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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
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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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
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.

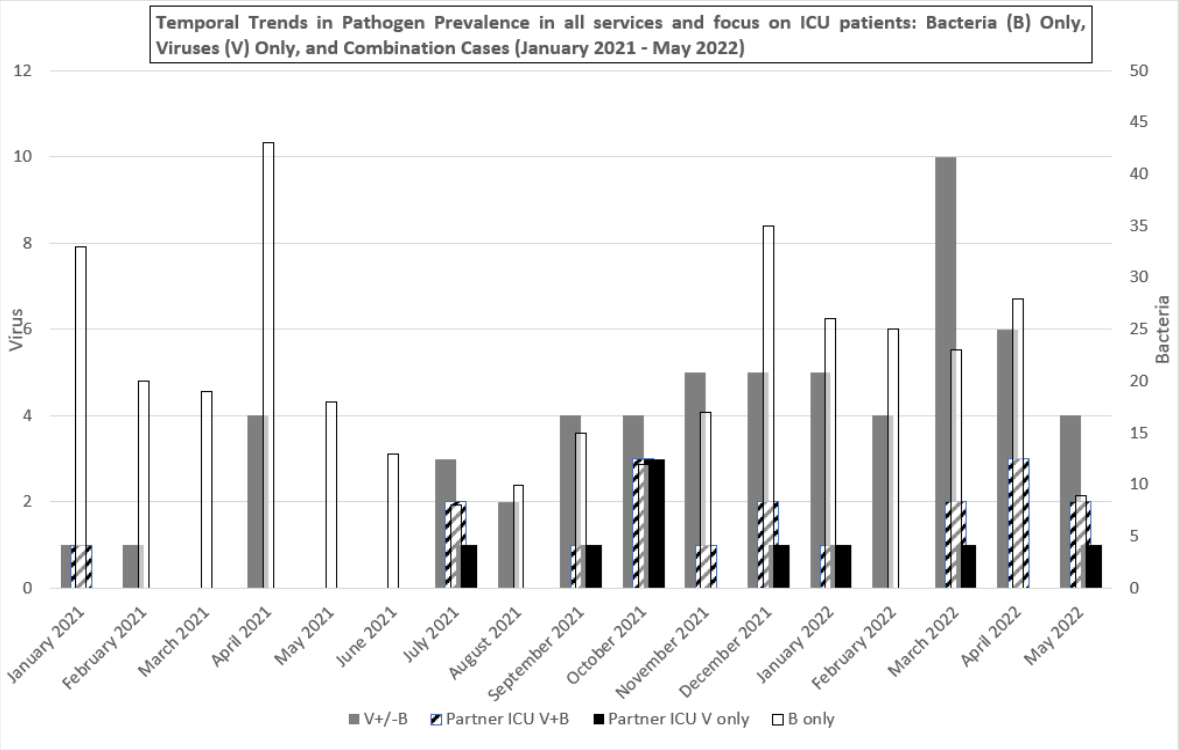

Figure 4: Temporal trends in infectious viruses (V) and/or bacteria (B) prevalence in all services and focus on ICU patients: Bacteria Only, Viruses Only, and Combination Cases (January 2021 - May 2022).

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.

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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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