. A systematic review carried out in 2010 showed that 0.3 million deaths caused by ARI occurred in hospitals among young children, and that 99% of these deaths occurred in developing countries [2]. In sub-Saharan Africa, ARI is the third leading cause of all-age mortality after cardiovascular disease

and neonatal conditions, and the leading cause of death in children aged 1–59 months [3].

In Senegal, ARI is endemic and the country's second leading cause of death for both sexes of all ages. In 2019, they were the cause of 7200 deaths in the general population (44.1 deaths per 100 000 inhabitants) of all ages; children under five were the most affected. ARI accounted for 45% of their causes of death [3]. Among these children in Senegal, ARI are the leading cause of death from infectious causes, and the leading cause of morbidity in level 3 hospital health facilities for children, with 18,487 cases in 2019 [4]. Several studies have been carried out in this population, which is the most affected. In these studies, high viral detection rates have been reported. Children infected with at least 1 virus ranged from 73% to 100% of cases. Rates of mono-infection have also been reported, ranging from 7% to 59% of cases, and viral co-infections from 23% to 49% of cases [5–7].

Most molecular characterization studies of respiratory viruses in Senegal have assessed the genetic diversity of viral strains in patients with influenza-like illness, based on sequencing of target gene fragments [8–11]. In children, the population most affected by ARI, apart from the genetic characteristics of respiratory syncytial virus recently described in children hospitalized for severe acute respiratory infection [12], there is a paucity of data on the molecular characterization of frequently detected classical respiratory viruses.

This study was conducted between November 2022 and November 2023 to investigate the genetic diversity of viral strains responsible for acute respiratory infections in urban children under 15 years of age in Senegal after the end of the four epidemic waves of covid-19. With the advent of next-generation sequencing (NGS) technologies, which are powerful and more informative tools, the study of the genetic diversity of viral strains was based in this study on whole-genome sequencing to better describe this diversity.

2 | Materials and Methods

2.1 | Presentation of the Study

This descriptive, cross-sectional study focused on nasopharyngeal swabs collected from children under 15 years of age received at the reception and emergency department of the Diamniadio children's hospital, located in the Rufisque department of the Dakar region. This level 3 hospital, located in the Diamniadio crossroads zone, is a benchmark facility for the care of young patients from the area, other regions of the country and even the sub-region. All the samples collected were subjected to biological analyses at the Institut de Recherche en Santé, de Surveillance Epidémiologique et de Formation (IRESSEF), Diamniadio, Senegal, and at the Institut Hospitalo-Universitaire Méditerranée Infection, Marseille, France, with molecular detection of various respiratory viruses and whole genome sequencing of the pathogens detected.

2.2 | Study Population, Sample and Data Collection

In this study, children under 15 years of age hospitalized for severe ARI or seen in curative consultation for ARI symptoms were recruited daily between November 2022 and November 2023. The inclusion criterion was based on the WHO case definition of an ARI [13]: a patient presenting with a fever ≥ 38°C or a history of fever, with the onset of one or more of the following symptoms and signs: rhinitis, cough, sore throat, wheezing, or dyspnea. The course of the illness should not exceed 10 days. Severe ARI was defined as an acute respiratory infection with a history of fever or a measured fever ≥ 38°C and cough occurring within the last 10 days, requiring hospitalization. For each patient included, a nasopharyngeal swab was taken by nurses trained in the nasopharyngeal sampling procedure. The swabs obtained were immediately placed in 3 mL viral transport medium (Kang Jian Medical, Jiangsu, China) and gently shaken in a circular motion before closing the cap. Samples were immediately stored at +4°C at the collection site, before being transported to IRESSEF, where the cold chain was maintained using an isothermal cooler and icepacks. Once at IRESSEF, samples were aliquoted and stored at -80°C pending biological analysis.

Sociodemographic and clinical data were collected from each patient participating in the study, using a standardized questionnaire administered to the legal representative.

2.3 | Laboratory Analysis

2.3.1 | Nucleic Acid Extraction and Detection of Respiratory Viruses by Multiplex PCR

Nucleic acids were extracted on the King Fisher extractor using the MagMAXTM Viral/Pathogen II Nucleic Acid Isolation kit (Thermofisher Scientific, Illkirch, France). In accordance with the manufacturer's instructions, viral nucleic acids were extracted from 200 µL of sample and eluted in 60 µL. During extraction, the FTD Respiratory pathogens 21 kit internal control (Siemens Healthineers, Courbevoie, France) was added to each sample and to the negative control to check extraction quality and any inhibitions during PCR.

The FTD Respiratory pathogens 21 kit (Siemens Healthineers) is a multiplex PCR test for the simultaneous amplification and qualitative detection of influenza A virus, the H1N1 subtype of influenza A virus, influenza B virus, human coronaviruses NL63, 229E, OC43 and HKU1, parainfluenza viruses (HPIV) 1, 2, 3 and 4, human metapneumoviruses (HMPV) A and B, human rhinovirus (HRV), human respiratory syncytial viruses (HRSV) A and B, human adenovirus (HAdV), enterovirus (EV), human parechovirus (HPeV), human bocavirus (HBoV) and *Mycoplasma pneumoniae*. The FTD Respiratory pathogens 21 test was performed according to the manufacturer's instructions (Siemens Healthineers). For multiplex RT-PCR, each of the 5 primer/probe mixes contained in the kit was prepared by mixing 12.5 µL of 2× RT-PCR Buffer, 1.5 µL of Primer/probe mix and 1 µL of 25× RT-PCR Enzyme mix. Then, in each well of the PCR plate containing this mix, 10 µL of nucleic acid extracts were added. Subsequently, multiplex real-time RT-PCR reactions were performed on the LightCycler480 system (Roche Diagnostics, Meylan, France) using the following program: reverse

transcription at 50°C for 15 min, initial denaturation and hot start DNA polymerase activation at 94°C for 1 min followed by 40 cycles of DNA strand denaturation at 94°C for 8 s and annealing at 60°C for 1 min. Ct values from FTD Respiratory pathogens 21 assays on the LightCycler480 system was analyzed using absolute quantification. Any sample showing a sigmoidal curve with a Ct value < 33 was considered positive.

2.3.2 | Genomic Sequencing of Detected Respiratory Viruses

Detected viruses were molecularly characterized by whole genome sequencing using NGS technologies.

Nucleic acid extraction was performed using the EZ1&2 Virus Mini Kit v2.0 (48) on an EZ1 Advanced XL automated system (Qiagen, Courtaboeuf, France). Viral nucleic acids were extracted from 200 µL of samples and eluted in 60 µL. The extraction followed the manufacturer's protocol. Extracts were stored at −80°C before sequencing.

With a view to sequencing the whole genome of various respiratory viruses, multiplex PCR systems using several pairs of primers targeting the whole genome and enabling the synthesis of short amplicons (home-made ARTIC-like) have been developed and tested at the IHU Méditerranée Infection [14].

For RNA viruses equipped with these primer systems, RT-PCR was performed using the SuperScriptTM III One-Step RT-PCR PlatinumTM Taq HiFi system (Invitrogen), before preparation of the sequencing libraries. This system enables cDNA synthesis and amplification in a single step. Amplicons were stored at −20°C pending sequencing.

For RNA viruses lacking these primer systems, RT-Klenow was performed before library preparation. RT-Klenow consisted of an initial annealing step to attach the hexamers to the RNA. Then, following this step, retrotranscription of the RNAs into complementary first strands of DNA (cDNA) was achieved using the SuperScript IV reverse transcription kit (Invitrogen, Illkirch, France). Synthesis of the second complementary strand of cDNA was performed without amplification using the DNA Polymerase I, Large (Klenow) Fragment kit (New England Biolabs Inc. Evry, France) under the following incubation conditions: 37°C for 60 min. After this step, samples were purified using Agencourt AMPure XP beads (Beckman Coulter, Villepinte, France). Pure samples were stored at −20°C until sequencing.

For DNA viruses, their nucleic acid extracts were used directly for the preparation of sequencing libraries.

All samples were quantified using the Qubit dsDNA HS (High Sensitivity) assay (Invitrogen). After quantification, a dilution was performed to obtain the input concentration of 0.2 ng/µL for the preparation of sequencing libraries.

Sequencing libraries were prepared using the Nextera XT Libary Prep Kit (Illumina, Evry, France). After enzymatic tagging of the genomic DNA, DNA amplification was carried out, enabling the addition of the indexes and adapters required for cluster formation. The libraries were then purified using Agencourt AMPure XP beads (Beckman Coulter, Villepinte, France) to eliminate very short fragments. The size distribution of the libraries was determined using the Agilent 2100 Bioanalyzer (Agilent BioTechnologies). After this step, the libraries were normalized to ensure equal representation of the libraries in the final pool. This final pool of libraries was obtained by combining equal volumes of normalized libraries. Respiratory virus genome sequences were obtained by Illumina sequencing technology using the Nextera XT paired-end strategy on MiSeq and NovaSeq. 6000 instruments (Illumina Inc.). For one sample, the MiSeq platform generated two paired fragments of 250 base pairs (bp) each, and the NovaSeq platform two paired fragments of 150 bp each. The raw data was demultiplexed into FastQ files. Minimap2 (version 2.28) was used to map the FastQ files to the respective reference genomes. Sequence duplicates were removed using samtools (version 1.21). Consensus genomes were generated using Sam2consensus [15].

2.3.3 | Genomic and Phylogenetic Analysis

For Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), respiratory syncytial virus and influenza A virus, the whole-genome consensus sequences obtained were analyzed with the NextClade web application (v3.8.2) (https://clades.nextstrain.org/) against the reference genomic sequences NC045512.1, NC001803.1 and A/California/07/2009/H1N1, respectively, for genotype determination. For the remaining respiratory viruses, using a multiple sequence alignment (MAFFT v7.505), consensus genomic sequences were aligned with reference sequences and sequences with high homology and uploaded from Genbank. From the resulting alignment, a phylogenetic tree was reconstructed using IQ-TREE2 with the GTR + I + G model and 1000 ultrafast bootstrap repeats (http://www.iqtree.org). The generated tree was stored in a treefile format. This file was then uploaded to the online Interactive Tree Of Life (iTOL) software (https://itol.embl.de/) (Leptonic and Bork, 2016) for tree visualization and annotation. Genotypes were determined based on the lineage distribution of phylogenetic trees based on complete genomes. To assess nucleotide identity, amino acid identity, and mutational diversity, genomic sequence analysis with a sequencing coverage of 50% or higher was performed using the Genome Detective Virus Tool (v2.83) [16].

2.4 | Statistical Analysis

Quantitative variables are reported as medians and ranges, while categorical data are presented as frequencies and percentages. Fisher's exact test was used to compare differences between groups for qualitative variables, while the Mann-Whitney U test was applied for quantitative variables. Additionally, the phi coefficient, a measure of association for binary variables, was calculated to quantify the strength of the relationships between the viruses. To identify risk factors associated with viral infections, we first performed univariate analyses using Fisher's exact test. Variables with a $p < 0.1$ in the univariate analyses were included in a multivariable logistic regression model with backward selection ($p < 0.010$ for retention). Due to the relatively small sample size ($n = 156$) and the low counts for many viruses ($n < 10$), we grouped the detected viruses by family. Additionally, we conducted exact logistic regression, an appropriate method for modeling binary outcomes in small

TABLE 1 | Socio-demographic and clinical characteristics of participants ($n = 156$).

Characteristics	Infected[†]						
	No ($n = 41$)		Yes ($n = 115$)			All ($n = 156$)	
	n	%	*n*	%	*p* value[‡]	*n*	%
Sex (%boys)	25	61.0	65	56.5	0.714	90	57.7
Sex (% girls)	16	39	50	43.5		66	42.3
Age in month: Median (std), Q1 - median - Q3	40.0 (43.9)	5–21–72	26.8 (33.6)	5–12–36	0.316	30.3 (36.9)	5–12–36
Age group (years)							
< 1	18	43.9	54	47.0	0.855	72	46.2
[1–5]	12	29.3	48	41.7	0.192	60	38.5
> 5	11	26.8	13	11.3	0.024	24	15.4
Hospitalization (%yes)	10	24.4	46	40.0	0.089	56	35.9
Vaccination (%yes)	37	90.2	112	97.4	0.078	149	95.5
Signs or symptoms							
Fever	41	100.0	115	100.0	—	156	100.0
Cough	34	82.9	110	95.7	0.015	144	92.3
Sore throat	2	4.9	0	0.0	0.680	2	1.3
Diarrhea	8	19.5	24	20.9	1.000	32	20.5
Vomiting	6	14.6	28	24.4	0.271	34	21.8
Dyspnea	38	92.7	101	87.8	0.562	34	21.8
Lethargic or unconscious	1	2.4	5	4.4	1.000	6	3.9
Convulsion	1	2.4	5	4.4	1.000	6	3.9
Stridor	8	19.5	44	38.3	0.034	52	33.3
Unable to drink or breastfeed	2	4.9	4	3.5	0.654	6	3.9
Intercostal traction	10	24.4	57	49.6	0.006	67	43.0
Severity	10	24.4	46	40.0	0.089	56	35.9

[†]Children infected with at least one virus.
[‡]Fisher's exact test for qualitative variables; Mann-Whitney U test for quantitative variables.

sample sizes. All analyses were performed using SAS 9.4 statistical software (SAS Institute, Cary, NC).

2.5 | Ethical Considerations

This study was approved by the ethics committee of the Institut de Recherche en Santé, de Surveillance Epidémiologique et de Formations du Sénégal (code of ethics and scientific CEI/IRESSEF 002/23); and was conducted in accordance with the guidelines of the Declaration of Helsinki. The children's parents or legal guardians were all informed of the study objectives. Free and informed consent was obtained from the parents or legal guardians of participating children before nasopharyngeal sampling.

3 | Results

3.1 | Patient Characteristics

In our study, 164 children under 15 years of age were included. Of these 164 children, 8 were excluded from the analysis for lack of information on clinical and sociodemographic data. Data from 156 patients were therefore analyzed. Of these 156 children, 90 (57.6%) were boys. The median age was 1 year, ranging from 0 to 14 years. Most children were under 5, with 72 (46.1%) under 1, 60 (38.4%) between 1 and 5, and 24 (15.3%) over 5. The Dakar region was the most represented with 121 (77.5%) children, followed by the Thiès region with 17 (27.3%) children and the Diourbel region with 3 (1.9%) children. There were 100 (64.1%) children seen in curative consultations for symptoms of ARI and not hospitalized, and 56 (35.9%) hospitalized for severe ARI. Most children (95.5%) were vaccinated through Senegal's Expanded Program on Immunization. All children included in this study presented with fever or a history of fever. Among the 156 children included, the most frequent symptoms were cough (92.3%), dyspnea (89.1%), intercostal indrawing (42.9%) and stridor (33.3%). Symptoms of vomiting and diarrhea were recorded in 21.7% and 20.5% of cases, respectively. All the signs and symptoms documented during this study are presented in Table 1.

3.2 | Prevalence of Respiratory Viruses

Among the 156 children tested, 115 (73.7%) were positive for at least one pathogen, and 41 (26.2%) were negative for all pathogens

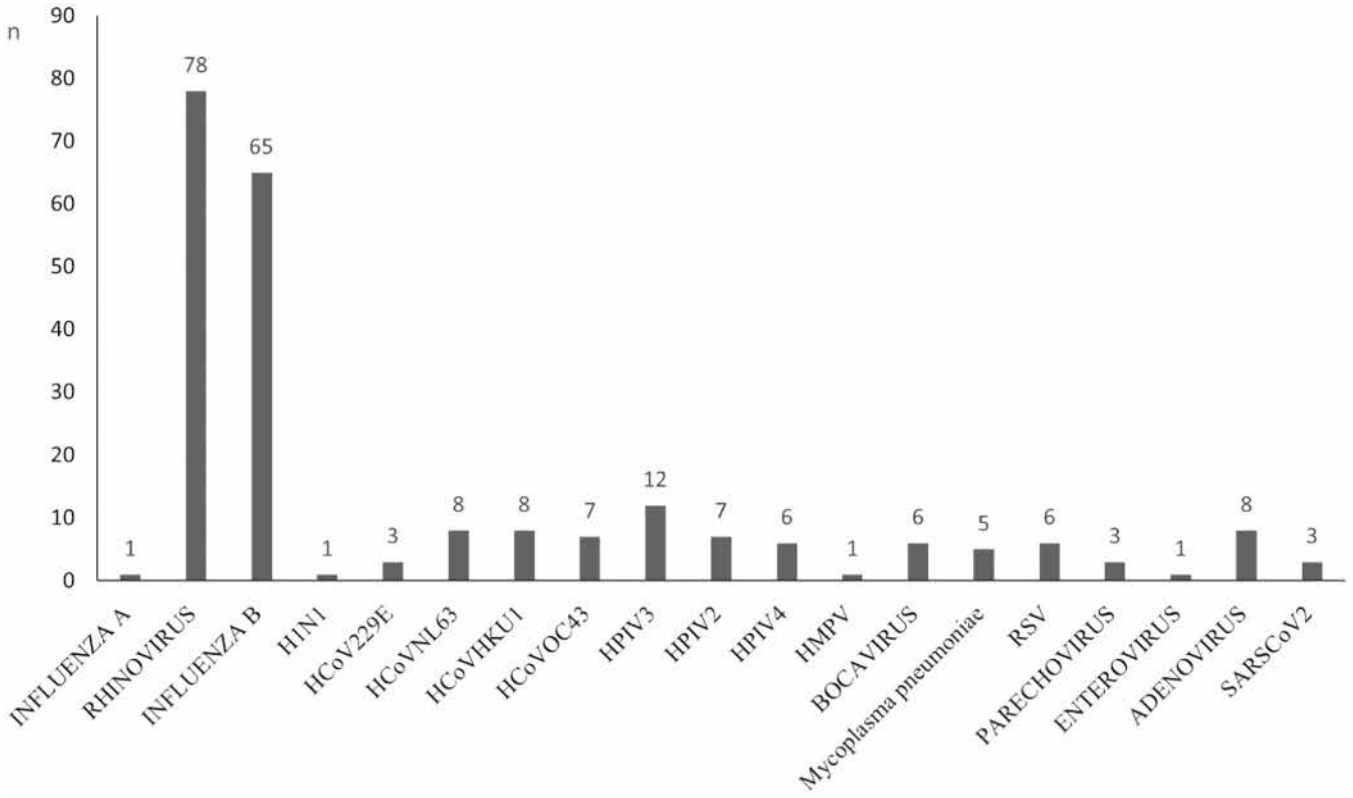


FIGURE 1 | Prevalence of detected pathogens. Number of different viruses detected. 156 children were tested; 1 child could be infected with several viruses at the same time. H1N1, hemagglutinin1 neuraminidase1; HPIV-2, human parainfluenza virus 2; HPIV-4, human parainfluenza virus 4; HPIV-3, human parainfluenza virus 3; HMPV, human metapneumovirus; HRSV, human respiratory syncytial virus; HCoV, human coronavirus.

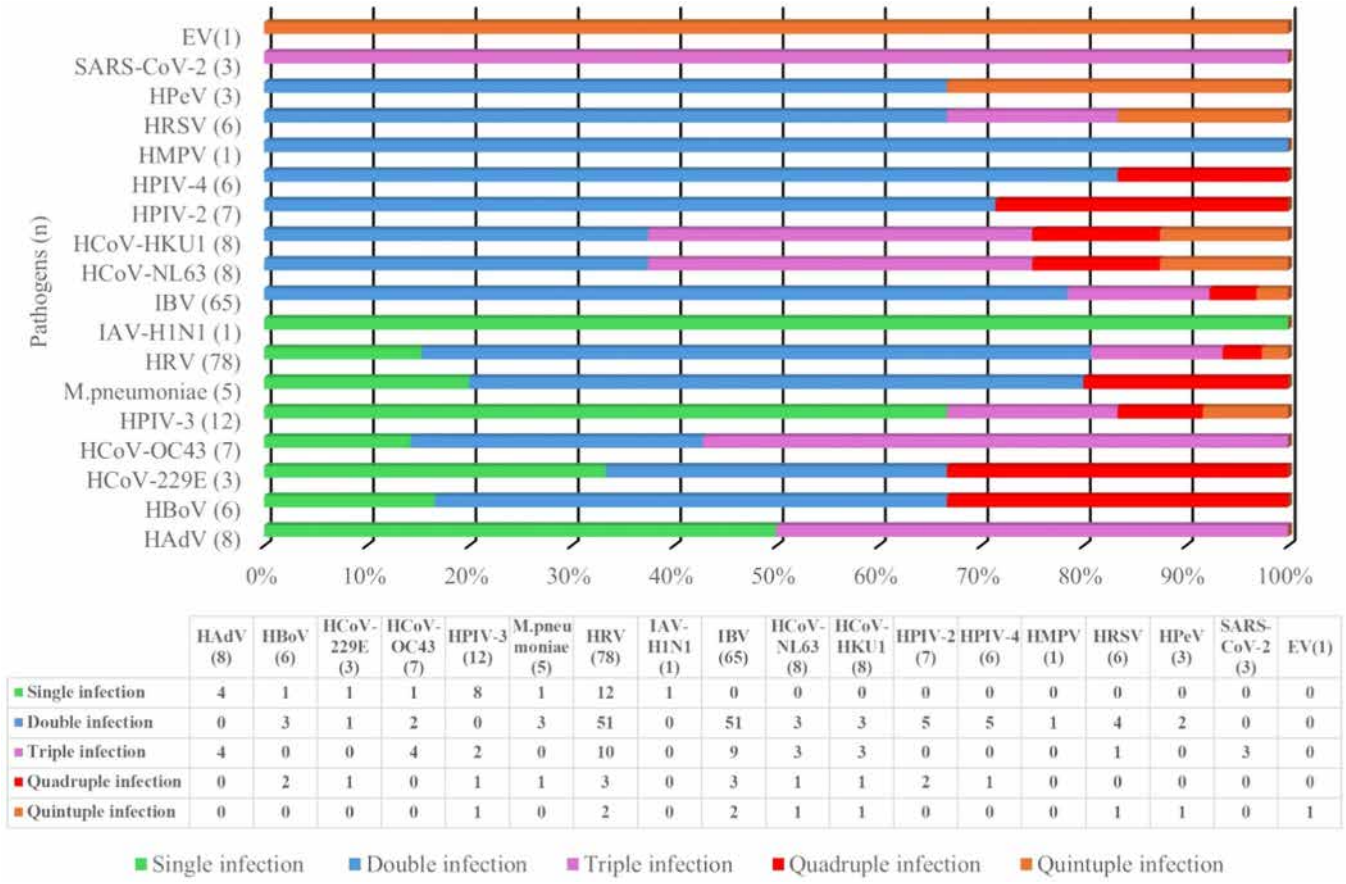
included in the test panel. Single infections were detected in 29 (18.5%) children; co-infections with 2 pathogens in 67 (42.9%) children; co-infections with 3 viruses in 13 (8.3%) children; co-infections with 4 viruses in 4 (2.5%) children; and co-infections with 5 viruses in 2 (1.2%) children. The most frequently detected virus was rhinovirus (50.0%, 78/156), followed by influenza B virus (41.6%, 65/156) and human parainfluenza virus type 3 (7.6%, 12/156) (Figure 1). In single infections, rhinovirus, parainfluenza virus type 3, and adenovirus were the most frequently detected, while in co-infections, rhinovirus and influenza B virus were the most common co-infecting viruses (Figure 2). The combinations of rhinovirus/influenza B, parainfluenza virus type 2/parainfluenza virus type 4, and rhinovirus/influenza B/adenovirus were the most frequently identified. For some co-infecting viruses, a statistically significant association was found as indicated in the matrix (Data S1). A statistically significant association was detected between rhinovirus and influenza B virus ($p < 0.001$), between HCoV-NL63 and HCoV-HKU1 ($p < 0.001$), between HCoV-NL63 and HCoV-OC43 ($p = 0.043$), between HCoV-HKU1 and HCoV-OC43 ($p = 0.043$), between HPIV-2 and HPIV-4 ($p < 0.001$), between bocavirus and HMPV ($p = 0.039$), between HRSV and parechovirus ($p < 0.001$) and between HRSV and enterovirus ($p = 0.039$) (Data S1).

3.3 | Distribution of Pathogens in Different Age Groups

The children included in this study were divided into three age groups. Pathogens were found in all age groups. The highest positivity rate was observed in children aged 1–5 years, with an 80% positivity rate, followed by the group of children under 1 year old with a 75% detection rate (Table 1). All detected pathogens predominated in children under 1 year old except for bocavirus and adenovirus, which were more common in children aged 1–5 years. Rhinovirus and influenza B virus were mostly detected across all age groups, with a predominance in children under 1 year old at 43.5% and 46.1%, respectively. In the group of children over 5 years old, fewer viruses were detected, with only rhinovirus, influenza B virus, and human coronavirus OC43 present. Co-infections were more frequent in children aged < 1–5 years (Table 2). Although detection rates varied among the detected pathogens, none showed a statistically significant difference in distribution between the different age groups.

3.4 | Viral Detection and Clinical Symptoms

Children infected with rhinovirus, influenza B virus, and bocavirus exhibited all the signs and symptoms associated with severe acute respiratory infection as recorded at the inclusion of this study. The absence of an inability to drink or breastfeed was recorded in children infected with HCoV-NL63, HCoV-HKU1, parainfluenza virus types 2 and 3, respiratory syncytial virus, adenovirus, and SARS-CoV-2. In children infected with HCoV-229E, the absence of diarrhea and vomiting was noted among the reported symptoms. No cases of diarrhea or inability to drink or breastfeed were recorded in children infected with HCoV-OC43. For children positive for HPIV-4, no vomiting or inability to drink or breastfeed was reported among the



	HAdV (8)	HBoV (6)	HCoV-229E (3)	HCoV-OC43 (7)	HPIV-3 (12)	M.pneumoniae (5)	HRV (78)	IAV-H1N1 (1)	IBV (65)	HCoV-NL63 (8)	HCoV-HKU1 (8)	HPIV-2 (7)	HPIV-4 (6)	HMPV (1)	HRSV (6)	HPeV (3)	SARS-CoV-2 (3)	EV(1)
Single infection	4	1	1	1	8	1	12	1	0	0	0	0	0	0	0	0	0	0
Double infection	0	3	1	2	0	3	51	0	51	3	3	5	5	1	4	2	0	0
Triple infection	4	0	0	4	2	0	10	0	9	3	3	0	0	0	1	0	3	0
Quadruple infection	0	2	1	0	1	1	3	0	3	1	1	2	1	0	0	0	0	0
Quintuple infection	0	0	0	0	1	0	2	0	2	1	1	0	0	0	1	1	0	1

FIGURE 2 | Proportion of single infections and co-infections ($n = 156$). Rhinovirus, parainfluenza virus type 3, and adenovirus were the most frequently detected, while in co-infections, rhinovirus and influenza B virus were the most common co-infecting viruses. H1N1, hemagglutinin1 neuraminidase1; HPIV-2, human parainfluenza virus 2; HPIV-4, human parainfluenza virus 4; HPIV-3, human parainfluenza virus 3; HMPV, human metapneumovirus; HRSV, human respiratory syncytial virus; HCoV, human coronavirus.

TABLE 2 | Distribution of pathogens in different age groups ($n = 156$).

Virus	< 1 year *n* (%)	1–5 years *n* (%)	> 5 years *n* (%)	*p* value[†]
RHINOVIRUS	34 (43.5)	31 (39.7)	13 (16.7)	0.843
INFLUENZA B	30 (46.1)	26 (40.0)	9 (13.9)	0.906
HCoV-229E	2 (66.7)	1 (33.3)	0 (0.0)	1.000
HCoV-NL63	7 (87.5)	1 (12.5)	0 (0.0)	0.079
HCoV-HKU1	7 (87.5)	1 (12.5)	0 (0.0)	0.079
HCoV-OC43	4 (57.1)	2 (28.6)	1 (14.3)	0.874
HPIV-3	7 (58.3)	5 (41.7)	0 (0.0)	0.359
HPIV-2	4 (57.1)	3 (42.9)	0 (0.0)	0.762
HPIV-4	3 (50.0)	3 (50.0)	0 (0.0)	0.745
Bocavirus	2 (33.3)	4 (66.7)	0 (0.0)	0.982
Mycoplasma pneumoniae	2 (40.0)	3 (60.0)	0 (0.0)	0.703
HRSV	5 (83.3)	1 (16.7)	0 (0.0)	0.213
Parechovirus	2 (66.7)	1 (33.3)	0 (0.0)	1.000
Adenovirus	2 (25.0)	6 (75.0)	0 (0.0)	0.129
SARS-CoV-2	3 (100.0)	0 (0.0)	0 (0.0)	0.282

[†] Fisher's exact test.

TABLE 3 | Clinical symptoms and viral detection ($n = 156$).

	Rhinovirus		Influenza B		HCoV-229E		HCoV-NL63		HCoV-HKU1	
	No ($n = 78$) %	Yes ($n = 78$) %	No ($n = 91$) %	Yes ($n = 65$) %	No ($n = 153$) %	Yes ($n = 3$) %	No ($n = 148$) %	Yes ($n = 8$) %	No ($n = 148$) %	Yes ($n = 8$) %
Cough	85.9	98.7***	87.9	98.5**	92.8	66.7	91.9	100.0	91.9	100.0
Fever	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Diarrhea	23.1	18.0	23.1	16.9	20.9	0.0	20.3	25.0	20.3	25.0
Vomiting	19.2	24.4	18.7	26.2	22.2	0.0	21.6	25.0	21.6	25.0
Dyspnea/respiratory difficulties	92.3	85.9	91.2	86.2	89.5	66.7	88.5	100.0	88.5	100.0
Stridor	29.5	37.2	28.6	40.0	32.7	66.7	32.4	50.0	32.4	50.0
Unable to drink or breastfeed	6.4	1.3	5.5	1.5	3.3	33.3	4.1	0.0	4.1	0.0
Intercostal traction	37.2	48.7	37.4	50.8	42.5	66.7	41.9	62.5	41.9	62.5
Other symptoms[†]	26.9	26.9	26.4	27.7	26.8	33.3	28.4	0.0	28.4	0.0
Severity	30.8	41.0	30.8	43.1	35.3	66.7	36.5	25.0	36.5	25.0

	HCoV-OC43		HPIV3		HPIV2		HPIV4		BOCAVIRUS	
	No ($n = 149$) %	Yes ($n = 7$) %	No ($n = 144$) %	Yes ($n = 12$) %	No ($n = 149$) %	Yes ($n = 7$) %	No ($n = 150$) %	Yes ($n = 6$) %	No ($n = 150$) %	Yes ($n = 6$) %
Cough	92.0	100.0	91.7	100.0	92.0	100.0	92.0	100.0	92.7	83.3
Fever	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Diarrhea	21.5	0.0	19.4	33.3	20.8	14.3	20.7	16.7	19.3	50.0
Vomiting	22.2	14.3	20.8	33.3	22.2	14.3	22.7	0.0	21.3	33.3
Dyspnea/respiratory difficulties	89.9	71.4	88.2	100.0	88.6	100.0	88.7	100.0	88.7	100.0
Stridor	32.9	42.9	31.9	50.0	32.9	42.9	32.7	50.0	31.3	83.3**
Unable to drink or breastfeed	4.0	0.0	4.2	0.0	4.0	0.0	4.0	0.0	2.7	33.3**
Intercostal traction	43.0	42.9	41.7	58.3	43.0	42.9	42.7	50.0	41.3	83.3
Other symptoms[†]	26.9	28.6	27.8	16.7	26.9	28.6	27.3	16.7	28.0	0.0
Severity	36.2	28.6	35.4	41.7	34.9	57.1	35.3	50.0	34.7	66.7

	Mycoplasma pneumoniae		HRSV		Parechovirus		Adenovirus		SARS-CoV-2	
	No ($n = 151$) %	Yes ($n = 5$) %	No ($n = 150$) %	Yes ($n = 6$) %	No ($n = 153$) %	Yes ($n = 3$) %	No ($n = 148$) %	Yes ($n = 8$) %	No ($n = 153$) %	Yes ($n = 3$) %
Cough	92.1	100.0	92.0	100.0	92.2	100.0	93.2	75.0	92.2	100.0
Fever	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Diarrhea	20.5	20.0	20.7	16.7	20.3	33.3	21.0	12.5	19.6	66.7
Vomiting	21.9	20.0	22.0	16.7	21.6	33.3	21.0	37.5	20.9	66.7

(Continues)

TABLE 3 | (Continued)

	Mycoplasma pneumoniae		HRSV		Parechovirus		Adenovirus		SARS-CoV-2	
	No ($n = 151$) %	Yes ($n = 5$) %	No ($n = 150$) %	Yes ($n = 6$) %	No ($n = 153$) %	Yes ($n = 3$) %	No ($n = 148$) %	Yes ($n = 8$) %	No ($n = 153$) %	Yes ($n = 3$) %
Dyspnea/respiratory difficulties	88.7	100.0	88.7	100.0	88.9	100.0	90.5	62.5**	89.5	66.7
Stridor	32.5	60.0	34.0	16.7	34.0	0.0	33.8	25.0	33.3	33.3
Unable to drink or breastfeed	3.3	20.0	4.0	0.0	3.9	0.0	4.1	0.0	3.9	0.0
Intercostal traction	41.7	80.0	42.7	50.0	42.5	66.7	43.2	37.5	42.5	66.7
Other symptoms[†]	27.8	0.0	27.3	16.7	27.5	0.0	26.4	37.5	27.5	0.0
Severity	35.1	60.0	36.0	33.3	36.0	33.3	35.8	37.5	36.0	33.3

*$p < 0.1$; **$p < 0.05$; ***$p < 0.01$ (Fisher's exact test).
[†]Rhinorrhea, nasal obstruction.

symptoms. Children infected with parechovirus did not exhibit stridor or an inability to drink or breastfeed. Patients infected with rhinovirus and influenza B virus had a significantly higher risk of presenting with a cough ($p = 0.005$ and $p = 0.015$, respectively). Patients infected with bocavirus had a significantly higher risk of presenting with stridor and an inability to drink/breastfeed ($p = 0.016$ and $p = 0.017$, respectively). A significantly lower risk of presenting with dyspnea ($p = 0.043$) was noted in patients infected with adenovirus (Table 3).

3.5 | Viral Detection in Different Clinical Diagnosis

In the overall study population, 44.2% (68/154) were diagnosed with bronchiolitis, 39.0% (60/154) with asthma exacerbation, and 3.9% (6/154) with pneumonia. Viral infections were recorded in 77.9% (53) of patients with bronchiolitis, 75.0% (45) of patients with asthma exacerbation, and 33.3% (2) of patients with pneumonia. The viruses detected across the different diagnostic groups were quite similar. Among children with bronchiolitis, there were 11 cases of single infections, 30 cases of double infections, 8 cases of triple infections, 3 cases of quadruple infections, and 1 case of quintuple infection. HPIV-3 was predominantly detected as a single infection pathogen in bronchiolitis cases, while rhinovirus and influenza B virus were predominant in mixed infections. For rhinovirus and influenza B, the incidence of bronchiolitis was similar between the negative and positive groups. HCoV-229E and SARS-CoV-2 viruses are associated with bronchiolitis in 100% of cases for patients testing positive

Among children with asthma exacerbation, there were 9 cases of single infections, 32 cases of double infections, 3 cases of triple infections and 1 case of quintuple infection. Rhinovirus and influenza B virus were predominant in mixed infections. Influenza A virus, HCoV-229E, HMPV, enterovirus, and SARS-CoV-2 were not detected in children with asthma exacerbation. Except for Rhinovirus, asthma exacerbation was generally less frequent in children positive for several viruses (Table 4).

Among children with pneumonia and viral infections, there was 1 case of single infection with HPIV-3 and 1 case of triple infection with rhinovirus, influenza B virus, and adenovirus. In the statistical analysis, respiratory distress syndrome was a very rare modality, with only 1 case in our sample ($n = 1$). Respiratory distress syndrome was included in the "other" group, which includes diagnoses with low numbers.

3.6 | Patient Characteristics and Virus Families

For the *Orthomyxoviridae* family, cough (OR = 10.61, 95% CI = 1.31–86.00, $p = 0.027$; see Table 5) and severe illness (OR = 2.05, 95% CI = 1.03–4.07, $p = 0.041$) were significant predictors in the multivariable logistic model. For the *Coronaviridae* family, younger children, particularly those under 1 year old, had a significantly higher probability of developing infections compared to older children. Although not statistically significant at the 5% level, there was a trend suggesting that patients with a sudden onset of illness had a lower probability of being infected with a virus from the *Paramyxoviridae* family

TABLE 4 | Diagnosis and viral detection ($n = 154$).

	Rhinovirus		Influenza B		HCoV-229E		HCoV-NL63		HCoV-HKU1	
	No ($n = 77$) %	Yes ($n = 77$) %	No ($n = 89$) %	Yes ($n = 65$) %	No ($n = 151$) %	Yes ($n = 3$) %	No ($n = 146$) %	Yes ($n = 8$) %	No ($n = 146$) %	Yes ($n = 8$) %
Other[†]	15.6	10.4	14.6	10.8	13.3	0.0	13.7	0.0	13.7	0.0
Bronchiolitis	45.5	42.9	43.8	44.6	43.1	100.0*	43.2	62.5	43.2	62.5
Pneumonia	6.5	1.3	5.6	1.5	4.0	0.0	4.1	0.0	4.1	0.0
Exacerbation of asthma	32.5	45.5	36.0	43.1	39.7	0.0	39.0	37.5	39.0	37.5

	HCoV-OC43		HPIV3		HPIV2		HPIV4		BOCAVIRUS	
	No ($n = 147$) %	Yes ($n = 7$) %	No ($n = 142$) %	Yes ($n = 12$) %	No ($n = 147$) %	Yes ($n = 7$) %	No ($n = 148$) %	Yes ($n = 6$) %	No ($n = 148$) %	Yes ($n = 6$) %
Other[†]	12.9	14.3	13.4	8.3	13.6	0.0	13.5	0.0	12.2	33.3
Bronchiolitis	43.5	57.1	43.0	58.3	43.5	57.1	43.9	50.0	44.6	33.3
Pneumonia	4.1	0.0	3.5	8.3	4.1	0.0	4.1	0.0	4.1	0.0
Exacerbation of asthma	39.5	28.6	40.1	25.0	38.8	42.9	38.5	50.0	39.2	33.3

	Mycoplasma pneumoniae		HRSV		PARECHOVIRUS		ADENOVIRUS		SARS-CoV-2	
	No ($n = 149$) %	Yes ($n = 5$) %	No ($n = 148$) %	Yes ($n = 6$) %	No ($n = 151$) %	Yes ($n = 3$) %	No ($n = 146$) %	Yes ($n = 8$) %	No ($n = 151$) %	Yes ($n = 3$) %
Other[†]	12.8	20.0	13.5	0.0	13.3	0.0	11.6	37.5*	13.3	0.0
Bronchiolitis	44.3	40.0	42.6	83.3*	43.7	66.7	44.5	37.5	43.1	100.0*
Pneumonia	4.0	0.0	4.1	0.0	4.0	0.0	3.4	12.5	4.0	0.0
Exacerbation of asthma	38.9	40.0	39.9	16.7	39.1	33.3	40.4	12.5	39.7	0.0

*$p < 0.1$; **$p < 0.05$; ***$p < 0.01$ (Fisher's exact test).
[†]Upper respiratory tract infections and one case of respiratory distress syndrome.

TABLE 5 | (a): Patient characteristics by Virus Family ($n = 156$). (b): Patient characteristics by virus family ($n = 156$).

	Orthomyxoviridae						*Coronaviridae*						*Paramyxoviridae*					
	No ($n = 90$)		Yes ($n = 66$)				No ($n = 138$)		Yes ($n = 18$)				No ($n = 131$)		Yes ($n = 25$)			
	n	%	*n*	%	OR 95% CI[†]	*p* value	*n*	%	*n*	%	OR 95% CI[†]	*p* value	*n*	%	*n*	%	OR 95% CI[†]	p-value
Demographics:																		
Age																		
Less than 1 year	42	46.7	30	45.5			58	42	14	77.8**	1	—	57	43.5	15	60		
1 to 5 years	33	36.7	27	40.9			57	41.3	3	16.7*	0.22 0.06-0.81	0.023	50	38.2	10	40		
More than 5 years	15	16.7	9	13.6			23	16.7	1	5.6	0.19 0.02-1.52	0.117	24	18.3	0	0.0**		NS
Sex (% male)	55	61.1	35	53			80	58	10	55.6			72	55	18	72		
Region																		
Dakar	69	76.7	52	78.8			106	76.8	15	83.3			102	77.9	19	76		
Thies	13	14.4	14	21.2			25	18.1	2	11.1			24	18.3	3	12		
Other	8	8.9	0	0.0**		NS	7	5.1	1	5.6			5	3.8	3	12		
Symptoms:																		
Cough (% Yes)	79	87.8	65	98.5**	10.61 1.31-86.00	0.027	127	92	17	94.4			119	90.8	25	100		
Diarrhea (% Yes)	21	23.3	11	16.7			28	20.3	4	22.2			25	19.1	7	28		
Vomiting (% Yes)	17	18.9	17	25.8			29	21	5	27.8			29	22.1	5	20		
Dyspnea/ Respiratory difficulties (% Yes)	82	91.1	57	86.4			124	89.9	15	83.3			114	87	25	100.0*		NS
Stridor (% Yes)	26	28.9	26	39.4			43	31.2	9	50			41	31.3	11	44		
Inability to drink/ breastfeed (% Yes)	5	5.6	1	1.5			5	3.6	1	5.6			6	4.6	0	0		
Intercostal retractions (% Yes)	34	37.8	33	50			56	40.6	11	61.1			54	41.2	13	52		
Other symptoms (% Yes)	24	26.7	18	27.3			40	29	2	11.1			38	29	4	16		
Up-to-date vaccination (% Yes)	85	94.4	64	97			132	95.7	17	94.4			125	95.4	24	96		
Antibiotic treatment at consultation (% Yes)	12	13.3	11	16.7			19	13.8	4	22.2			20	15.3	3	12		
Disease																		

(Continues)

TABLE 5 | (Continued)

	Orthomyxoviridae						*Coronaviridae*						*Paramyxoviridae*					
	No ($n = 90$)		**Yes ($n = 66$)**				**No ($n = 138$)**		**Yes ($n = 18$)**				**No ($n = 131$)**		**Yes ($n = 25$)**			
	n	**%**	***n***	**%**	**OR 95% CI[†]**	***p* value**	***n***	**%**	***n***	**%**	**OR 95% CI[†]**	***p* value**	***n***	**%**	***n***	**%**	**OR 95% CI[†]**	**p-value**
Onset																		
Sudden	15	16.7	13	19.7			26	18.8	2	11.1			27	20.6	1	4.0**	0.16 0.02-1.22	0.076
Progressive	75	83.3	53	80.3			112	81.2	16	88.9			104	79.4	24	96.0**	1	—
Diagnosis (nmissing = 2)																		
Bronchiolitis	39	43.3	29	43.9			56	40.6	12	66.7**	NS		52	39.7	16	64.0**	NS	
Asthma exacerbation	32	35.6	28	42.4			55	39.9	5	27.8			53	40.5	7	28		
Other	17	18.9	9	13.6			25	18.1	1	5.6			24	18.3	2	8		
Severity (% Yes)	27	30	29	43.9*	2.05 1.03-4.07	0.041	50	36.2	6	33.3			46	35.1	10	40		

b

	Picornaviridae						*Parvovriridae*						*Adenoviridae*						
	No ($n = 76$)		**Yes ($n = 80$)**		**OR 95% CI[†]**	***p* value**	**No ($n = 150$)**		**Yes ($n = 6$)**		**OR 95% CI[†]**	***p* value**	**No ($n = 148$)**		**Yes ($n = 8$)**		**OR 95% CI[†]**	***p* value**	**All ($n = 156$)**	
	n	**%**	***n***	**%**			***n***	**%**	***n***	**%**			***n***	**%**	***n***	**%**			***n***	**%**
Demographics:																				
Age																				
Less than 1 year	37	48.7	35	43.8			70	46.7	2	33.3			70	47.3	2	25			72	46.2
1 to 5 years	28	36.8	32	40			56	37.3	4	66.7			54	36.5	6	75.0*	NS		60	38.5
More than 5 years	11	14.5	13	16.3			24	16	0	0			24	16.2	0	0			24	15.4
Sex (% male)	46	60.5	44	55			87	58	3	50			86	58.1	4	50			90	57.7
Region																				
Dakar	58	76.3	63	78.8			115	76.7	6	100			116	78.4	5	62.5			121	77.6
Thies	11	14.5	16	20			27	18	0	0			25	16.9	2	25			27	17.3
Other	7	9.2	1	1.3**	NS		8	5.3	0	0			7	4.7	1	12.5			8	5.1
Symptoms:																				
Cough (% Yes)	65	85.5	79	98.8***	13.41 1.69-106.62	0.014	139	92.7	5	83.3			138	93.2	6	75			144	92.3
Diarrhea (% Yes)	17	22.4	15	18.8			29	19.3	3	50			31	21	1	12.5			32	20.5
Vomiting (% Yes)	14	18.4	20	25			32	21.3	2	33.3			31	21	3	37.5			34	21.8

(Continues)

TABLE 5 | (Continued)

b

	Picornaviridae						*Parvoviridae*						*Adenoviridae*						All ($n = 156$)	
	No ($n = 76$)		Yes ($n = 80$)		OR 95% CI[†]	*p* value	No ($n = 150$)		Yes ($n = 6$)		OR 95% CI[†]	*p* value	No ($n = 148$)		Yes ($n = 8$)		OR 95% CI[†]	*p* value		
	n	%	*n*	%			*n*	%	*n*	%			*n*	%	*n*	%			*n*	%
Dyspnea/ Respiratory difficulties (% Yes)	70	92.1	69	86.3			133	88.7	6	100			134	90.5	5	62.5**	0.18 0.04-0.82	0.027	34	21.8
Stridor (% Yes)	23	30.3	29	36.3			47	31.3	5	83.3**	10.70 1.13-101.24	0.039	50	33.8	2	25			52	33.3
Inability to drink/ breastfeed (% Yes)	5	6.6	1	1.3			4	2.7	2	33.3**	17.54 1.95-157.51	0.011	6	4.1	0	0			6	3.9
Intercostal retractions (% Yes)	28	36.8	39	48.8			62	41.3	5	83.3*	NS		64	43.2	3	37.5			67	43
Other symptoms (% Yes)	21	27.6	21	26.3			42	28	0	0			39	26.4	3	37.5			42	26.9
Up-to-date vaccination (% Yes)	71	93.4	78	97.5			144	96	5	83.3			141	95.3	8	100			149	95.5
Antibiotic treatment at consultation (% Yes)	12	15.8	11	13.8			20	13.3	3	50.0**	NS		22	14.9	1	12.5			23	14.7
Disease:																				
Onset																				
Sudden	12	15.8	16	20			28	18.7	0	0			27	18.2	1	12.5			28	18
Progressive	64	84.2	64	80			122	81.3	6	100			121	81.8	7	87.5			128	82.1
Diagnosis (missing = 2)																				
Bronchiolitis	34	44.7	34	42.5			66	44	2	33.3			65	43.9	3	37.5			68	43.6
Asthma exacerbation	24	31.6	36	45.0*	NS		58	38.7	2	33.3			59	39.9	1	12.5			60	38.5
Other	17	22.4	9	11.3*			24	16	2	33.3			22	14.9	4	50.0**	NS		26	16.7
Severity (% Yes)	24	31.6	32	40			52	34.7	4	66.7			53	35.8	3	37.5			56	35.9

*$p < 0.1$; **$p < 0.05$; ***$p < 0.01$ (Fisher's Exact Test).
[†]Adjusted odds ratios (95% CI). Exact multivariable logistic regression with backward selection ($p < 0.10$ for stay).

(OR = 0.16, 95% CI = 0.02–1.22, $p = 0.076$). Regarding the *Picornaviridae* family, cough was also a significant predictor (OR = 13.41, 95% CI = 1.69–106.62, $p = 0.014$). For the *Parvoviridae* family, stridor (OR = 10.70, 95% CI = 1.13–101.24, $p = 0.039$) and an inability to drink or breastfeed (OR = 17.54, 95% CI = 1.95–157.51, $p = 0.011$) were identified as significant predictors. Conversely, the probability of respiratory difficulties or dyspnea was significantly lower for patients infected with a virus from the *Adenoviridae* family (OR = 0.18, 95% CI 0.04–0.82, $p = 0.02$.

3.7 | Genomic Results

3.7.1 | Genotyping and Phylogenetic Analysis of Viruses in the *Coronaviridae* Family

Of the children tested, 29 were infected with at least 1 virus belonging to the *Coronaviridae* family: 3 HCoV-229E, 8 HCoV-NL63, 8 HCoV-HKU1, 7 HCoV-OC43 and 3 SARS-CoV-2. For genomic sequencing, we focused on samples with a threshold cycle less than or equal to 30. A total of 25 samples were sequenced. In all, 15 human coronavirus consensus sequences were obtained, including 2 HCoV-229E, 6 HCoV-NL63, 1 HCoV-HKU1, 5 HCoV-OC43 and 1 SARS-CoV-2.

For HCoV-229E, whole-genome phylogenetic analysis was performed with the complete genomes of HCoV-229E strains that showed the highest homology with Senegalese strains. The 2 HCoV-229Es in our study formed a subgroup with American strains identified between 2022 and 2023 (PQ037246.1; PQ037247.1; ON791801.1; ON791801.1) whose genogroup has not yet been assigned. However, this subgroup is genetically close to strains of the emerging HCoV-229E lineage (LC654446.1; ON554133.1; ON554132.1; KY684760.1), phylogenetically distinct from known genotypes, which emerged after genotype 6 and currently appears to be dominant worldwide [17]. This lineage is essentially composed of HCoV-229E strains from China and the United States (Data S2, Figure 1A).

For HCoV-NL63, the 6 sequences of the complete genome were found in 2 distinct genotypes. SN-IR-149-HCoV-NL63/SENEGAL/2023 and SN-IR-160-HCoV-NL63/SENEGAL/2023 were classified in genotype B and were close to Chinese strains and a Kenyan strain identified between 2016 and 2019. Sequences SN-IR-024-HCoV-NL63/SENEGAL/2023, SN-IR-040-HCoV-NL63/SENEGAL/2023, SN-IR-102-HCoV-NL63/SENEGAL/2023 and SN-IR-138-HCoV-NL63/SENEGAL/2023 were identified as belonging to genotype C. The SN-IR-040-HCoV-NL63/SENEGAL/2023 sequence was close to the 2018 Chinese strains and slightly further away from the other three Senegalese sequences. The other three Senegalese sequences classified as genotype C showed genetic proximity to Chinese, American and Japanese strains identified between 2017 and 2021 (Data S2, Figure 1B).

The only HCoV-HKU1 strain whose complete genome sequence was available was classified as genotype B. This Senegalese strain showed genetic proximity to Japanese Fukushima sequences identified in 2020 (Data S2, Figure 1C).

Phylogenetic analysis of whole-genome sequences showed that our HCoV-OC43 strains were probably very close genetically and belonged to genotype K. Phylogenetically, our Senegalese strains shared a strong similarity with other K-genotype strains from various regions, such as Canada, China and the USA, suggesting that the virus has spread across continents (Data S2, Figure 1D).

In our study, which took place during a period that can be considered post COVID-19, only 3 samples were positive for SARS-CoV-2. Based on our sequencing algorithm, the whole genome of one sample with a Ct below 30 was sequenced. This Senegalese strain was classified as an Omicron variant according to WHO nomenclature. The clade corresponding to our strain was clade 22 L according to Nextstrain, and according to the PANGOLIN nomenclature to the BA.2 sub-variant, more specifically lineage XBB.1.17.1, which is an evolution of lineage XBB, itself the result of recombination of two BA.2 sub-variants. Among the related genomes, American strains were the most frequent (Data S2, Figure 1E).

3.7.2 | Genotyping and Phylogenetic Analysis of Viruses in the *Paramyxoviridae* Family

Among the children tested, 32 were infected with at least 1 virus belonging to the *Paramyxoviridae* family: 7 HPIV-2, 12 HPIV-3, 6 HPIV-4, 1 HMPV and 6 HRSV. Whole genome sequencing was performed on samples with a threshold cycle less than or equal to 30. A total of 30 samples were sequenced and 11 *paramyxoviridae* consensus sequences were obtained, including 3 HPIV-2, 5 HPIV-3, 1 HPIV-4 and 2 HRSV. Phylogenetic analysis based on the complete genome was carried out with complete genomes that showed the highest homology with the complete genomes of Senegalese strains.

For HPIV-2, the Senegalese strains were classified in group G3, thus identifying themselves with “V98-like” sequences or clade 1. They are genetically very close to several other strains, mainly strains reported from Seattle, USA and Zagreb, Croatia, suggesting a common origin or recent genetic exchanges with strains from the USA or Europe (Data S2, Figure 2A).

For HPIV-3, the complete or nearly complete genomes of Senegalese strains showed significant genetic diversity. The SN-IR-004/HPIV-3/SENEGAL/2023 sequence showed a different evolutionary relationship to the other Senegalese sequences. It was found in subcluster C3, which is quite far from subcluster C2, where the other Senegalese sequences are grouped. The Senegalese HPIV-3s in this study were phylogenetically close to the American strains collected between 2009 and 2015 (Data S2, Figure 2B).

The only HPIV4 strain whose genome sequence was available clustered with subtype A strains, indicating its membership of this group. Phylogenetic analysis showed us that our strain appears to have close evolutionary relationships with US strains identified between 2018 and 2019 and Japanese strains from 2022 to 2023 (Data S2, Figure 2C).

For the respiratory syncytial virus, we obtained 2 complete genomes out of the 5 HRSVs sequenced in this study. The 2

sequences were identified as HRSV subtype A. According to the classification defined by Nextstrain, the 2 Senegalese strains were classified in clade A.D.1. According to the Goya et al. classification, the strains in our study fell into the GA2.3.5 subgenotype of the GA2 clade, corresponding to the ON1 genotype. Our Senegalese strains were closely related to the American strains collected in 2023 (Data S2, Figure 2D).

3.7.3 | Genotyping and Phylogenetic Analysis of Viruses in the *Parvoviridae* Family

In our study, only 1 virus of the *Parvoviridae* family infected 6 children: bocavirus. Of these 6, whole-genome sequencing was carried out on the 5 samples with a threshold cycle less than or equal to 30. We obtained 4 complete genomes of Senegalese strains. They were all identified as genotype 1 (HBoV-1), known to be strongly associated with acute respiratory infections. Our Senegalese sequences showed high percentages of nucleotide identity with strains from China, Japan and Tunisia. Phylogenetic analysis showed the Senegalese sequences to be in distinct clades, suggesting that they are genetically diverse. These Senegalese sequences are also closely related to strains from other parts of the world, indicating global circulation of the virus (Data S2, Figure 3).

3.7.4 | Genotyping and Phylogenetic Analysis of Viruses Belonging to the *Orthomyxoviridae* Family

In our study population, 66 children were infected by at least 1 virus belonging to the *Orthomyxoviridae* family: 1 by influenza A H1N1 virus and 65 by influenza B virus.

For the influenza A virus, we were able to obtain the complete genome sequence. To deduce the phylogenetic relationships of the hemagglutinin (HA) gene of the influenza A virus in our study, phylogenetic analysis based on the HA gene was carried out with the reference strain Influenza A virus (A/California/07/2009(H1N1)) and the strains showing the highest homology with our sequence. The Senegalese strain was classified as belonging to subclade 6B.1 A.5a.2a (Vaccine virus type A/Victoria/2570/2019), which emerged from clade 6B and was phylogenetically very close to an American strain collected in 2023 (Data S2, Figure 4). For the influenza B viruses detected predominantly in our study, no complete genome sequences could be obtained. This is probably due to the low viral load of our samples, which is not compatible with the metagenomic protocol used to generate complete genomes.

3.7.5 | Genotyping and Phylogenetic Analysis of *Adenoviridae* Family Viruses

Among the children tested, 8 were infected with adenovirus. According to our sequencing algorithm, whole genome sequencing was performed on 6 samples with a cycle threshold value of less than or equal to 30. Of the 6 samples, we obtained 1 sequence of a nearly complete genome. Phylogenetic analysis based on the complete genome was carried out with complete adenovirus genomes that showed the highest homology with the Senegalese strain. The latter grouped with human adenovirus type C1. The Senegalese strain shared close genetic proximity with other C1 adenovirus strains, mainly from the United States (US). Although classified under genotype C1, the Senegalese strain formed a distinct clade with a few US strains, which could suggest some evolutionary divergence from the other strains (Data S2, Figure 5).

3.7.6 | Identity Analysis

The nucleotide and amino acid identity percentages between the complete or nearly complete genomes from our study and their respective reference sequences were determined (Data S3). For the *Coronaviridae*, nucleotide identities of the 15 complete or nearly complete genomes with their respective reference strains ranged from 95% to 98%, and amino acid identities ranged from 96.9% to 98.9%. For the *Paramyxoviridae*, nucleotide identities of our 11 complete or nearly complete genomes with their respective reference strains ranged from 93.8% to 96.8%, and amino acid identities ranged from 96.5% to 98.2%. Nucleotide identities of the 4 bocavirus strains from our study compared to the reference strain NC_007455.1 ranged from 99.3% to 99.8%, and amino acid identities ranged from 99.5% to 99.9%. For the AH1N1 influenza virus strain, the 8 segments compared to the reference strain showed nucleotide identities ranging from 94.8% to 97.3%, and amino acid identities ranging from 93.8% to 99%. The nearly complete genome of HAdV from our study exhibited a nucleotide identity of 99.4% and an amino acid identity of 99.4% compared to the reference strain NC_001405.1.

3.7.7 | Mutation Analysis

Compared to the reference sequence NC_002645.1, a total of 1,024 single nucleotide polymorphisms (SNPs) were recorded across the two HCoV-229E genomes from our study. These SNPs were scattered throughout the genome. For nucleotide insertions, 23 were detected in one genome and 2 in the other. A total of 72 nucleotide deletions were identified in both genomes. Across the coding regions open reading frame 1a/1b (ORF1ab), spike (S), envelope (E), membrane (M), and nucleocapsid (N), these SNPs led to 186 and 159 nonsynonymous mutations in genomes SN-IR-080 and SN-IR-113, respectively, with 152 being common to both genomes. Additionally, both genomes exhibited amino acid insertions and deletions within their coding regions. Regarding amino acid insertions, only one genome presented seven in the ORF1ab region. For amino acid deletions, four and three deletions were observed in ORF1ab and the S gene for the two genomes, respectively.

For our HCoV-HKU1 strain, 1,051 single nucleotide variations were identified across the genome. Thirteen nucleotide insertions and 22 deletions were observed. These SNPs led to 296 nonsynonymous mutations in the coding regions ORF1ab, S, hemagglutinin-esterase (HE), E, M, and N, with the highest number in the S region. Compared to the reference sequence NC_006577.2, the Senegalese strain HCoV-HKU1 presented one amino acid deletion in the ORF1ab region and one deletion in the S gene.

For our six HCoV-NL63 strains, a total of 1,200 single nucleotide variations were recorded relative to the reference strain NC_005831.2. No nucleotide deletions were observed in any of the six genomes. However, 12 nucleotide insertions were observed in only two genomes. These SNPs resulted in 307 nonsynonymous mutations, with 35 common to two genomes, 22 to three genomes, 18 to four genomes, 8 to five genomes, and 4 to all six genomes. Nonsynonymous mutations were observed across all coding regions except for the envelope protein E gene. No amino acid deletions were recorded in any of the six genomes. Four amino acid insertions were observed in the M coding region of two genomes.

For the five HCoV-OC43 genomes obtained in this study, 1470 single nucleotide changes were observed compared to the reference sequence NC_006213.1. Only two genomes exhibited nucleotide insertions, with a total of 36. For amino acid deletions, 45 were recorded in two genomes. Across the coding regions ORF1ab, S, E, M, N, and HE, 560 nonsynonymous mutations were observed in the five genomes, with 10 common to two genomes, 49 to three genomes, 37 to four genomes, and 37 to all five genomes. These nonsynonymous mutations were observed in all coding regions. Twelve amino acid insertions were recorded in the S coding region of two genomes. One genome exhibited seven amino acid deletions in the S coding region.

For our SARS-CoV-2 strain, 95 single nucleotide changes were observed relative to the reference sequence NC_045512.2. No nucleotide insertions were observed, while 77 deletions were recorded. These SNPs resulted in 74 nonsynonymous mutations across the major coding regions ORF1ab, S, E, M, and N, with the highest number in the S region. No amino acid insertions were recorded, while amino acid deletions were observed in ORF1ab (3 deletions), S (11 deletions), and N (3 deletions).

For the three HPIV-2 genomes from Senegal, 1359 single nucleotide variations were recorded compared to the reference sequence NC_003443.1. Thirty nucleotide insertions and eight deletions were recorded across the three genomes. Across the NP (Nucleocapsid Protein), P (Phosphoprotein), M (Matrix Protein), F (Fusion Protein), HN (Hemagglutinin-Neuraminidase), and L (Large Protein) genes, these SNPs resulted in 230 nonsynonymous mutations, with 14 common to two genomes and 58 common to all three genomes. One genome exhibited an amino acid deletion in the L gene. Amino acid insertions were observed in the NP and L genes, with 4 and 6 insertions, respectively.

For the two HPIV-3 genomes from Senegal, with at least 50% of the genome covered, 580 single nucleotide variations were recorded compared to the reference sequence NC_075446.1. No nucleotide deletions were observed in these two genomes, and only one nucleotide insertion was found in one genome. These SNPs resulted in 116 nonsynonymous mutations across the NP, P, M, F, HN, and L coding regions. One amino acid insertion was observed in the NP coding region of one genome.

For our two HRSV genomes, 774 single nucleotide changes were observed relative to the reference sequence NC_001803.1. One nucleotide insertion was observed in one genome, and 19 nucleotide deletions were recorded across the two genomes. These SNPs resulted in 98 nonsynonymous mutations across the n (nucleoprotein), p (phosphoprotein), m (matrix protein), f (fusion Protein), g (attachment protein), l (rna-dependent rna polymerase), sh (dmall hydrophobic protein), and m2 (m2-1 and m2-2) genes, with 35 nonsynonymous mutations common to both genomes. No amino acid deletions were recorded, while one amino acid insertion was observed in the rna-dependent rna polymerase coding region of one genome.

For the four human bocavirus genomes, 66 single nucleotide variations were observed compared to the reference sequence NC_007455.1. Two genomes exhibited 4 nucleotide insertions and 4 deletions. These SNPs across the Nonstructural Protein 1 (NP1), Nonstructural Proteins (NS), Viral Protein 1 (VP1), and Viral Protein 2 (VP2) genes resulted in 20 nonsynonymous mutations, with 4 common to two genomes. No amino acid insertions or deletions were recorded in these bocavirus genomes.

For our Influenza A H1N1 strain, the hemagglutinin (HA) and neuraminidase (NA) genes were used to assess mutation diversity. Compared to the reference sequence NC_026433.1, the HA gene presented 88 single nucleotide variations leading to 35 nonsynonymous mutations. No nucleotide insertions or deletions were observed in the HA gene. For the NA gene, 60 single nucleotide variations were recorded relative to the reference sequence NC_026434.1, resulting in 27 nonsynonymous mutations. Similarly, no nucleotide deletions or insertions were observed in the NA gene.

For our type C adenovirus strain, compared to the reference sequence NC_001405.1, 157 single nucleotide variations were observed, leading to nonsynonymous mutations in both early and late phase replication genes. Two nucleotide insertions and 29 deletions were identified in this genome. A total of 25 nonsynonymous mutations were observed across all early phase genes, except for the Early Region 3 (E3) gene. For the five late phase genes, nonsynonymous mutations were observed only in the Late Region 2 (L2) and Late Region 4 (L4) genes, with 5 and 8 nonsynonymous mutations, respectively. No amino acid insertions were recorded, while 10 amino acid deletions were observed in the Early Region 1B (E1B) gene.

All data on the mutational analysis are available in Data S4.

4 | Discussion

In our study, 73.7% of children were infected with at least 1 respiratory virus. This prevalence is like that reported in other studies conducted in Senegal [5, 7, 18]. Other studies conducted among outpatients in Senegal recorded lower prevalences than our study [18–20]. A study carried out in inpatients obtained a higher prevalence than our study [6]. In our study, the most frequently detected respiratory viruses were rhinovirus (50.0%), influenza B virus (41.6%) and human parainfluenza virus type 3 (7.6%), whereas in previous studies carried out in Senegal among hospitalized patient's adenovirus constituted the most frequently detected group (50.0%), after influenza viruses (45.6%). In these studies, however, rhinoviruses were found in

significant proportions [5, 6]. These differences may reflect differences in the populations studied, the age range of the participants, the study periods as well as the sampling matrices used.

This high prevalence of respiratory virus detection obtained in our study after the end of the 4 epidemic waves of COVID-19 in Senegal and after the attenuation of non-pharmaceutical measures to contain the pandemic was also found in other countries. A study carried out in Italy around the same time as our study, and after the social distancing associated with COVID-19, reported an unprecedented resurgence of respiratory viruses, with a viral detection rate of 88% [21]. A study in Hong Kong reported a rebound in respiratory viruses and an increase in multi-virus infections after the lifting of COVID-19 restrictions [22]. This same increase in the detection rate of respiratory pathogens was documented by a study comprehensively describing the epidemiological data of respiratory pathogens in southern China [23]. A study carried out in Qatar demonstrated an increase in hospitalizations for viral ARI and a resurgence of respiratory viruses following the COVID-19 pandemic in young children [24]. In terms of virus-associated clinical symptoms, in our study, children infected with rhinovirus and influenza B had a significantly higher risk of presenting with a cough. Various studies have demonstrated a statistically significant association between cough and rhinovirus and influenza infections [25–27]. Bocavirus-infected patients in our study had a significantly higher risk of stridor and inability to drink/breathe. The association between wheezing and bocavirus in children has been demonstrated in several studies [28–30]. Although digestive symptoms are sometimes associated, the inability to drink or suckle is not specifically linked to bocavirus according to available data [31]. In our study, children infected with adenovirus had a significantly higher risk of developing respiratory difficulties and/or dyspnea. However, it has been reported in a systematic review and meta-analysis in China that the clinical presentation of adenovirus infections varies according to age group and virus type, and that children are more likely to experience respiratory difficulties [32]. However, it is important to note that despite the statistically significant associations reported by some studies, none of the clinical features are specific to a particular pathogen [33]. Molecular characterization studies of respiratory viruses in Senegal have made it possible to assess the genetic diversity of strains of viruses that predominate only in patients with influenza-like illness. This is the first study in Senegal to document whole-genome sequencing of human parainfluenza and bocavirus viruses from samples collected from children suffering from acute respiratory infections.

Analysis of viruses belonging to the coronaviridae family showed that the HCoV-229E strains identified in this study belonged to genogroup 6, HCoV-NL63 strains to genotypes B and C, HCoV-HKU1 strain to genotype B, HCoV-OC43 strains to genotype K and SARS-CoV-2 to the Omicron variant (Supporting data S2). These results differ from a recent study carried out in Senegal, mainly on patients with influenza-like illness. In this study, HCoV-229E belonged to genogroup 4, HCoV-NL63 to genotypes A and B, HCoV-HKU1 to genotypes A and B and HCoV-OC43 to genotypes F G and H [34]. This study used target fragments of the S gene, which may account for some of the differences with the genotypes in our study, whose lineage determination was based on the complete genome. As for SARS-CoV-2, the variant detected in our study is similar to that circulating in the country at the time of collection.

The analysis of viruses belonging to the Paramyxoviridae family showed that the HPIV-2 strains identified in this study were classified in group G3, our HPIV-3 strains in subclusters C3 and C2, our HPIV-4 strains in subtype A, and the HRSV strains in genotype GA2.3.5 (Data S2). In Africa, there is very limited data on the circulating genotypes of human parainfluenza virus in children. A study on the analysis of human parainfluenza virus type 3 genomes on a global scale highlighted that the circulating strains in South Africa were classified in groups B and C [35]. The HRSV-A genotype found in our study is the same as that identified in a recent study conducted in Senegal, which used NGS for whole genome sequencing of HRSV in patients with ARI [12].

The analysis of viruses belonging to the Parvoviridae family showed that the bocavirus strains identified in this study were classified as genotype 1 (Supporting data S2). In Senegal, circulating genotypes have not yet been reported in the literature. However, the genotype found in our study corresponds to the Bocavirus genotype that is frequently associated with ARI. Studies conducted in Japan and France using nasopharyngeal swabs have demonstrated the predominance of bocavirus genotype 1 [14, 36]. However, a study conducted in Brazil revealed the presence of Bocavirus genotype 1 in stool samples from children with gastroenteritis [37].

The analysis of viruses belonging to the Orthomyxoviridae family showed that the single complete genome sequence of Influenza A H1N1 identified in this study was classified in subclade 6B.1 A.5a.2a, which emerged from clade 6B (Data S2). Clade 6B is currently considered the predominant circulating clade [38]. A study conducted in Senegal among pilgrims of the Grand Magal of Touba presenting with fever and cough showed that the genomes of Influenza A H1N1 virus detected in these pilgrims were classified in subclade 6B.1 A.5a.1a [38]. It was reported that this lineage had been circulating since at least August 2020 in various countries, then significantly declined (or almost disappeared) by the end of 2022 [39]. This could explain the classification of our strain in subclade 6B.1 A.5a.2a, as the sample was collected in 2023. A recent post-Coronavirus Disease (post-COVID) study involving the complete genome sequencing of 1,723 H1N1 influenza viruses showed that all belonged to subclade 6B.1 A.5a.2a [40].

In our study, the adenovirus strain was classified as genotype C type 1 (Data S2). The most common adenovirus species causing respiratory infections in children are serotypes B (HAdV-B3 and B7) and C (HAdV-C1, C2, and C5) [41]. In Senegal, adenoviruses have frequently been detected at high prevalence in hospitalized pediatric patients and are often associated with influenza-like illness. The presence and predominance of HAdV-C species in Senegal was demonstrated by a study conducted as part of an influenza-like illness surveillance program. Based on the characterization of a hexon gene fragment, this study showed that 81.5% of the isolates belonged to species C [9]. In Egypt, adenovirus species typing also revealed a

predominance of species C among patients with influenza-like illness [42]. In contrast to this predominance of species C, a study conducted in Cameroon identified HAdV-B as the predominant species among pediatric patients with acute respiratory infections [43]. These studies conducted in Africa suggest that species C may be associated with influenza-like illness. Although our study was conducted in pediatric patients with acute respiratory infections (ARI), the very limited number of HAdV sequences in our study does not allow us to correlate species C with ARI in Senegal.

The main limitation of our study was the sample size. Our study population was small and did not extend across the different regions of Senegal, which may introduce bias in the interpretation of respiratory viruses frequently detected in various areas. Additionally, the duration of our study was a limitation, as it only spanned 1 year; this did not allow us to fully assess the seasonality of the viruses detected in relation to the diseases presented by the children. In addition, our study does not necessarily reflect the general distribution of viral ARI in Senegal, as our study population was limited to children who were seen in consultation or hospitalized for ARI. Another limitation of our study was the absence of a protocol for enriching viral nucleic acids for human rhinovirus and influenza B. This led to the inability to identify the genotypes of these two viruses, which were the most frequently detected in our study.

5 | Conclusion

Our study provided significant insights into the genetic diversity of respiratory viruses circulating among children in Senegal, using whole-genome sequencing. Our findings highlight the prevalence of rhinoviruses, influenza B virus, and human parainfluenza viruses in this pediatric population, with the presence of co-infections. Our results also revealed the global circulation patterns of these viruses, as the strains identified in our study share close genetic relationships with those circulating in other parts of the world, particularly North America and Asia. This study represents an important step toward a better understanding of the molecular epidemiology of respiratory viruses in Senegal. It underscores the importance of continuing genomic surveillance to inform public health strategies aimed at reducing the burden of acute respiratory infections among children, particularly in resource-limited settings like Senegal.

Author Contributions

Designed research: A.J.S.N, G.L. Performed research: A.J.S.N, G.L, I.K, V.B, N.L, A.P. Analyzed data: A.J.S.N, M.B, S.C, L.D. Data acquisition: N.R.D. Writing original draft: A.J.S.N. Review and editing: All.

Acknowledgments

The authors are grateful to the Institut de Recherche en Santé, de Surveillance Epidémiologique et de Formation (Diamniadio, Senegal) and the Institut Hospitalo-Universitaire Méditerranée Infection (Marseille, France) that contributed to success of this study.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.