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Research article

Respiratory pathogens circulation among non-reactive sars-cov2 patients during the covid-19 pandemic in morocco

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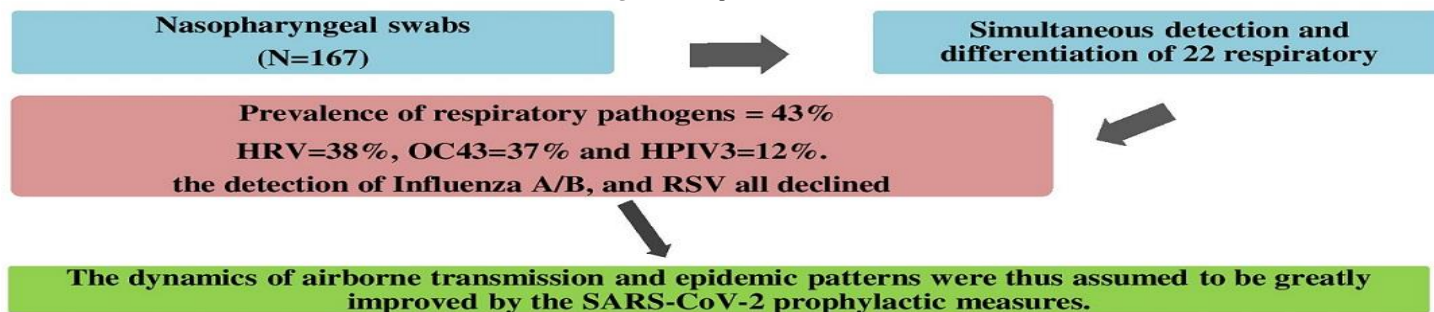
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ABSTRACT

The SARS-CoV-2 pandemic has prompted the implementation of stringent measures to curb viral transmission, including mask mandates, hand hygiene practices, and social distancing. These interventions may also have a substantial impact on the prevalence of other respiratory infections, such as influenza and severe acute respiratory illness. This study aimed to assess the incidence of respiratory pathogens among SARS-CoV-2 negative patients admitted to healthcare facilities during the COVID-19 outbreak. From February to July 2021, nasopharyngeal swabs from 167 adults presenting with COVID-19 syndrome symptoms and negative SARS-CoV-2 tests were collected from seven regional sites across Morocco. Multiplexed nucleic acid testing was employed to simultaneously detect and differentiate 22 respiratory pathogens. Our findings revealed a 43% (n=72) overall prevalence of respiratory pathogens, with Human Rhinovirus (HRV) accounting for 38%, Human Coronavirus OC43 HCoV OC43) for 37%, and Human Parainfluenza3 (HPIV3) for 12%. In contrast to the decline in Influenza A/B, Respiratory Syncytial Virus (RSV) and Human Metapneumovirus (HMPV) detection, a significant increase was observed in HRV and HCoV OC43 prevalence. These observations suggest that SARS-CoV-2 prophylactic measures have significantly altered the dynamics of airborne transmission and epidemic patterns for respiratory pathogens. Our findings provide valuable insights into the impact of COVID-19 containment strategies on respiratory infection epidemiology and can inform future prevention and management strategies.

Keywords: COVID-19, SARS-CoV-2, Respiratory viruses, Respiratory bacteria, Acute respiratory infections.

Figure 1: Graphical abstract



INTRODUCTION

Viral acute respiratory infections (ARI) pose a substantial public health concern, particularly among the adult population. Research has consistently demonstrated that viruses play a major role in the etiology of ARI, accounting for a significant proportion of these infections^[1]. The impact of viral ARIs on adult health is multifaceted, encompassing both mortality and morbidity^[2]. This is particularly evident in elderly individuals and those with underlying comorbidities, who are at an increased risk of severe complications^[3]. Moreover, viral ARIs incur substantial annual economic costs worldwide, further highlighting their significant impact on healthcare systems.^[4]

While viruses are the primary culprits behind ARIs, bacterial infections can also play a role, depending on patient exposure and underlying risk factors^[5]. Despite advancements in diagnostic techniques, differentiating between viral and bacterial ARIs remains a formidable clinical challenge^[4]. This difficulty stems from the overlapping clinical presentation of these infections, with both often manifesting with similar symptoms such as cough, fever, headache, and complications like pneumonia, bronchitis, and acute respiratory failure^[3]. This diagnostic ambiguity can lead to inappropriate antibiotic prescriptions, contributing to antimicrobial resistance and escalating healthcare costs^[3].

The first cases of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) were discovered in Wuhan in China in December 2019^[6]. But in March 2020, the illness was officially declared a pandemic due to its quick global spread. Within the Moroccan context, the first case of COVID-19 was confirmed on March 2nd, 2020, concomitant with the end of the annual influenza epidemic^[7].

Like the majority of other SARS-CoV-2-affected nations, the Moroccan government encouraged the deployment of serious epidemiological controls to prevent the virus dissemination, such as facemask use, social distancing, hand washing, and surface disinfection. Additionally, a nationwide lockdown was implemented by the Moroccan Ministry of Health, which was successful in stopping the spread of the initial pandemic wave^[8]. However, new outbreaks struck several countries, and the epidemic continued to spread fast and globally^[9] generating a real public health issue and impacting the mental health of healthcare workers with many experiencing symptoms of anxiety, depression, and post-traumatic stress disorder^[10,11].

The illness is characterized by a broad spectrum of clinical symptoms that can range from asymptomatic or mild symptoms like fever, coughing, myalgia, and headache) to serious illnesses like pneumonia, acute respiratory distress, multiple organ failure, and death^[9,12]. Meanwhile, COVID-19 symptoms have been stated in several respiratory viral and bacterial diseases, not just SARS-CoV-2^[12].

Thus, the ability to recognize which microorganism causes ARI is essential for patient monitoring and recovery as well as defining therapeutic decisions so as to develop appropriate public health strategies^[13].

At the beginning of the COVID-19 pandemic, reductions in respiratory infections, especially those caused by the influenza virus and respiratory syncytial virus, were particularly noticeable, and they persisted to variable degrees through succeeding waves of SARS-CoV-2 infections^[14]. Worldwide variations in respiratory virus infections caused by different virus strains have been linked to the COVID-19 pandemic preventive measures^[15]. Additionally, the Moroccan government's preventive measures, such as mandated mask use, social withdrawal, and the cessation of schools to stop the spread of SARS-CoV-2, might significantly impact the incidence and epidemiology of respiratory pathogens^[16]. There is a lack of information on the spread of respiratory pathogens during the COVID-19 epidemic in Morocco. Therefore, in this study we aimed to estimate the prevalence of respiratory viruses: Influenza A (Inf A), Influenza AH1N1pdm09 (AH1N1pdm09), Influenza AH1N1(AH1N1), influenza AH3N2 (AH3N2), Influenza B (Inf B), human Adenovirus (HadV), Respiratory Syncytial Virus (RSV), human Parainfluenza Virus (HPIV 1, 2, 3 and 4), Human Rhinovirus/Enterovirus (HHRV), human Metapneumovirus (HMPV), Middle East Respiratory Syndrome Coronavirus (MERS-CoV) and human Coronavirus (HCoV NL63, 229E, OC43 and HKU1) as well as respiratory bacterial pathogens (*Bordetella parapertussis*, *Bordetella pertussis*, *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*) in samples investigated for SARS-Cov-2 during the COVID-19 pandemic among adults. The data obtained may be important to understand the dynamic of other respiratory pathogens outside the main epidemic area of COVID19.

MATERIAL AND METHODS

Study setting, patient's characteristics and specimens

The study was conducted at the National Influenza Center (NIC), located at the National Institute of Hygiene in Morocco. The samples were collected as part of the influenza surveillance system approved by the Moroccan Ministry of Health. Patient confidentiality is respected, and verbal consent was obtained from patients. Therefore, formal ethical review was not required for the study.

Patients aged over 18 years old were recruited from seven geographically distinct Moroccan sites: Rabat, Salé, Kenitra, Sidi Kacem, Sidi Slimane, Oujda, and Berkane.

From February to July 2021, 167 nasopharyngeal swabs were collected and shipped to the NIC. They were received for SARS-CoV2 inquiry in the context of managing COVID-19 at healthcare facilities. Eligible samples for the study were non-reactive to nucleic acid testing for SARS-CoV2 and consistent with the following case definition:

- Acute onset of fever and cough or acute onset of any three or more of the following signs or symptoms: Fever, cough, general weakness, headache, myalgia, sore throat, coryza, dyspnea, anorexia, nausea, vomiting, diarrhea, and impaired mental status [17].
- A patient responding to a case definition of severe acute respiratory illness (SARI): ARI with a history of fever or fever > 38°C, cough, onset of symptoms within the last 10 days; and requiring hospitalization [17].

Samples were stored in the laboratory refrigerator at +4 within a period not exceeding 48 hours before processing. The biosafety measures in force were highly respected.

Methods

RNA extraction

Nucleic acid extraction was performed on Gene pure Pro nucleic acid purification system NPA-32P using a Maga-Bio plus Virus RNA Purification Kit II (HangzhouBioertehchnology, China) from 200ul of respiratory specimens according to the manufacturer's instructions.

SARS-CoV2 RNA detection

The purified viral RNA is reverse transcribed into cDNA and amplified according to the manufacturer's instructions using the TaqPath™ COVID-19 CE-IVD RT-PCR Kit, and run on the QuantStudio™ 5 Real-Time PCR Instrument (Thermofisher, USA).

In the process, probes anneal to three target sequences specific to SARS-CoV-2:ORF1ab, N and S genes. Non-reactive samples are sorted and tested for other respiratory viruses and bacteria.

Respiratory pathogens detection

All SARS-CoV2 negative samples (n = 167) were tested using the molecular multiplex assay; namely FilmArray Respiratory Panel 2 (RP2) (Biomerieux, USA), targeting 22 pathogens including 18 viruses: Inf A, Inf AH1N1pdm09, Inf H1N1, inf AH3N2, Inf B, HadV, RSV, HPIV (1, 2, 3, 4), HRV, HMPV, MERS-CoV and HCoV (NL63, 229E, OC43 and HKU1) and 4 bacteria (Bordetella parapertussis, Bordetella pertussis, Chlamydia pneumoniae and Mycoplasma pneumonia).

The testing was performed on approximately 300 ul of specimen per the manufacturers instructions [18]. The FilmArray RP2 test takes about 45 minutes and includes automated nucleic acid extraction, reverse transcription, nucleic acid amplification, and result interpretation. For accuracy, each target tested is recorded as "detected" or "not detected" by the FilmArray software, which performs automatic result analysis. The FilmArray software aims at controlling the device and interpreting the results [19]. Accordingly, when internal control fails, the software generates an "invalid" result for all panel analytes.

All sample processing was conducted in accordance with a biosafety cabinet class II, and appropriate protective equipment. For

this reason, samples were processed one at a time, and the work areas cleaned per the manufacturer's recommendations [18].

Statistical analysis

The laboratory information system (Kalisil), which creates an Excel file, was used to record clinical, virological, and demographic data. The results were analyzed by the CDC's Epi-info version 7.1 software (CDC; Atlanta, USA). When appropriate, the Fisher exact test or the Pearson Chi-square tests were used to estimate group comparisons. P-values for interactions below 0.05 were considered statistically significant. The same program was used to perform all statistical analyses, including proportions and means.

RESULTS

Table 1: Demographic characteristics of patients investigated for respiratory pathogens, Morocco, February to July 2021.

Age groups (years)	Total	Positives	Negatives	P-value
	N = 167 n (100%)	N = 73 n (43.71%)	N = 94n (56.29%)	
18-39	91 (54.49)	42 (57.53)	49 (52.13)	<0.01
40-59	51 (30.54)	21 (28.77)	30 (31.91)	
>60	25 (14.97)	10 (13.70)	15 (15.96)	
Gender				0.563
Female	37 (50.68)	58 (61.70)		
Male			0.015	
Town				NA
Rabat	14 (8.38)	7 (9.58)	7 (9.58)	
Sale	38 (22.75)	18 (24.66)	20 (21.28)	
Kenitra	37 (22.15)	15 (20.55)	22 (23.40)	
Sidi Slimane	4 (2.40)	3 (4.11)	1 (1.06)	
Sidi Kacem	13 (7.78)	1 (1.37)	12 (12.77)	
Oujda	60 (35.93)	29 (39.73)	31 (32.98)	
Berkane	1 (0.60)	0 (0)	1 (1.06)	

Demographic characteristics

From February to July 2021, a total of 167 specimens collected from 7 geographically distinct sentinel hospitals in Morocco were analyzed in this study. The notification forms were reviewed, and all patients met the World Health Organisation's (WHO) case definition of COVID-19 syndrome and were negative for SARS-CoV-2. Overall, the study included more male than female specimens. The sex ratio (male/female) was 1.35 (96/71). The age of the enrolled patients ranged from 18 to 80 years (median age 37 years). Most specimens were from adults aged 18 to 39 (55%); 31% were from the age group between 40 and 60 years, and 15% were from adults aged above 60 years. Among the seven sentinel sites, the largest number of specimens collected was 36%, 23%, and 22%, in Oujda, Sale, and Kenitra, respectively (Table 1).

Three simultaneous infections with two pathogens were detected in this study. Of the 167 samples, 5 (3%) cases were co-infected with other respiratory pathogens. The most prevalent co-infectious agents were HRV/Enterovirus and Bordetella parapertussis, HRV/Enterovirus and HCoV OC43, then HRV/ Enterovirus and RSV with frequencies of 2% (3/167), 0,6% (1/167) and 0,6% (1/167) respectively (Table 3).

During our study period, the largest number of positive cases was observed in March, with a predominance of HCoV OC43,

followed by the HRV/Entero. Thereafter, these positive cases decreased and still with the predominance of HRV and HCoV OC43 that continued to circulate until the end of July (Figure 2).

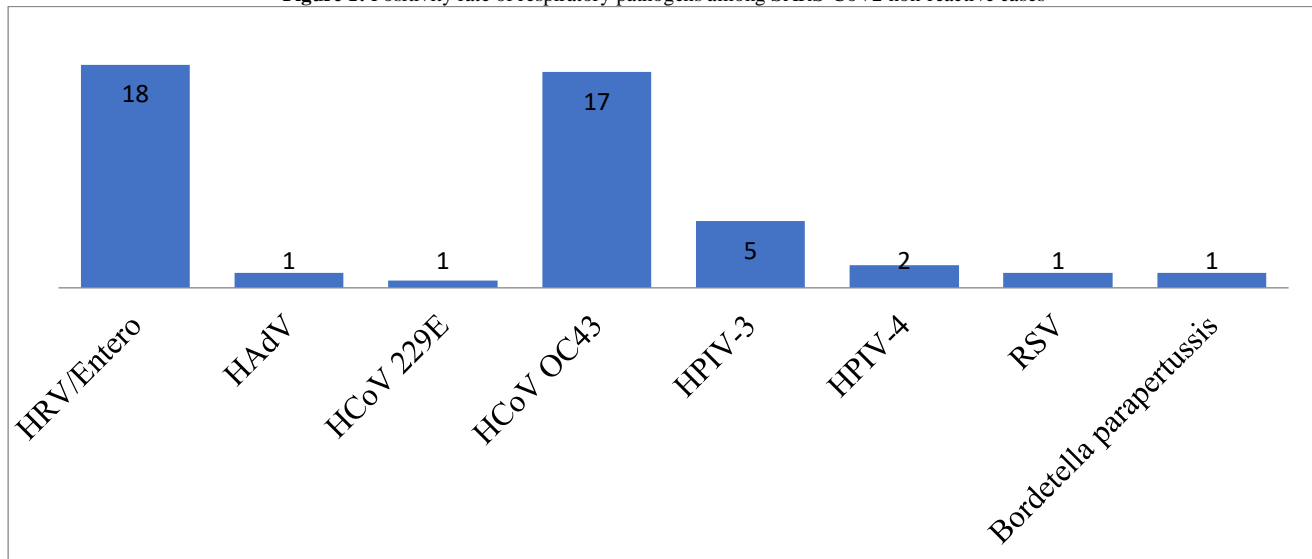
Table 3: Co-infections detected in samples enrolled. Morocco, Feb. to July 2021.

Pathogens	Co-Infectious Pathogens	Number of cases (%)
HRV/Entero + HCoV OC43	2	1 (0.6%)
HRV/Entero + Bordetella parapertussis	2	3 (2%)
HRV/Entero + RSV	2	1 (0.6%)

Table 2: Distribution of respiratory pathogens detected by age group. Morocco, February to July 2021.

Pathogens	Age group		
	18-39 (%)	40-59 (%)	≥ 60 (%)
HAdV	1 (0.6)	1 (0.6)	0
HCoV 229E	1 (0.6)	0	0
CoV OC43	18 (11)	8 (5)	3 (2)
HRV/Entero	15 (9)	10 (6)	5 (3)
HPIV-3	5 (3)	1 (0.6)	3 (2)
HPIV-4	2 (1)	1 (0.6)	0
RSV	1 (0.6)	1 (0.6)	0
Bordetella parapertussis	0	1 (0.6)	1 (0.6)
Total of detection	43 (26)	23 (14)	12 (7)

Figure 1: Positivity rate of respiratory pathogens among SARS-CoV2 non-reactive cases



*Percentage of viruses detected equals the number of detection of each virus /total number of specimen(n=167).

DISCUSSION

In China's Wuhan, the COVID-19 virus was discovered for the first time in late December 2019. The virus outbreak initially moved slowly across the country, but suddenly it began to spread quickly across numerous other countries [20]. Due to their severe clinical symptoms and transmission patterns, respiratory infections substantially risk human health during the influenza seasonal peak [21]. Accordingly, clinicians must urgently and properly identify the cases affected by COVID-19 so as to treat them effectively. However, patients with ARIs and COVID-19 are difficult to distinguish from one another, mostly because of the similarity of their first clinical symptoms[9]. Meanwhile, some common respiratory pathogens have been identified as the primary causes of ARIs, despite the disease's complex etiology [22].

This study assessed the prevalence of common respiratory pathogens, the leading causes of ARIs [1] among adults who had

performed a nasopharyngeal swab for suspected Covid-19 infection using the Biofilm Array assay method. The results show that a total of 73 patients

were confirmed to have respiratory infections, with a positive detection rate of 46.70%. These findings suggest that, even during the COVID-19 outbreak, we should also pay close attention to ARIs patients affected by respiratory pathogens along with SARS-CoV-2 [24]. Among the 22 types of common respiratory pathogens, the most prevalent was HRV/Entero (18%, 30/167), which also represents one of the most significant etiological pathogens for respiratory tract infection [22]. Additionally, HRV/Entero accounts for 38.46 % of detected respiratory pathogens among the positive cases.

This finding matches with the overall patterns described in other studies, which showed that the positive rate of HRV/Entero was the most prevalent virus circulating during the COVID-19

pandemic [24]. However, RSV was one of the least detected viruses in our study (2.56%), while Inf A and B were not detected. Although it was observed that before the pandemic, these two pathogens were among the most prevalent viruses causing respiratory infections during the winter season [25,26]. In fact, several studies reported that during the COVID-19 pandemic, the prevalence of influenza and other respiratory viruses, including the HMPV, HPIV as well as HCoV were considerably decreased [15,27,28]. It is worth noting that HRV are unique pathogens whose circulation is not affected by cataclysms such as epidemics and pandemics [29]. Although over 100 serotypes of HRV exist, there is little immunological protection provided to humans by their prior infection [29]. This accounts for the high incidence of subsequent infections [29]. However, a study revealed that the Influenza viruses A, Influenza B and RSV circulated at very low levels and only a few cases were detected in 2021 season [30]. The positivity rate sharply dropped during the lockdown and remained low throughout the interseason circulation [31–33].

The decrease of respiratory pathogens activity which was transmitted in a similar route to SARS-CoV-2 during the 2020 and 2021 seasons resulted from strict control such as social distancing, wearing face masks and increased hand hygiene that were established to mitigate the spread of COVID-19 [34]. It has not been proved yet whether the non-pharmaceutical measures implemented to limit the spread of SARS-CoV-2 will have a long-term effect on decreasing the circulation of other respiratory pathogens. However, the low levels of some pathogens' circulation, like Influenza viruses and RSV, after the COVID-19 outbreak may impact the severity of respiratory infections. This is due to a decreased population immunity as a result of the absence of natural exposure to those respiratory pathogens [35].

Our study results necessitate consideration within the context of several limitations. Firstly, the absence of comprehensive epidemiological and clinical data regarding patients posed challenges in the implementation of evidence-based therapeutic and preventive strategies. Additionally, the sample population lacked representativeness at the national level, and the constrained sample size precluded an in-depth exploration of the interconnections between clinical, epidemiological, and risk factors concerning the identified pathogens. Nevertheless, notwithstanding these limitations, the findings remain valuable for informing public health interventions in the context of respiratory infections.

CONCLUSION

The COVID-19 pandemic has shed new light on the effectiveness of non-pharmaceutical interventions (NPIs) in curbing the spread of respiratory viruses. Our research findings suggest that NPIs, such as mask-wearing, social distancing, and hand hygiene, have exerted a discernable impact on the seasonal circulation of predominant viral patterns, including RSV and Influenza viruses.

However, it was observed that these interventions did not exhibit any significant influence on HRV circulation during the COVID-19 pandemic. These findings highlight the need for a more nuanced understanding of the differential effects of NPIs on various respiratory viruses.

Despite the observed lack of impact on HRV circulation, the overall effectiveness of NPIs in reducing respiratory virus transmission is undeniable. Consequently, it is imperative to implement and maintain these measures as cornerstones of public health strategies, particularly during public health emergencies and pandemics. Additionally, the burgeoning public health burden associated with respiratory viruses underscores the importance of ongoing surveillance and characterization of their epidemiology. This will enable the development of more targeted and effective prevention and control strategies. The expanding utility of multiplex molecular respiratory virus tests holds great promise for enhancing our understanding of the epidemiology of respiratory viruses. By simultaneously detecting and differentiating multiple viruses from a single clinical sample, these tests can provide valuable insights into viral co-infections and seasonal circulation patterns. This enhanced knowledge can inform the development of more comprehensive and effective public health interventions.

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