fortune Journals

Research Article

ARCHIVES OF
CLINICAL AND
BIOMEDICAL RESEARCH

ISSN: 2572-5017

How to Make the Most in Molecular Monitoring of Key Respiratory Viruses in Patients Cared in a Regional University Hospital, from the Period of COVID-19 Emergence

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Abstract

For patients with lower respiratory tract infections, optimized molecular monitoring of key respiratory viruses along with the COVID-19 emergence is needed. The aims of this retrospective study were to investigate (i) epidemiological viral patterns among patients hospitalized for acute respiratory failure, (ii) the use of multiplex infectious respiratory PCR panel along with COVID-19, (iii) exemplary clinical scenarios within a subgroup of 18 patients in intensive care unit (ICU).

From January 2021 to June 2022, during the very high SARS-CoV-2 circulation in France, 2,383 respiratory samples from 1,614 patients who underwent multiplex PCR were retrospectively analyzed in a regional university hospital, either a panel for upper respiratory tract samples (19 viruses/4 bacteria) or for the lower respiratory tract samples (8 viruses/18 bacteria). The 'upper panel' detected viruses in 45.5% of 990 samples, including enterovirus/rhinovirus (n=168) and SARS-CoV-2 (n=99), while the 'lower panel' showed positivity in 60.4% of 1,393 samples, including 9.6% that were positive for viruses alone. For the 18 well documented ICU patients with at least one virus in the lower panel, seven received direct antiviral treatment, mainly against influenza viruses. The main findings showed (i) the frequency of entero/rhinovirus infections, with severe clinical forms; (ii) the interest in expanded molecular detection of viral respiratory infections along with the SARS-CoV-2 emergence to learn about co-circulation of viruses in patients and their dissemination; (iii) the usefulness of syndromic panel-based assays in an exemplary ICU for adapted direct antiviral treatment.

Keywords: Severe viral respiratory tract infections; Syndromic panel-based assays; SARS-CoV-2; Epidemiological patterns

Abbreviations: AL: Bronchoalveolar lavage; BMI: Body mass index; CHRU: Centre hospitalier régional universitaire; COVID-19: Coronavirus disease; CRA: Cardiorespiratory arrest; ETA: Endotracheal aspirate; EV/HRV: Enterovirus/rhinovirus; F: Female; FA: FilmArray; FA-PP: FilmArray® Pneumonia Panel plus; h-CoV: Human coronaviruses; hMPV: Human metapneumovirus; hPIV: Human parainfluenza; IAV: Influenza A virus; ICCA: IntelliSpace Critical Care and Anesthesia; ICU: Intensive care unit; IQR: Interquartile range; LIS: Laboratory Information System; M: Male; MERS-CoV: Middle East respiratory syndrome-related coronavirus; NAATs: Nucleic acid amplification tests; NPSs: Nasopharyngeal swabs; OTAS: Orthopedic, traumatology and arthroscopy surgery; PCR: Polymerase chain reaction; PDS: Projected distal sample; RICU: Respiratory intensive

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Citation: Yasmina Sayed, Antoine Kimmoun, Véronique Venard, Eliane Albuisson, Raphaël E. Duval, Evelyne Schvoerer. How to Make the Most in Molecular Monitoring of Key Respiratory Viruses in Patients Cared in a Regional University Hospital, from the Period of COVID-19 Emergence. Archives of Clinical and Biomedical Research. 8 (2024): 433-443.

Received: November 26, 2024
Accepted: December 03, 2024
Published: December 12, 2024

Sayed Y, et al., Arch Clin Biomed Res 2024
DOI:10.26502/acbr.50170425

care unit; RP2.1 plus: Respiratory Panel 2.1 plus; RSV: Respiratory syncytial virus; RT-PCR: Reverse Transcriptase – Polymerase chain reaction; SARS: Severe acute respiratory syndrome; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2

Introduction

Severe respiratory infections have been a major cause of mortality worldwide during the COVID-19 pandemic. These COVID-19 infections have led to major disruptions in global health systems and economies, with more than 758 million confirmed cases and 6.88 million deaths worldwide as of March 2023, while SARS-CoV-2 is establishing itself in lasting circulation with very diverse clinical forms [1].

Beyond the predominant SARS-CoV-2, a range of other easily spread respiratory pathogens can cause severe illness, particularly in immunocompromised individuals, infants and older adults. Rapid and etiologic diagnosis of respiratory infections is crucial for effective clinical management, infection control, and public health surveillance [2]. In response to this urgent need, syndromic panel-based assays targeting several respiratory infectious agents have gained prominence in recent years, offering high sensitivity, specificity, and fast and large-scale screening for respiratory pathogens, particularly in vulnerable patients during the current COVID-19 pandemic [3]. Notably, nucleic acid amplification tests (NAATs), particularly PCR multiplex assays, have gained widespread acceptance in clinical laboratories for the etiologic diagnosis of respiratory infections [4]. These assays, which can simultaneously detect multiple respiratory pathogens, including bacteria, viruses, and fungi, in a single biological sample, have advantages such as a swift turnaround time and the ability to adapt to emerging infectious diseases [5]. The expeditious diagnosis facilitated by multiplex PCR assays can help physicians in crucial aspects of patient care, including isolation measures, tailored treatments, and discharge planning, thereby mitigating antibiotic overuse and fostering effective antiviral interventions, thus reducing the risks of antimicrobial resistance and associated side effects [6].

Through a retrospective analysis of the use of syndromic panel-based assays in the real-life conditions of a regional university hospital, the present study aimed (i) to determine the prevalence of non-COVID-19 illnesses during SARS-CoV-2 circulation over an 18-month period, (ii) to specifically investigate a well-characterized and representative patients subgroup in an intensive care unit (ICU) and (iii) to discuss on optimized molecular monitoring of respiratory viruses for epidemiology or clinical practice along with the COVID-19 emergence and subsequent viral co-circulations.

Materials and Methods

This retrospective study was conducted over an 18-month period, from January 2021 to June 2022, and included all 2,383 different respiratory samples collected from 1,614 hospitalized patients analyzed with the BioFire® FilmArray® pneumonia panels (Biomérieux, France). The decision to conduct multiplex PCR testing in addition to standard bacterial culture was guided by clinician judgment. Biological assay results were anonymized, focusing on a standard-of-care patient population, thereby providing an epidemiological overview of respiratory pathogen co-circulation during the SARS-CoV-2 emergence across the French hospital network. Ethical guidelines for human studies, as outlined in the Declaration of Helsinki, were strictly followed (nb 2023PI112-478, ethical committee CHRU Nancy, France S-n° 440).

For nasopharyngeal swabs (NPSs), the BioFire® Respiratory Panel 2.1 *plus* (RP2.1 *plus*) panel was used. Standard collection methods were used, and NPSs were immediately placed in viral transport media (i.e., UTM); viral infections/coinfections were enumerated using this panel (Table 1). For sputum samples, endotracheal aspirates, bronchoalveolar lavages and protected distal samples, the BioFire® Pneumonia Panel *plus* was applied.

The results are presented according to reports generated by the software that is incorporated into the FilmArray® equipment. Then they were extracted by using the Laboratory Information System (SIL) of the Nancy University Hospital (GLIMS, edited by MIPS, v9) and then sorted and analyzed with Excel software.

Moreover, the usefulness of FilmArray® technology in medical practice was explored for severe virus-induced pneumonia in critical care patients hospitalized in an ICU. For the period of interest, the 18-month period, the corresponding multiplex PCR panel analysis results were retrospectively collected (FilmArray® respiratory panels, Biomérieux), the population data and occurrence of infectious pathogens were examined. A descriptive analysis was done according to variables distribution: percentages, occurrence, mean, median. Additionally, the clinical conclusion reports of 18 ICU patients infected with respiratory viruses, well-characterized by the collaborative ICU team, were examined (Table 2) - among a total of 58 patients with 135 samples that were positive for at least one virus by the FilmArray® Pneumonia Panel *plus* (Figure 1).

The inclusion criteria for the latter analyses on the 18 well-characterized ICU patients were as follows: patients in the collaborative ICU who underwent lower respiratory tract sampling for a BioFire® FilmArray® Pneumonia Panel *plus* that had detected at least one virus. For the 18 ICU patients with available detailed clinical files (exploratory data search), their representativeness was examined for age, sex, sampling

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period over the 1.5-year, based on the laboratory information system (LIS) data, in relation to the above mentioned total of 58 patients. Finally, clinical data were extracted from different sources, namely, the IntelliSpace Critical Care and Anesthesia (ICCA), and then sorted and analyzed with Excel software.

Concerning the variables, they included the microbiological approach:

- result of the multiplex respiratory test on lower respiratory tract samples (viruses and/or bacteria)
- radiological data, age, sex, and body mass index (BMI), clinical characteristics (justification and duration of hospitalization and comorbidities: cancer, cardiac/cardiovascular disease, chronic renal/hepatic/respiratory disease, diabetes, hypertension, immunosuppression, neurological lesions, and obesity).

For the medical conclusion, we included confirmation of infectious pneumonia, antibiotic protocols, and oxygen therapy modalities, disease evolution and any complications/mortality due to any cause.

Thus, the present retrospective study aimed to (i) give a global descriptive analysis according to variables distribution of viral respiratory viruses and patients: percentages, occurrence, mean, median (with IQR), then discussed according to previous scientific publications on viral respiratory infections in hospitalized patients and their dissemination along with COVID-19 emergence, (ii) perform a systematic examination of the clinical conclusion reports for 18 ICU patients infected with respiratory viruses, to give a detailed analysis on medical practices for severe viral pneumonia in the hospital intensive care team.

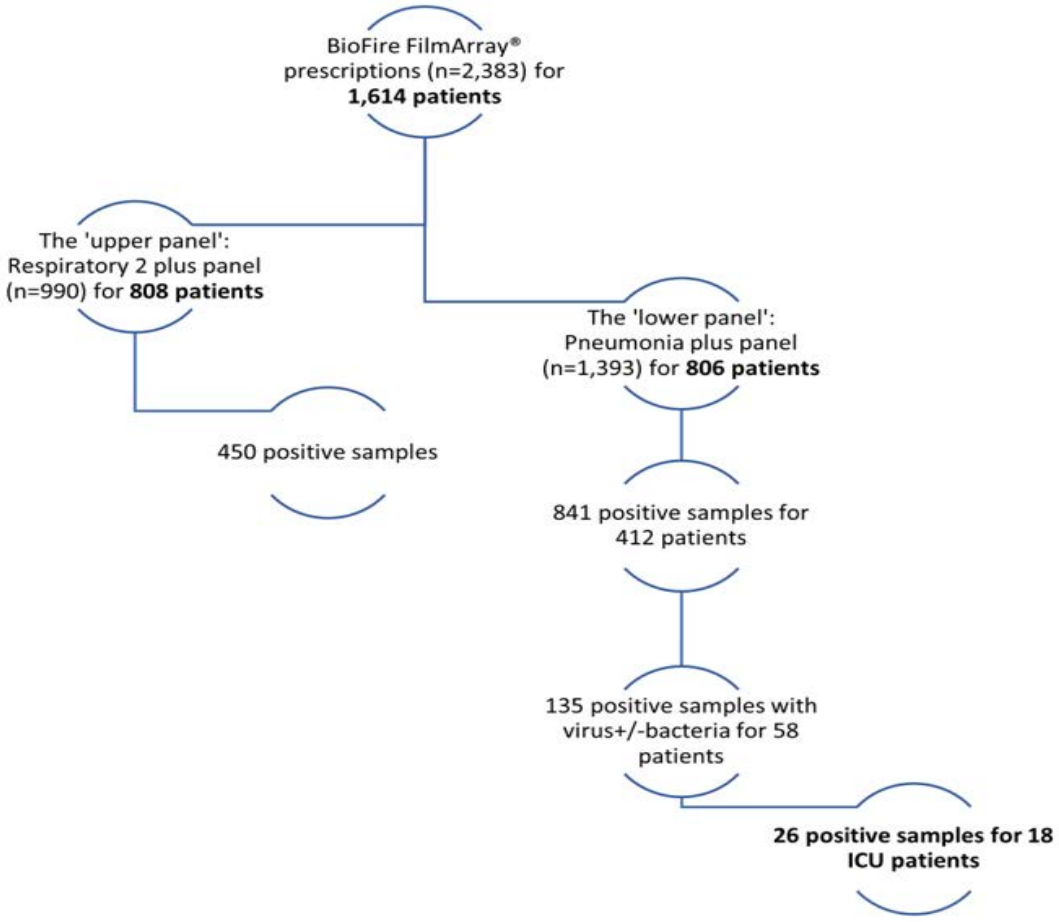

Figure 1: Study selection flowchart summarizing the study selection process and distribution of the viruses detected with the FilmArray® Pneumonia test in the exemplary intensive care unit (n=18). The initial study recorded data from a total of 2,383 multiplex PCR assays, including 990 samples analyzed with the FilmArray® Respiratory 2.1 *-plus* panel (RP2.1, corresponding to the upper respiratory tract samples) and 1,393 samples analyzed with the FilmArray® Pneumonia Panel *plus* (FA-PP, corresponding to the lower respiratory tract samples). Among these, 841 tests detected a pathogenic organism, including 135 with at least one virus. A subgroup of 18 patients from the intensive care unit were included in the last part of the detailed bioclinical study.

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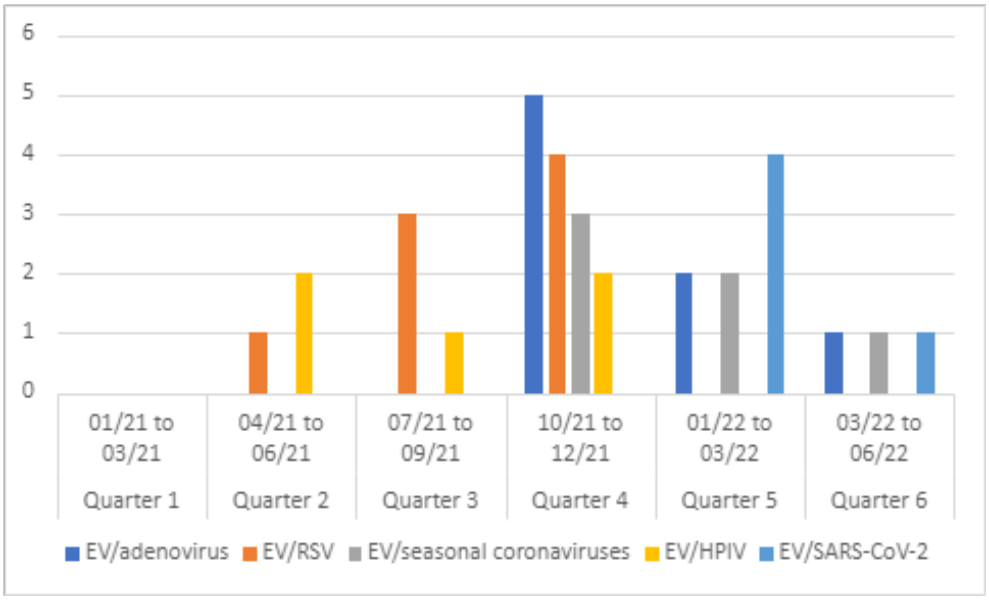
Table 1: Prevalence of coinfections with respiratory viruses detected with the BioFire® FilmArray®Pneumonia Panel *plus*. For the 41 patients, the detailed chronology of the viral coinfections from April 2021 to mid-2022 is shown.

Viral coinfections	Number of positive FilmArray® Pneumonia assays
Entero/Rhinovirus + Adenovirus	8
Entero/Rhinovirus + RSV	8
Entero/Rhinovirus + seasonal coronavirus	6
Entero/Rhinovirus + Parainfluenza virus	5
Entero/Rhinovirus + SARS-CoV-2	5
Entero/Rhinovirus + Human Metapneumovirus	4
RSV + Adenovirus	1
Human Metapneumovirus + Adenovirus	1
RSV + Parainfluenza virus	1
RSV + coronavirus 229E	1
Parainfluenza virus 3 + Adenovirus	1
	TOTAL: 41



Results

A flowchart summarizing the study selection process is shown in Figure 1.

The first part of the study (Figure 2) focused on the results of 990 nasopharyngeal samples tested by using the FilmArray® RP2.1-*plus* panel. The male-to-female ratio was 1.6, and the median age was 38 years (7-67 years). The most requested medical services were hematology (31.3%), intensive care (25%), pediatrics (17.6%) and the pneumology department (10.8%). Viruses were detected in 450 samples corresponding to an overall positivity rate of 45%. The isolated viruses are represented in Figure 2. Enterovirus/rhinovirus (EV/HRV) was most common (n=168), followed by SARS-CoV-2 (n=99), seasonal coronaviruses (H-CoV) (n=36), adenovirus (n=36), respiratory syncytial virus (RSV) (n=34), human parainfluenza (hPIV) (n=31), influenza A and B viruses (n=23), human metapneumovirus (hMPV) (n= 21), and two atypical bacteria. No cases of MERS-CoV have been detected.

For the second part of the study, the BioFire® FilmArray® Pneumonia Panel *plus* detected at least one virus and/or bacteria in 841 out of 1,393 samples (60.4%) (Figure 1), with 299 samples containing at least two bacteria and 54 containing both a virus and a bacterium. Respiratory virus coinfection was found in 9% of the patients, corresponding to 41 patients, detailed in Table 1. *Thus, for the second part* of the study on Pneumonia, a total of 1,393 lower respiratory samples were analyzed with

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the BioFire® FilmArray® Pneumonia Panel *plus*. Samples were collected mainly from adults (97.6%) and patients in intensive care (87%). The median age of the patients was 63 years (IQR: 54-71 years), with a male/female ratio of 2.32. Among the 1,393 expectorations, bronchoalveolar lavages, endotracheal samples, and projected distal aspirates analyzed by the BioFire® FilmArray® Pneumonia Panel *plus*, a total of 93 were positive for the following pathogens: influenza A and B viruses (n=11), respiratory syncytial virus (n=3), seasonal coronaviruses (n=11), human parainfluenza (n=12), enterovirus/rhinovirus (n=43), human metapneumovirus (n=5), adenovirus (n=8), and MERS-CoV (n=0) (Figure 3).

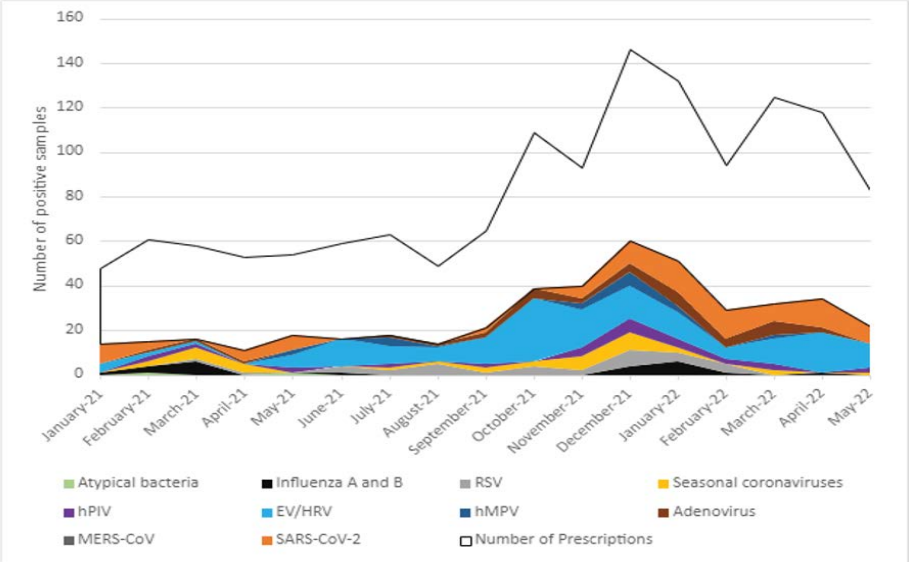

Figure 2: A total of 990 nasopharyngeal samples were analyzed with BioFire® FilmArray® Respiratory 2,1 -plus panel. The data corresponds to the number of positive samples for each of the viruses (total positive, n=450); black curve, total number of tests; light green, atypical bacteria (n=2); purple, hPIV (n=31); MERS-CoV, dark green (n=0); influenza A and B, black (n=23); EV/HRV, blue (n=168); SARS-CoV-2, orange (n=99); RSV, green (n=34); hMPV, dark blue (n=21); seasonal coronaviruses (no SARS-CoV-2), yellow (n=36); adenovirus, brown (n=36).

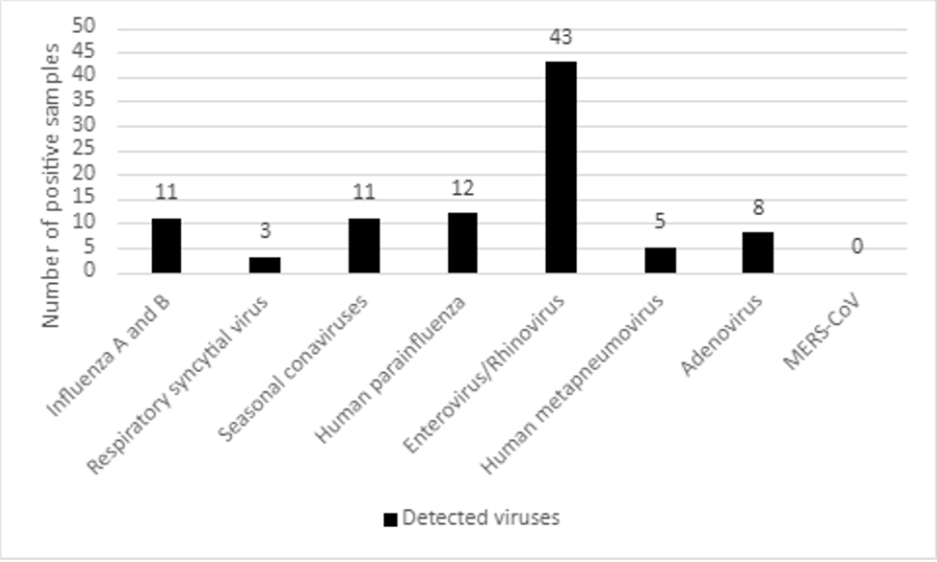

Figure 3: A total of 1,393 expectorations, bronchoalveolar lavages, endotracheal samples, and projected distal aspirates were analyzed with the BioFire® FilmArray® Pneumonia Panel *plus* from January 1, 2021, up to June 1, 2022. **The results are presented as the number of samples (total positive n=93) positive for each of the following identified viruses**: influenza A and B (n=11); respiratory syncytial virus (n=3); seasonal coronavirus (n=11); human parainfluenza (n=12); enterovirus/rhinovirus (n=43); human metapneumovirus (n=5); adenovirus (n=8); and MERS-CoV (n=0). MERS-CoV, Middle East respiratory syndrome-related coronavirus.

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Among the patients who were tested for SARS-CoV-2 infection within 15 days before or after, a BioFire® FilmArray® Pneumonia Panel *plus* test was performed (405 out of 806 patients had both tests performed), 136 were positive for SARS-CoV-2 infection. Additionally, 67 bacterial codetections were identified in SARS-CoV-2-positive patients. Among them, 38 patients were positive for SARS-CoV-2, with an average of 7 days before the detection of bacteria by the FilmArray®, as mentioned above. For 10 patients, the analyses were conducted on the same day, while for 19 patients, a positive result was obtained from FilmArray®, on average, two days before SARS-CoV-2 was detected by RT–PCR.

A total of 18 patients from the ICU showed positivity for a virus, either isolated or as a co-detection. The median age of these patients was 60 years (IQR: 36-65 years), and 14 of them had comorbidities, including arterial hypertension and chronic smoking. These patients were hospitalized for an average of 13 days, and none of them were known to have a positive SARS-CoV-2 test. Among the detected viruses, the most common were enterovirus/rhinovirus (n=10), followed by influenza A virus (n=4), adenovirus (n=2), parainfluenza virus (n=1), and seasonal coronavirus (n=1) (Table 2). We then analyzed the conclusions drawn by the intensive care physicians according to the FilmArray® Pneumonia results. The findings indicated that six patients suffered from viral pneumonia, with influenza A virus being the causative agent in four of them. In addition, one immunocompromised patient was positive for an adenovirus, while the asthma of one patient was complicated by an entero-rhinovirus infection. Five additionally positive FilmArray® results showed entero/rhinovirus positivity. For two patients, a bacterial pneumonia occurred in addition to the presence of entero-rhinovirus and adenovirus according to the FilmArray® results. One patient was diagnosed with polymicrobial pneumonia due to the detection of four pathogens, including entero/rhinovirus and parainfluenza virus. Finally, for the remaining three patients, pneumonia was also attributed to noninfectious causes. The four patients with influenza A virus infections received adapted oseltamivir, while the immunocompromised patient with an adenovirus infection was treated with cidofovir.

Table 2: Patients hospitalized in the intensive care unit from January 2021 to June 2022 whose respiratory tract samples were positive for virus signals according to the FilmArray® Pneumonia Panel *plus* assay.

PATIENT	SEX	AGE	HOSPITALIZATION	SAMPLE	RÉSULTS FA	BACTERIAL CULTURE	HOSPITALIZATION DURATION (J)	STATUS	COMORBIDITIÉS (Y/N)	CONCLUSION VIRAL PNEUMONIA	ANTI-INFECTIOUS TREAITMENT ANTIINFECTIEUX	ANTI-INFECTIOUS APPROACH	ANTIBIOTHÉRAPY DURATION	OXYGÉNOTHÉRAPIY(Y/N)	IMAGERY (Y/N)	COVID PCR
1	F	65	CRA	ETA (2)	EV/RV (2)	Flora < 2x10*4 UFC/mL (2)	9	Death	Y	N	N	/	/	NR	N	NEG
2	F	28	SARS on severe acute asthma	ETA	EV/RV	Neg	4	To Pneumo-logy Dpt, Good evolution	NR	Y	N	/	/	Y	N	NEG
3	M	34	SARS on pneumonia in an immunocom-promised patient	ETA (2) + BAL	Adenovirus (4)	Flora < 2x10*4 UFC/mL (4)	38	Death	Y	Y	Y	MEROPENEM BACTRIM SPIRAMYCINE VORICONAZOLE VALACICLOVIR	NR	Y	Y	NEG
4	M	61	Cholecystec-tomy + perforation of the gallbladder	ETA	HCoV	Flora < 2x10*4 UFC/mL	9	Toward digestive surgery, Good evolution	Y	N	N	/	/	Y	N	NEG
5	M	61	fall with head trauma without loss conscious-ness	ETA	EV/RV	Neg	4	Transfer OTAS. Good evolution	Y	N	Y	CEFOXITIN SPIRAMYCIN (3j) -> LEVOFLOXACIN (7j)	NR	Y	N	NR

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6	M	37	SARS secondary to progression of metastatic lung carcinoma	BAL	EV/RV	Neg	26	Death	N	N	Y	CEFOTAXIME SPIRAMYCIN	NR	Y	Y	NR
7	M	73	Cardiogenic shock + progressive heart failure	ETA	EV/RV	Neg	9	Toward Cardiolo-gy Dpt	Y	N	Y	CEFTRIAXONE	5j	NR	N	NEG
8	M	63	Septic shock on post-aspiration pneumo-pathy	BAL	EV/RV (2)	C. freundii 10^3	15	Death	N	N	O	PIPERACILLIN/ TAZOBACTAM	7j	Y	NR	NEG
9	F	36	Cardiogenic shock on pheochromocy-toma	PDS + ETA	Adenovirus + P. aeruginosa 10^5 Adenovirus + P. aeruginosa 10^7	P. aeruginosa 10^3 and P. aeruginosa 10^6	47	Death	N	N	Y	LINEZOLID	NR	Y	Y	NEG
10	M	78	Acute hypercapnic respiratory failure	PDS	Influenza virus A + S. aureus 10^6	S. aureus 10^3	7	Toward another hospital	N	Y	Y	OSELTAMIVIR	5j	N	NR	NR
11	M	68	SARS	ETA	Influenza virus A + H. influenzae 10^4 + M. catarrhalis 10^4 + S. agalactiae 10^4	Non applicable	9	Toward another ICU	Y	Y	Y	CEFOTAXIME OSELTAMIVIR	6j + 5j	Y	NR	NEG
12	M	52	Refractory CRA	ETA AET	EV/RV + H. influenzae 10^5	Neg (flore commensale)	31	Death	Y	N	Y	AMOXICILLIN/ CLAVULANIC ACID	7j	Y	Y	NEG
13	M	29	SARS	LBA	EV/RV + P. aeruginosa 10^4	P. aeruginosa 10^2	7	Toward Pneumo-logy Dpt	Y	N	Y	VORICONAZOLE CIPROFLOXACIN	NR + 7j	Y	Y	NEG

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14	M	59	Cardiogenic shock secondary to septic shock	LBA	Parainfluenza + S. aureus 10^4	Neg	2	Death	Y	N	Y	IMIPENEM/ CILASTATIN LINEZOLID	NR	Y	N	NEG
15	M	8	Cardiogenic shock on myocarditis	ETA	Influenza virus A + S. aureus 10^4	Neg (commensal flora)	1	Good evolution	N	Y	Y	OSELTAMIVIR (1j) -> ZANAMIVIR CEFOTAXIME	NR	Y	NR	NEG
16	M	64	Acute respiratory failure on influenza A complicated by atrial fibrillation	ETA	Influenza virus A + M. catarrhalis 10^5 H. influenzae 10^6	Neg	3	Toward Pneumo-logy	Y	Y	Y	OSELTAMIVIR + CEFOTAXIME	3j + 7j	Y	Y	NEG
17	F	76	CRA of undetermined etiology	PDS	EV/RV	Neg	7	Death	Y	N	Y	AMOXICILLIN/ CLAVULANIC ACID	5j		Y	NEG
18	F	64	Hypercapnic respiratory failure	ETA	EV/RV Parainfluenza E. coli 10^4 + Proteus 10^4	Neg	9	Hospital discharge Good evolution	Y	Y	Y	PIPERACILLIN/ TAZOBACTAM	7j	Y	Y	NR

F: female; M: male; FA: FilmArray®; BAL: bronchoalveolar lavage; PDS: projected distal sample; ETA: endotracheal aspirate; EV/RV: enterovirus/rhinovirus; HCoV: seasonal coronavirus; SARS: severe acute respiratory syndrome; CRA: cardiorespiratory arrest; N: no; O: yes; NR: not filled in; Neg: negative; RICU: respiratory intensive care unit; OTAS: orthopedic, traumatology and arthroscopy surgery.

To assess the representativeness of the partner ICU patients' group (n=18) compared to all ICU patients positive for virus +/- bacteria (n=58), a comparison between patient ages was conducted using a two-tailed Student's t-test (a significance level of 5%) and a χ^2 test (p-value<0.001) to compare the percentage of males between these two groups. There was no significant difference between the ages and the male percentage of the two groups. When analyzing all patients evaluated by the 'Lower panel,' the percentage of male patients among cases positive for viruses (+/- bacteria) (75.9%) appeared higher compared to those positive for bacteria only (69.8%) – (χ^2 test, p-value<0.001). The same trend was observed among the patients hospitalized in the ICU unit (84.9% of male in the virus-positive group compared to 68.7% in the bacteria-only positive group) (χ^2 test, p-value<0.01). For the partner ICU, the trend was similar, with a slightly higher proportion of male patients among cases positive for viruses (+/- bacteria) (77.8%) compared to cases positive for bacteria only (69.6%) (χ^2, p-value<0.05).

At last, for the distribution of cases according to time, positive for bacteria only, viruses only, and a combination of bacteria and viruses over different periods ranging from January 2021 to mid-2022, seasonal fluctuations in the prevalence of various pathogens were observed. For instance, winter months appeared to correlate with an increase in cases positive for bacteria only, while positive cases for viruses or a

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combination of bacteria and viruses may also exhibit seasonal variations, albeit less pronounced, as observed in the Figure 4. Months such as November and December 2021 exhibited frequent cases positive for a combination of bacteria and viruses, for all evaluated patients including those admitted to ICUs.

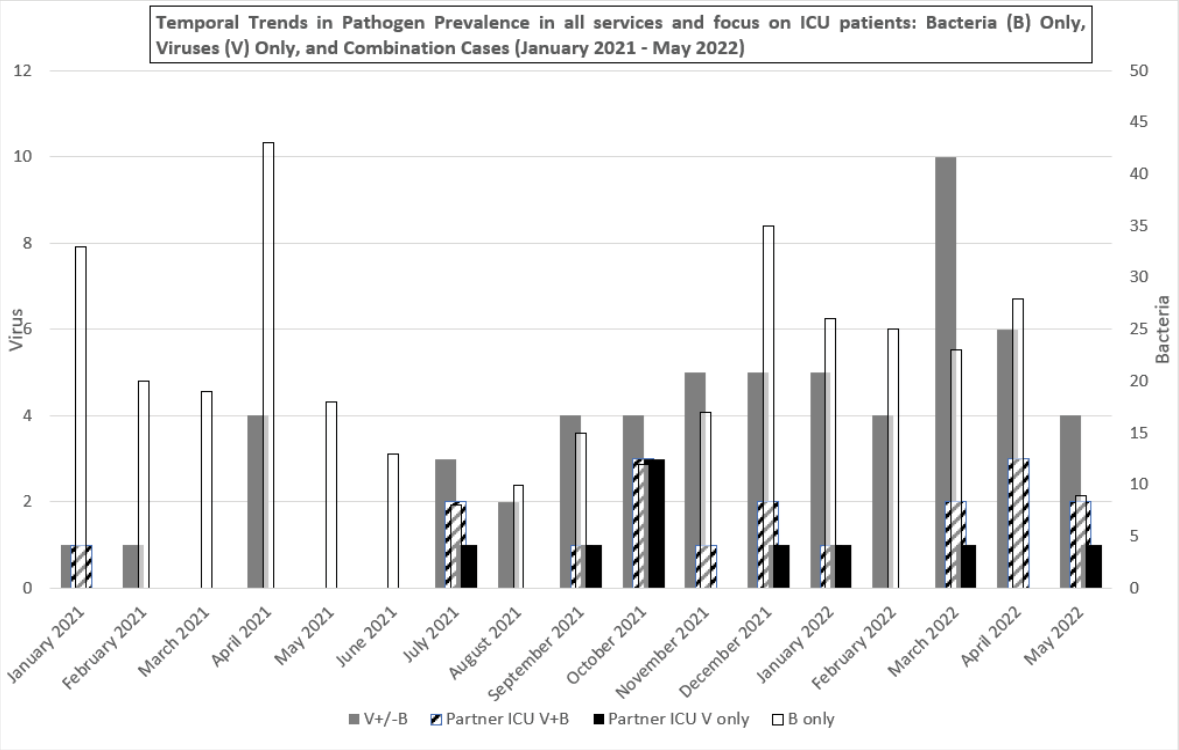

Figure 4: Temporal trends in infectious viruses (V) and/or bacteria (B) prevalence in all services and focus on ICU patients: Bacteria Only, Viruses Only, and Combination Cases (January 2021 - May 2022).

Discussion

The COVID-19 pandemic has highlighted the importance of rapid and accurate methods for the diagnosis of respiratory infections in hospitalized patients. Indeed, multiplex PCR approaches have emerged as valuable tools for the diagnosis of severe respiratory infections in hospitalized COVID-19 patients. The pandemic allowed us to confirm the potential of other respiratory viruses to cause significant morbidity and mortality, particularly in vulnerable populations such as elderly people and those with comorbidities. Furthermore, the co-circulation of multiple respiratory viruses, including SARS-CoV-2, can lead to challenges in the interpretation of multiplex PCR results.

Overall, our findings showed highly frequent enterovirus/rhinovirus infections in hospitalized patients, potentially in severe respiratory diseases, the interest in molecular diagnosis of viral respiratory infections along with the SARS-CoV-2 co-circulation and the usefulness during intensive care of direct antiviral treatment according to lower panel multiplex PCR results.

Moreover, our retrospective analysis reported that in the well-characterized exemplary intensive care unit group, the four patients with influenza A virus infections received adapted oseltamivir, while the immunocompromised patient with an adenovirus infection was treated with cidofovir. Accurately diagnosing respiratory tract infections in their early stages is crucial for proper patient management, appropriate antiviral or antibacterial therapy, implementing effective infection control measures, and reducing the length of hospital stay. To manage outbreaks of respiratory infections, conduct epidemiological surveillance, determine antimicrobial susceptibility, laboratory diagnoses must incorporate both bacteria and viruses [7]. The use of respiratory molecular panel assays enables the identification of a diverse set of targets, including some that would otherwise be missed. Researchers have demonstrated the widespread presence of RSV and the involvement of hMPV in severe illness [8].

The clinical implications of identifying multiple agents, with a reported coinfection rate of approximately 10%, remain to be investigated. Viral factors, such as viral tropism, replication efficiency, and virulence, play a critical role in the

pathogenesis of respiratory virus infections, as do host-related factors, such as age, immune status, and underlying medical conditions. Immune-mediated pathology can contribute to severe respiratory illness in some individuals infected with respiratory viruses [9]. Despite being a widespread pathogen, the pathogenesis of rhinovirus is still not fully understood and is potentially underestimated by physicians. Recent studies have shown that these infections can lead to more severe outcomes [10,11].

Several studies have shown that custom-ordered multiplex panels can decrease unnecessary testing and reduce patient expenses [12]; other studies have suggested that the use of respiratory panels in diagnostic workflows may improve clinical outcomes due to the timely adoption of targeted therapy [5,8,13,14]. A study estimated that multiplex panels could favor earlier antibiotic adjustments in 70.7% of patients, with de-escalation or discontinuation in 48.2% and an average of 6.2 antibiotic days saved per patient [15]. Moreover, it is pertinent to note that extensive research has been conducted among COVID-19 patients [16-18] but relatively little research has been conducted among those presenting with other viral respiratory infections [19].

While this study sheds light on the clinical implications of viral respiratory infections, it is essential to acknowledge certain limitations. The retrospective nature of the study and the reliance on data from a single regional university hospital may limit the generalizability of the findings. Larger, diverse cohorts and multicenter studies will provide a comprehensive understanding of the epidemiology and clinical outcomes of viral respiratory infections. Other approaches such as wastewater surveillance could predict the epidemiology of these viral diseases: peaks of influenza A virus (IAV) H3:N2 in February-March 2022 and RSV in winter 2021 were observed in Spain, matching the chronological incidence of infections recorded in the Catalan Government clinical database [20]. Elsewhere, recent observations argued in favor of possible false positive results for seasonal coronaviruses on the multiplex Pneumonia assay to be checked by a second technique, while rhinoviruses investigated by next-generation sequencing in immunocompromised hosts progressing from upper to lower respiratory tract infections, with rhinovirus capsid proteins showing a high variability [21].

In summary, the COVID-19 pandemic has accentuated the need for swift respiratory infection diagnostics. Multiplex PCR, particularly in ICU patients, has revealed frequent enterovirus/rhinovirus infections in hospitalized patients, the interest in molecular diagnosis of viral respiratory infections along with the SARS-CoV-2 co-circulation and the usefulness for adapted direct antiviral treatment. Further exploration is needed to deepen our understanding of viral respiratory infections and guide diagnostic and therapeutic advancements.

Acknowledgments

We would like to thank the Microbiology Laboratory, University Hospital of Nancy, for the assistance**.** We also thank Springer Nature for language editing and our local ethical committee, both from the University hospital of Nancy, for providing appropriate institution ethical approval.

Funding: There was no specific funding, as the work was performed in the context of standard-of-care approaches at the University Hospital of Nancy, France.

Transparency declarations: None to declare.

Highlights

- The frequency of enterovirus/rhinovirus infections including severe clinical forms, has been documented in this study.
- The multiplex molecular diagnosis of viral respiratory infections can be informative in epidemiology and clinical practice along with the COVID-19 emergence and subsequent viral co-circulations.
- In intensive care context, antiviral treatments can be adapted to multiplex PCR results, useful for available therapeutics and antiviral molecules in development.

Authors contributions:

Yasmina Sayed, Antoine Kimmoun, Véronique Venard and Evelyne Schvoerer were involved in data curation, formal analysis, investigation; Eliane Albuisson and E Schvoerer were responsible for conceptualization, methodology, project administration; Y Sayed wrote the original draft; Raphaël E Duval and E Schvoerer were in charge of writing, review & editing.

Conflict of interest: No

Ethic statement: Ethical guidelines for human studies, as outlined in the Declaration of Helsinki, were strictly followed - ID approval nb 2023PI112-478, ethical committee CHRU Nancy, France S-n° 440.

Data availability statement:

Data partially presented at the ESCV congress, Poster 056 and Oral talk, Milano Italy 2023.

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