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Research Article

Codetections of SARS-CoV-2 with Other Respiratory Viruses among Patients with COVID-19 in Senegal

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Abstract

The emergence of SARS-CoV-2 and the subsequent COVID-19 pandemic have sparked renewed interest for viral respiratory infections. Respiratory viruses other than SARS-CoV-2 are known for their widespread involvement in acute respiratory infections, as well as their endemicity with seasonal peaks. As was observed in various areas, co-infections in COVID-19-positive patients may increase the risk of disease severity. In Senegal, the prevalence of respiratory viral coinfections in patients infected with SARS-CoV-2 has not fully been assessed. In this retrospective study conducted in an urban environment, we investigated the occurrence and prevalence of viral respiratory coinfections in COVID-19-positive patients during the first and second epidemic waves of COVID-19 in Senegal. Using the FTD Respiratory pathogens 21 multiplex PCR kit, 437 nucleic acid extracts from laboratory-confirmed COVID-19 positive samples between May 2020 and May 2021 were analyzed. Of these, 15 samples (3.43%) were coinfected with at least 1 additional virus. Nine different respiratory viruses were detected among these fifteen samples. The most common respiratory viruses were adenovirus (n =6, 40%) and rhinovirus (n =4, 26.67%). Interestingly, only 1 case of SARS-CoV-2 co-detection involving influenza B was detected in the study samples. The median age of coinfected patients was 40.5 years; 73.33% identified themselves as female and 26.67% as male. Respiratory viral coinfections were more frequent in the 30-60 age group. Our results are likely to prompt more advanced studies of respiratory viral coinfections associated with SARS-CoV-2 during the four epidemic waves experienced by Senegal, to assess their evolutionary dynamics during the COVID-19 pandemic.

Keywords: Viral co-infection; Acute infection; Respiratory viruses; Rhinovirus; Adenovirus; Multiplex PCR

Introduction

In December 2019, a new coronavirus was associated with cases of pneumonia of unknown etiology in a group of individuals in Wuhan, Hubei, China (WHO 2020). This new coronavirus was named Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) and the pneumonia it caused, COVID-19. Subsequently, the COVID-19 pandemic has spread to every continent. As of January 02, 2024, 773,449,299 confirmed cases of COVID-19 infection and 6,991,842 deaths have been reported worldwide [1]. COVID-19 pneumonia shares common symptoms and mode of transmission with other respiratory viruses such as influenza viruses, respiratory syncytial virus (RSV), rhinoviruses, enteroviruses, adenoviruses, and human coronaviruses [2,3]. In many countries, the circulation of respiratory viruses other than SARS-CoV-2 is endemic, with occasional seasonal peaks [4,5]. In viral respiratory infections, coinfections and superinfections are frequent [6,7] and generally cause increased severity in co-infected patients [8]. Thus, during the COVID-19 pandemic, several studies were carried out to assess the proportion of COVID-19 patients coinfected simultaneously with other respiratory viruses, and to describe co-pathogens. These studies have been carried out mainly in Asia, Europe and North America, and their analysis has resulted in a pooled prevalence of respiratory viral coinfections of 5.01% [9]. In patients with COVID-19, influenza A virus, rhinovirus and adenovirus are the most frequently identified co-pathogens. However, a marked decrease in the incidence of these respiratory co-circulating viruses has been noted overall [10].

In Senegal, the first case of COVID-19 was reported on February 26, 2020. Within a month of circulation, the disease spread slowly throughout the country, with an absence of severe cases, a high cure rate and a low incidence [11]. This slow spread of the virus possibly resulted from the non-pharmaceutical measures put in place early such as social distancing, compulsory wearing of face masks, border closures and a ban on intercity travel. This evolutionary dynamic was disrupted by the appearance of SARS-CoV-2 variants of concern [12] and the attenuation of the measures put in place. This led to an increase in severe cases and deaths [13]. As of January 02, 2024, Senegal has experienced 4 epidemic waves of variable intensity, with 89,033 confirmed cases and 1,971 deaths [1].

In Senegal, respiratory viruses other than SARS-CoV-2 are widely implicated in influenza-like illnesses and acute respiratory infections. They circulate endemically with different patterns depending on the viral type [14]. It has also been shown that a large proportion of the population are asymptomatic carriers of these viruses [15]. During the COVID-19 pandemic, the implementation of social distancing and other physical barriers was associated with a decline in rates of influenza and RSV during 2020 to 2021 [16]. However, in the following year, respiratory viruses re-emerged and circulated in the population. To date in Senegal, data on the circulation of other respiratory viruses during the COVID-19 pandemic are scarce. The aim of our study was to assess co-pathogens and the prevalence of respiratory viral coinfections in SARS-CoV-2-positive patients during the first and second epidemic waves of COVID-19 in Senegal.

Materials and methods

Sampling

In this study, we used a panel of 437 residual aliquots of respiratory specimens (nasopharyngeal or oropharyngeal) from original respiratory specimens initially tested positive for SARS-CoV-2 by Real Time Polymerase Chain Reaction (RT-PCR) (Table 1). A SARS-CoV-2 positive sample was defined as any sample in which viral RNA was detected by a SARS-CoV-2 RT-PCR technique with a threshold cycle (Ct) lower than 35. This COVID-19 diagnosis had been carried out at the molecular biology platform of the Institut de Recherche en Santé, de Surveillance Epidémiologique et de Formation (IRESSEF), Diamniadio, Senegal. Socio-demographic data were provided by the patients themselves, using a questionnaire that they completed at the time of sampling [17]. The samples came mainly from the Dakar and Thiès regions, the 2 cities in Senegal most affected by COVID-19 [18]. Tested samples had been taken from suspected cases, confirmed cases and contact cases, as well as from travellers leaving Senegal. This systematic screening was part of the obligatory measures taken by the Senegalese government. For the search for other respiratory viruses, sample aliquots were transferred to the Institut Hospitalo-Universitaire Méditerranée Infection (Marseille, France) in biohazard containers (DHL, Dakar, Senegal) [17].

Real-time PCR system	Country	Regulation	Target genes	Limit of detection (copies/mL)	Thermal cycler
Abbott RealTi*m*e SARS-C0V-2 assay (Abbott)	USA	US-IVD	**RdRp et N**	100	System m2000sp/rt
Allplex™2019-nCoV Assay (Seegene)	South Korea	CE-IVD	E, N, et RdRp	4,167	Real-time PCR detection system CFX96 Touch, BIO-RAD
Detection Kit for 2019 Novel Coronavirus (2019-nCoV) RNA (PCR-Fluorescence Probing (DAAN GENE)	China	CE-IVD	ORF1ab et N	500	Amplix 16/Amplix NG48 system
Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (Sansure Biotech)	China	CE-IVD	ORF1ab et N	200	Real-time PCR detection system CFX96 Touch, BIO-RAD

E: Envelope; N: Nucleocapsid; RdRp: RNA dependent RNA polymerase; nCoV: novel coronavirus; USA: United States of America; US-IVD: United States-In Vitro Diagnostics; CE-IVD: European Certification; sp: sample preparation; rt: real-time; PCR: Polymerase Chain Reaction

Table 1: RT-PCR kits used for the diagnosis of COVID-19included in this study.

Detection of respiratory viruses other than SARS-CoV-2 by multiplex RT-PCR

Nucleic acids were extracted using a King Fisher extractor and 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 the sample and eluted in 80 µl. During extraction, the FTD Respiratory pathogens 21 kit (Siemens Healthineers, Courbevoie, France) internal control was added to each sample and to the negative control to check the quality of the extraction and any inhibition during PCR.

The FTD Respiratory pathogens 21 kit (Siemens Healthineers), a multiplex PCR assay for the simultaneous amplification and qualitative detection of influenza A virus, influenza A virus subtype H1N1, 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 assay was performed according to the manufacturer's instructions (Siemens Healthineers). Briefly, each of the 5 primer and probe mixes contained in the kit was prepared by mixing 12.5 µl of 2x RT-PCR Buffer, 1.5 µl of Primer/probe mix and 1 µl of 25x 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 a LightCycler®480 thermal cycler (Roche Diagnostics, Meylan, France) using the following program: reverse transcription at 50°C for 15 min, initial denaturation at 94°C for 1 minute, primer hybridization at 94°C for 8 seconds and enzyme elongation at 60°C for 1 minute. Primer hybridization and enzyme elongation were repeated for 40 cycles.

Data analysis

Ct values from Respiratory pathogens 21 tests were analyzed using absolute quantification. Any sample showing a sigmoidal curve with a Ct value <35 was considered positive. Co-infection was determined by detecting SARS-CoV-2 with one or more additional respiratory pathogens in the same sample. RT-PCR test results and socio-demographic data were entered into a database using Microsoft Excel. All data were saved in a Microsoft Excel database. Descriptive analysis was performed using a series of formulas in Excel (Microsoft) and SPSS version 16.0 (Chicago SPPS Inc).

Ethical statement

The National Health Research Ethics Committee of Senegal (opinion 000159/MSAS/CNERS/SN) approved the protocol concerning the samples used in this study.

Results

Patient characteristics

Four hundred and thirty-seven patients were included in the study. Of these, 78.03% (341/437) lived in the Thiès region, 21.74% (95/437) in the Dakar region and 0.23% (1/437) in the Matam region. According to the epidemic waves in Senegal, 18.76% (82/437) of samples were collected between May and July 2020 during the first wave, and 81.24% (355/437) between February and May 2021, coinciding with the second wave during which the highest case-fatality rate was recorded [13]. The mean age was 46 years, with a range of 8 to 93 years. According to gender, 59.73% of patients (261/437) were males, 39.82% (174/437) were females and no information was available for 0.46% (2/437). Clinical data were only available for patients in the second wave. Of the 355 patients in the second wave, information on symptoms was available for 352. Of these, 287 (81.53%) were asymptomatic. For symptomatic subjects, cough was the most frequent symptom (64/65, 98.46%), followed by fever (29/65, 44.62%) and sore throat (28/65, 43.08%). Other symptoms were headache (25/65, 38.46%), myalgia (19/65, 29.23%), diarrhea (7/65, 10.77%), dyspnea (5/65, 7.69%) and vomiting (2/65, 3.08%) (Table 2).

Characteristics	n, (%)
Gender	
Female	174 (39.82)
Male	261 (59.73)
Median age	46 8-93
Age groups	
<15	15 (3.43)
15-29	63 (14.42)
30-60	239 (54.69)
>60	111 (25.40)
No information	9 (2.06)
Patients with or without symptoms	
Symptomatic	65 (14.87)
Asymptomatic	287 (65.67)
No clinical data	85 (19.45)
Signs or symptoms	
Cough	64 (98.46)
Fever	29 (44.62)
Sore throat	28 (43.08)
Headache	25 (38.46)
Myalgia	19 (29.23)
Diarrhea	7 (10.77)
Dyspnea	5 (7.69)
Vomiting	2 (3.08)

Table 2: Characteristics of COVID-19 positive patients.

Detection of respiratory viruses other than SARS-CoV-2

Of 437 COVID-19-positive patients tested with the FTD Respiratory pathogens 21 kit, 15 (3.43%) were infected with at least 1 virus (Table 3). Of these 15 patients, 13 (86.67%) were infected with 1 additional virus, 1 (6.67%) with 2 additional viruses and 1 (6.67%) with 4 additional viruses.

Co-infection	n (%)
≥ 1 Respiratory virus	15 (3.43)
Rhinovirus	4 (26.67)
HCoV-229E	2 (13.33)
HCoV-NL63	1 (6.67)
HCoV-OC43	1 (6.67)
HCoV-HKUl	1 (6.67)
HPIV-4	2 (13.33)
Bocavirus	1 (8.33)
Adenovirus	6 (40.00)
Influenza B virus	1 (6.67)
Co-infection	
One (01) respiratory virus	13 (86.67)
Two (02) respiratory viruses	01 (6.67)
Four (04) respiratory viruses	01 (6.67)

Table 3: Co-infections in COVID-19 positive patients and other identified respiratory pathogens.

Among these 15 patients, rhinovirus was detected in 4 patients (26.67%), HCoV 229E in 2 patients (13.33%), HCoV-NL63 in 1 patient (6.67%), HCoV-OC43 in 1 patient (6.67%), HCoV-HKU1 in 1 (6.67%) patient, HPIV-4 in 2 patients (13.33%), bocavirus in 1 patient (6.67%), adenovirus in 6 patients (40.00%) and influenza B virus in 1 patient (6.67%) (Figure 1).

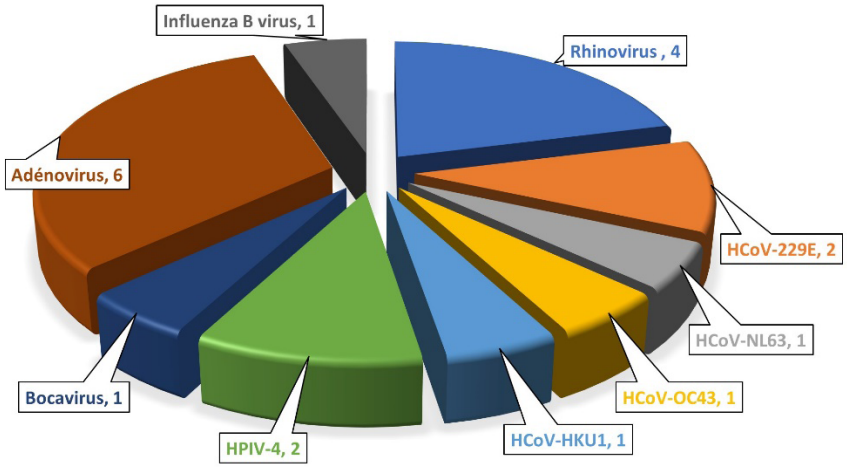

Figure 1: Number of other respiratory viruses detected in coinfected COVID-19-positive patients. Adenovirus was the most frequently detected, followed by rhinovirus, HPIV-4 and human coronavirus 229E. Influenza B virus, bocavirus and the 3 other human coronaviruses were each detected only once.

The COVID-19 positive patient with 2 additional viruses detected, was infected with rhinovirus and influenza B virus. The COVID-19 patient with 4 additional viruses detected was infected with all 4 human coronaviruses (HCoV 229E, HCoV NL63, HCoV OC43 and HCoV HKU1, Table 4). The threshold cycle (Ct) values of these detected respiratory pathogens ranged from 31 to 35.

	Rhinovirus	HCoV-229E	HCoV-NL63	HCoV-OC43	HCoV-HKU1	HPIV-4	Bocavirus	Adenovirus	Influenza B
SARS-CoV-2	1								
SARS-CoV-2	1								
SARS-CoV-2	1								
SARS-CoV-2	1								1
SARS-CoV-2		1							
SARS-CoV-2		1	1	1	1				
SARS-CoV-2						1			
SARS-CoV-2						1			
SARS-CoV-2							1		
SARS-CoV-2								1	
SARS-CoV-2								1	
SARS-CoV-2								1	
SARS-CoV-2								1	
SARS-CoV-2								1	
SARS-CoV-2								1	

Table 4: Respiratory virus combinations identified in COVID-19-positive patients.

Of the 15 patients with respiratory viral co-infections, 73.33% (n=11) were women, while 26.67% (n=4) were men. The median age was 40.5 years, ranging from 13 to 93 years. Of the 15 coinfected patients, clinical signs were available for only 9. Of these, 6 were asymptomatic and 3 symptomatic (Table 5). The most frequent symptom was cough (n=3), followed by fever (n=2 each), diarrhea and myalgias (n=1 each).

Characteristics	n, (%)
Gender	
Female	11 (73.33)
Male	04 (26.67)
Median age	40.5 8-93
Age groups	
<15	2 (13.33)
15-29	3 (20.00)
30-60	7 (46.67)
>60	3 (20.00)
Symptoms	
Symptomatic	3 (20.00)
Asymptomatic	6 (40.00)
No clinical data	6 (40.00)

Table 5: Characteristics of COVID-19 patients with viral co-detection.

Discussion

Since December 2019, COVID-19 infection has been a real global health priority. Given the clinical and symptomatological similarity with infections caused by other respiratory viruses, studies assessing the prevalences of viral respiratory coinfections in COVID-19 patients have been conducted across continents [9].

Between March 2020 and October 2023, Senegal experienced 4 COVID-19 waves of varying intensity [13]. Prior to this outbreak of COVID-19 in the country, acute respiratory infections caused by other respiratory pathogens were the second leading cause of death in both sexes and all ages [19]. Co-circulation of SARS-CoV-2 and common respiratory viruses seems inevitable. However, in Senegal, there are limited data on the prevalence of respiratory viral coinfections in people who have contracted COVID-19. The aim of this study was to investigate viral respiratory coinfections in samples collected from COVID-19-positive patients during the first and second waves of the epidemic in Senegal. Detection of respiratory pathogens by multiplex PCR provided information on the prevalence of these viral respiratory coinfections [20]. In our study, the proportion of COVID-19-positive patients coinfected simultaneously with other respiratory viruses was 3.43%. This rate is comparable to those of studies carried out at the start of the pandemic (March-April 2020) in Italy, Chicago, and California, where rates of 3.66%, 3.7% and 3.3% respectively were recorded in COVID-19 positive patients. This low prevalence found in our study shows the persistence of the low prevalence of viral respiratory coinfections found at the start of the pandemic [21-23]. The measures implemented in the country following the detection of the first case of COVID-19, i.e., mandatory masking in public places, a ban on public gatherings, the closure of international flights and the introduction of a curfew, could also be responsible for this low proportion of circulation of other respiratory viruses. In prevalence studies of respiratory viral co-infections like ours, the search was carried out in high-risk patients suspected of COVID-19 and presenting with respiratory and flu-like symptoms [21-23]. These inclusion criteria were comparable to our own. In the literature, COVID-19-positive patients have also been found to have lower rates of respiratory viral coinfections than our study [24-26]. Although it has been shown that coinfections with SARS-CoV-2 and other respiratory virus were rare at the onset of the pandemic and even more so a year after the first COVID-19 case [10], high rates of respiratory viral coinfections were found in COVID-19-positive patients. In their study of patients with hypoxemic pneumonia due to COVID-19, Allou, et al. obtained a prevalence of viral respiratory co-infection of 9.68% [27]. A rate of 12.20%, higher than in our study was recorded in COVID-19-positive patients hospitalized for acute respiratory distress syndrome in Brazil [28]. This seems to show that rates of viral respiratory co-infections are higher in patients with more severe infections. However, although the impact of respiratory viral co-infections on COVID-19 disease severity remains poorly understood, it has been demonstrated based on multivariate analyses that co-infection is a risk factor for mortality and disease worsening [29,30]. In a systematic review of respiratory viral coinfections in COVID-19-positive patients, it was reported that rates of SARS-CoV-2 and other respiratory viral co-infections varied from 0% to 56% [9]. This variability may be due to the seasonality of respiratory viruses and the timing of the studies, to the impact of non-pharmaceutical measures, and to the number of viral respiratory pathogens included in the detection tests.

In our study, adenovirus and rhinovirus were the viruses most frequently detected in SARS-CoV-2 positive patients. Adenovirus was found in 40% of samples, and rhinovirus in 26.67%. It should be stressed that in Senegal, there is a high prevalence of adenovirus respiratory infections [31,32]. Our results concur with those found in studies conducted in the south-eastern United States, the Republic of Korea and Canada, where rhinovirus and adenovirus were the most common respiratory pathogens among patients testing positive for SARS-CoV-2 [33-35]. This simultaneous predominance of adenoviruses and rhinoviruses was not, however, identified in studies carried out in Singapore and California. In these studies, rhinovirus predominated, and no adenovirus was found in SARS-CoV-2 positive samples [36,37]. This could potentially be linked to the short study period of these 2 studies, which did not coincide with the period of adenovirus circulation.

In our study, only 1 influenza B virus was detected in coinfected COVID-19-positive patients. No influenza A viruses were detected. The proportion of SARS-CoV-2 and influenza co-infection varies from country to country, as does the seasonal nature of influenza [38]. In Senegal, the circulation of SARS-CoV-2 has had a notable impact on local influenza circulation. In 2020, unusually low levels of influenza were observed between the time of detection of the first case of COVID-19 and the peak of the rainy season. In 2021, abnormally low levels of influenza and the absence of an epidemiological peak were observed during the first few months [39]. This period, corresponding to the date of sample collection in our study, may explain our low influenza detection rate.

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In our study, respiratory viral co-infections linked to SARS-CoV-2 were more frequent in patients aged between 30 and 60 than in patients aged under 15 and over 60. Our findings are consistent with a study describing respiratory pathogen co-infections in COVID-19 cases in China [46]. However, in the published literature, it was reported in a meta-analysis of 59 studies of respiratory viral coinfections that the rate of co-infection was significantly higher in paediatric patients than in adult patients [9]. An immature immune system and more frequent interaction could be linked to the higher detection of respiratory coinfections in children, and viruses have always been the respiratory pathogens most frequently implicated in acute respiratory infections in children [47].

We acknowledge that our study has certain limitations. First, our analysis was limited to the detection of respiratory pathogens included in the FTD multiplex PCR panel. Second, our cohort selected from the first and second epidemic waves in Senegal was small compared with the population of COVID-19-positive patients recorded during these waves. The samples tested came mainly from 2 regions of Senegal, which was not representative and does not allow us to extrapolate our microbiological results and apply them to the entire of the whole country. Further large-scale studies are needed to assess the true prevalence of viral respiratory coinfections during the COVID-19 pandemic in Senegal. The cross-sectional nature of our study did not allow us to investigate the seasonality of other respiratory viruses and their dynamic changes during the COVID-19 pandemic in Senegal. Clinical data and the course of infection in co-infected patients were not sufficiently provided, so we were unable to establish correlations between the severity of coinfection with SARS-CoV-2 and other respiratory viruses. In addition, it is important to note that detecting other pathogens in an infection does not prove causality with symptomatology. Despite these limitations, our study provides information on the circulation of other respiratory pathogens during the COVID-19 pandemic in Senegal, reinforcing our knowledge of viral respiratory coinfections, which until now have been little studied in the country.

Conclusion

In this retrospective analysis, we report viral co-infections in 3.43% of patients infected with SARS-CoV-2 during the first and second COVID-19 waves in Senegal. Viral co-infections were mainly caused by adenoviruses and rhinoviruses. Only one case of influenza B was detected in our patient population. Viral co-detections were found in all age groups. In conclusion, even at low detection rates, respiratory viruses other than SARS-CoV-2 continued to circulate during the COVID-19 pandemic in Senegal.

Author Contribution Statement

Designed research: A.J.S.N, I.K, C.G, G.L, S.M, P.E.F; performed research : A.J.S.N, A.P, C.K.D, G.L, I.K, A.S, D.N, N.L; analyzed data : A.J.S.N, M.B, I.K; data acquisition : A.M; Writing original draft: A.J.S.N; review & editing: All. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement

Data generated during this study are available from the corresponding author on reasonable request.

Conflict of Interest Statement: The authors declare no conflict of interest.

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