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Original Article

Bacterial and Viral Respiratory Co-infections With SARS-CoV-2 in Deceased Patients of North Khorasan, Iran, During 2020-2023

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Abstract

Introduction: Co-infections play a significant role in **exacerbating** COVID-19 complications, especially among hospitalized patients. Many respiratory bacteria and viruses can cause co-infection or superinfections.

Methods: We evaluated bacterial and viral co-infections in 480 samples from deceased patients and 480 randomly selected samples from surviving SARS-CoV-2-positive patients. Samples were tested for respiratory bacterial pathogens, including *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Mycoplasma pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, as well as important respiratory viral pathogens, including influenza virus, human metapneumovirus, parainfluenza virus (A–D), adenovirus, respiratory syncytial virus (RSV), and bocavirus using polymerase chain reaction (PCR) and reverse transcription PCR (RT-PCR) assays.

Results: We evaluated 8846 samples for SARS-CoV-2 from May 1, 2020 to March 21, 2023. Of these, 2919 (33%) were SARS-CoV-2 positive, and 5927 (67%) had negative RT-PCR results. Four hundred eighty cases (16.44%) of positive patients died. *A. baumannii* was the most prevalent bacterium in deceased patients compared with surviving patients ($P=0.043$), and *S. pneumoniae* was the most abundant in surviving patients ($P=0.062$). Influenza A virus was the most common virus in both groups ($P<0.001$). The most common underlying conditions in deceased patients were diabetes and asthma ($P<0.05$), while in the surviving patients they were obesity and hypertension ($P=0.071$). Most coinfected patients were older than 60 years.

Conclusion: This study highlights the significant impact of bacterial and viral coinfections on the clinical course and outcomes of SARS-CoV-2 infection. We recommend active screening for co-infections upon hospital admission, promotion of influenza vaccination, and strengthening of hospital infection-control measures, especially against multidrug-resistant organisms such as *A. baumannii*.

Keywords: SARS-CoV-2, Coinfection, Bacteria, Virus

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Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus responsible for the coronavirus disease 2019 (COVID-19) pandemic, has had a profound impact on global health, causing significant morbidity and mortality worldwide. Although initial research primarily focused on understanding the pathogenesis and clinical manifestations of SARS-CoV-2, accumulating evidence suggests that coinfections with bacterial and viral pathogens may substantially influence the course and outcomes of COVID-19. Coinfections occur when an individual is simultaneously or sequentially infected with multiple pathogens, including bacteria and other respiratory viruses. These coinfections can modify clinical presentations, disease severity, and treatment outcomes in SARS-CoV-2 infection, posing challenges for accurate diagnosis, management, and infection control strategies.

Bacterial coinfections in COVID-19 have been widely reported, with several studies highlighting pathogens such as *Streptococcus pneumoniae*, *Staphylococcus aureus*, and

Haemophilus influenzae. These bacterial infections can lead to secondary pneumonia, exacerbating respiratory symptoms and increasing the risk of complications and mortality. Additionally, antibiotic use in COVID-19 management may further contribute to the emergence of drug-resistant bacterial strains, posing an additional challenge in patient care.

Furthermore, viral coinfections have also been documented in individuals infected with SARS-CoV-2. Other respiratory viruses, such as influenza viruses, respiratory syncytial virus (RSV), and human metapneumovirus (hMPV), alongside SARS-CoV-2, can complicate the clinical picture and impact disease severity. Such coinfections may produce synergistic effects, resulting in a more severe respiratory illness and potentially increasing hospitalizations and mortality rates. In our previous study, we evaluated the viral coinfections in SARS-CoV-2-infected patients in North Khorasan, northeastern Iran (1). We found a high prevalence of co-infection with influenza A virus (H1N1), especially in deceased patients. That study provided data from the beginning of the pandemic until the end of 2020.

This study aimed to provide an updated analysis of bacterial and viral co-infections in SARS-CoV-2 patients in North Khorasan from May 2020 to March 2023. The primary objectives were: (1) to determine the prevalence of specific bacterial and viral pathogens in deceased and surviving COVID-19 patients; (2) to identify the most common underlying conditions associated with mortality; and (3) to assess the age distribution of co-infections.

Methods

Study Population and Design

We evaluated bacterial and viral co-infections in 480 samples from deceased patients and 480 samples from surviving SARS-CoV-2-positive patients. The surviving patients were randomly selected from a pool of 2439 survivors using a computer-generated random number sequence to ensure a sample size comparable to that of the deceased group. The most frequent reason for hospitalization was acute respiratory syndrome. Inclusion criteria were: (1) laboratory-confirmed SARS-CoV-2 infection by reverse transcription polymerase chain reaction (RT-PCR); (2) availability of a respiratory sample for co-infection testing; and (3) complete clinical data. Exclusion criteria were: (1) patients under 18 years of age (unless included in a specific pediatric analysis); (2) samples of insufficient quality or quantity for additional testing; and (3) patients with incomplete medical records. Patients presented with a wide variety of signs and symptoms, including decreased smell, cough, fever, gastrointestinal involvement, and muscle and joint pain. Laboratory findings and clinical presentation were collected from electronic medical records.

Laboratory Methods

Sample Collection. Nasopharyngeal and throat swabs were collected from patients upon hospital admission or within the first 48 hours for SARS-CoV-2 and co-infection testing. We reviewed laboratory records for respiratory sample culture results. All samples were tested to detect respiratory pathogens, including *S. pneumoniae*, *H. influenzae*, *Klebsiella pneumoniae*, *Mycoplasma pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *S. aureus*, using routine bacteriological methods. Important respiratory viral pathogens, including influenza virus, human metapneumovirus, parainfluenza virus (A–D), adenovirus, RSV, and bocavirus, were detected using PCR and RT-PCR tests (1, 2).

Nasopharyngeal and throat swabs from suspected patients were evaluated for COVID-19 using the LightMix Modular SARS-CoV-2 probe and primers (TIB Molbiol, Berlin, Germany) and AddBio one-step RT master mix (ADD BIO Inc., Daejeon, Republic of Korea). Other respiratory bacterial and viral pathogens were evaluated using previously described methods (1-6). For nucleic acid extraction, we used the AddPrep Viral Nucleic Acid Extraction Kit (AddBio, South Korea) based on the manufacturer's protocol. This kit could extract both viral RNA and DNA. To detect DNA viruses (adenovirus and bocavirus), we used Add-Prob Taq Master 2x (AddBio, South Korea), and for RNA viruses (influenza virus, hMPV, parainfluenza virus [A–D], and RSV), we used the AddScript RT-PCR kit. Primer and probe sequences are listed in Table 1.

Data Collection and Statistical Analysis

Additional information, such as laboratory test results, chest computed tomography scan results, and physical examination data, was obtained from patients' records. For statistical analyses, depending on sample sizes in each group and variables under investigation, we used Fisher's exact test, the chi-square test, and the t-test. Continuous data, such as age and duration of hospitalization, are presented as mean ± standard deviation. Categorical data, such as pathogen prevalence, are presented as counts and percentages with 95% confidence intervals, where appropriate. Statistical analyses were performed using SPSS version 26. The ethics approval code from North Khorasan University of Medical Sciences was IR.NKUMS.REC.1399.020.

Results

We evaluated 8846 samples for SARS-CoV-2 from May 1, 2020 to March 21, 2023 for SARS-CoV-2. Of these, 2919 (33%) were SARS-CoV-2 positive, and 5927 (67%) had negative SARS-CoV-2 RT-PCR test results. Four hundred eighty cases (16.44%) of positive patients died, and 2439 (83.56%) survived. Figure 1 shows the prevalence of respiratory bacterial pathogens in both groups, and Figure 2 presents the incidence of viral pathogens in nasopharyngeal swabs and respiratory samples from all SARS-CoV-2 positive patients. *A. baumannii* was the most prevalent bacterium in deceased patients

Table 1. The Primer and Probe List

RSV	F	TAGCCCATCCAAACAACC
	R	TAGTGGTTCTGCCTTTGG
	Probe	ACAACCACATCACCAATCCAC
hMPV	F	ATGTCTTGGAAAGTGGTAATC
	R	CCTCAGTTATAGTGCTACATG
	Probe	TGCTAATAACACCTCAACACGG
Inf A	F1	CAAGACCAATCYTGTCACCTCTGAC
	R1	GCATTYTGGACAAAVCGTCTACG
	F2	CAAGACCAATYCTGTCACCTYTGAC
	R2	GCATTTTGGATAAAGCGTCTACG
	Probe	TGCAGTCCTCGCTCACTGGGCACG
adeno	F	GGAGTATCAGCAGGTTGG
	R	TTACTGGAGAATGGGATGC
	Probe	TTCTTCACCAGCATAACAACTCA
Boca	F	GAATATGAAAGACAAGCATCG
	R	TGCTCCTGTGATGAGTTG
	Probe	TGCCAGTGTCTCTTCCTCTC
Para inf1	F	GARGCAGCATACACTACTACATC
	R	GGATACTTGTCTTGAACAACATAGG
	Probe	CACTCATTTCGGRAAGGGCTACTGCT
Para inf2	F	ATCGATTTGCTGGAGCYTTTC
	R	TGAATCCGGTAGTATTTARKAGGG
	Probe	TGAGTCCAACCGAACYAATCCCACA
Para inf3	F	ARTGGCATCGGATAATAYYAATG
	R	AATTCTGKACATGACTCTGAATTG
	Probe	AAGAAGCCTTGTRTTCACTCCTGACTGT
Para inf3	F	AATTGAAATCGCCCCAAGACC
	R	TTGRTAGACTCCYGTTATACATTC
	Probe	TGGGCAAGCYTTATTYCCTGGACAATCTTT
IC	F	AGATTTGGACCTGCGAGCG
	R	GAGCGGCTGTCTCCACAAGT
	Probe	TTCTGACCTGAAGGCTCTGCGCG

Note. RSV: Respiratory syncytial virus; hMPV: Human metapneumovirus; Inf A: Influenza A virus; adeno: Adenovirus; Boca: Human bocavirus; Para inf1: Parainfluenza virus type 1; Para inf2: Parainfluenza virus type 2; Para inf3: Parainfluenza virus type 3; IC: Internal control.

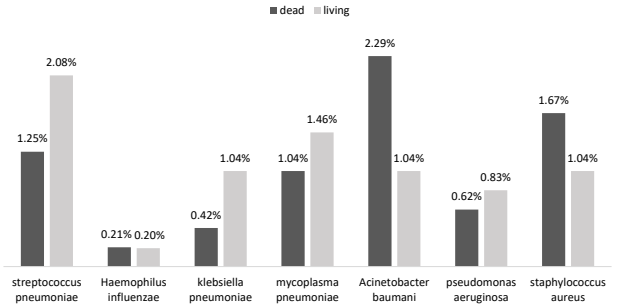

Figure 1. The Rate of Positive Cases of Bacterial Respiratory Pathogens in SARS-CoV-2 Positive Dead and Living Patients
Note. SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2

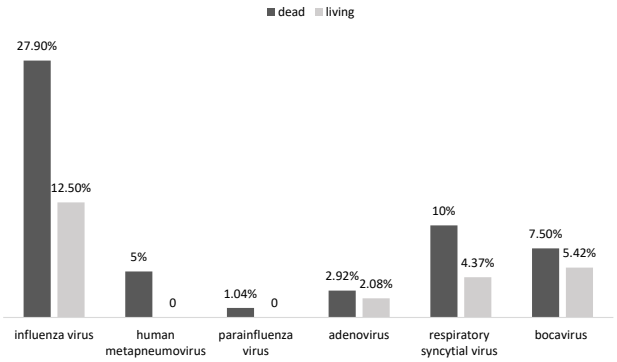

Figure 2. Prevalence of Positive Cases of Viral Respiratory Pathogens in SARS-CoV-2 Positive Dead and Living Patients
Note. SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2

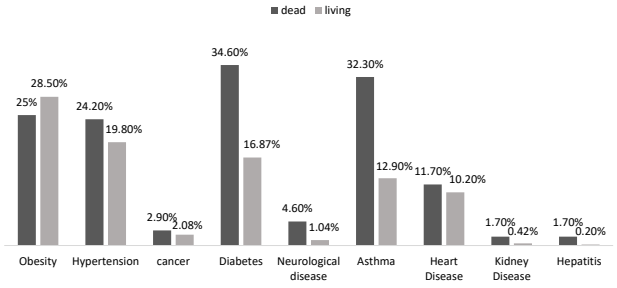

Figure 3. The Distribution of Underlying Diseases in SARS-CoV-2 Positive Dead and Living Patients
Note. SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2

($P=0.043$), whereas *S. pneumoniae* was most abundant in surviving patients ($P=0.062$), as depicted in Figure 1. The influenza virus was the most common virus in both deceased and surviving patients ($P<0.001$). Other viruses with significantly higher prevalence in the deceased patient group included RSV ($P=0.001$) and Human metapneumovirus ($P<0.001$), as illustrated in Figure 2.

The most common underlying conditions in deceased patients were diabetes and asthma ($P<0.05$), while in the surviving patients they were obesity and hypertension ($P=0.071$), as shown in Figure 3. Most coinfected patients were older than 60 years (Table 2). In the deceased patient group, influenza and RSV had significantly higher prevalence in individuals over 60 years ($P<0.05$). Similarly, in the surviving patient group, influenza was also significantly more prevalent among those older than 60 years ($P<0.05$). Other bacteria and viruses did not demonstrate significant differences in distribution between the surviving and deceased patient groups ($P>0.05$).

Discussion

In our previous study, we evaluated the prevalence of viral coinfections in deceased COVID-19 patients (1). The current study evaluated the bacterial respiratory pathogens alongside viral agents. The findings of this three-year evaluation highlight the considerable prevalence of bacterial and viral coinfections among SARS-CoV-2-positive deceased patients in North Khorasan, Iran. Bacterial coinfections, particularly with *A. baumannii*, *S. aureus*, and *S. pneumoniae*, were more frequent than other respiratory pathogens and were associated with increased disease severity and adverse outcomes (7). Considering

Table 2. The Prevalence of Respiratory Bacteria and Viruses in Various Age Ranges of Dead and Living Patients

Organism	Age (n/%)							
	Dead				Living			
	0-14	14-40	40-60	>60	0-14	14-40	40-60	>60
Streptococcus pneumoniae	4(66.7)	0	0	2(33.3)	5(50)	0	2(20)	3(30)
Haemophilus influenzae	0	0	0	1(100)	0	0	0	1(100)
Klebsiella pneumoniae	0	0	1(50)	1(50)	1(20)	0	2(40)	2(40)
Mycoplasma pneumoniae	1(20)	0	1(20)	3(60)	2(28.6)	1(14.3)	1(14.3)	3(42.8)
Acinetobacter baumani	2(18.2)	0	0	9(81.8)	0	0	0	5(100)
Pseudomonas aeruginosa	0	1(33.3)	0	2(66.7)	0	1(25)	1(25)	2(50)
Staphylococcus aureus	2(25)	1(12.5)	1(12.5)	4(50)	1(20)	1(20)	1(20)	2(40)
Influenza virus	17(12.7)	5(3.7)	24(17.9)	88(65.7)	12(20)	3(5)	16(26.7)	29(48.3)
Metapneumovirus	20(83.3)	1(4.2)	0	3(12.5)	0	0	0	0
Parainfluenza virus	2(40)	0	0	3(60)	0	0	0	0
Adenovirus	0	1(7.1)	3(21.3)	10(71.4)	2(20)	1(10)	1(10)	6(60)
Respiratory Syncytial Virus	3(6.2)	1(2.1)	11(22.9)	33(68.8)	2(9.5)	4(19)	6(28.6)	9(42.9)
Bocavirus	11(30.5)	2(5.5)	9(25)	14(38.9)	7(26.9)	0	9(34.6)	10(38.5)

that *A. baumannii* is a common cause of hospital-acquired infections and was more frequent in deceased patients than in surviving patients in the present study ($P<0.05$), the hospital may represent an important source of infection acquisition among deceased patients. Viral coinfections, such as the influenza virus, RSV, and bocavirus, were also frequent among deceased COVID-19 patients.

Our findings are consistent with global trends. Secondary bacterial infections can lead to pneumonia and exacerbate respiratory symptoms, thereby increasing the risk of complications and mortality (7, 8). These viruses, like bacteria, can complicate the clinical course of COVID-19 and may worsen respiratory illness (9). Coinfections may also influence treatment response, prolong recovery time, and increase hospitalization needs (10).

Azimi et al. evaluated bacterial and viral respiratory infections in the COVID-19 era in Tehran and reported a lower prevalence of influenza A virus (4.1%) and adenovirus (1.4%) compared to those observed in our study. They also documented bacterial prevalence as follows: *S. pyogenes* (2%), *M. pneumoniae* (0.7%), *H. influenzae* (0%), and *B. pertussis* (0%). In contrast, the prevalence of influenza A virus and adenovirus was considerably higher in our study. Moreover, comparison of the prevalence of shared bacteria in our study and this study, such as *H. influenzae* and *M. pneumoniae*, indicates a higher overall bacterial prevalence in our study. It should be noted that we analyzed co-infections, but this study evaluated all patients, which may partly explain the observed differences (11). Other studies examining concurrent bacterial and viral infections in COVID-19 patients in Iran have generally reported lower prevalence rates than those identified in the present study (12, 13). It should be noted that most of these studies assessed different viruses and bacteria compared to the present study.

Accurate and timely diagnosis of coinfections in SARS-CoV-2 cases poses significant challenges (14). The overlap of clinical manifestations between COVID-19 and other bacterial or viral respiratory infections makes it difficult to identify causative pathogens based solely on symptoms (15). Molecular diagnostic techniques, such as multiplex PCR assays, can facilitate detection by simultaneously identifying multiple pathogens (16, 17). However, the availability and accessibility of such tests may vary across healthcare settings. Therefore, improved diagnostic strategies, including the integration of advanced technologies and point-of-care testing, are warranted to enable rapid identification of coinfections and to guide appropriate therapeutic decisions (18, 19).

The widespread use of antibiotics in the management of COVID-19 patients, including those with suspected or confirmed bacterial coinfections, raises concerns regarding antimicrobial resistance (20, 21). Inappropriate or excessive antibiotics can contribute to the selection and dissemination of drug-resistant bacterial strains, further complicating patient care (22, 23). It is crucial to implement antimicrobial stewardship programs and adhere to evidence-based prescribing guidelines to ensure appropriate antibiotic use while minimizing resistance development (24, 25). A limitation of our study is the lack of specific antibiotic resistance data for the detected bacterial pathogens; future work should include such analyses. Additionally, strengthened surveillance of antibiotic resistance patterns in coinfections is needed to inform treatment strategies and optimize empirical therapy (26).

The presence of bacterial and viral coinfections in SARS-CoV-2 cases has significant implications for patient management and infection-control strategies. Early recognition and appropriate treatment of coinfections, such as initiating timely antibiotic therapy for bacterial infections, are essential to mitigate disease progression and improve clinical outcomes (27, 28). Healthcare providers

should remain vigilant for signs suggestive of coinfections, especially in severe or worsening cases of COVID-19. Furthermore, effective infection-prevention and control measures, including strict hand hygiene, respiratory precautions, and vaccination against common respiratory pathogens such as influenza, can help reduce the risk of coinfections and their associated complications (29).

This study has several limitations. The available medical information was incomplete for some evaluated patients, which may have affected data interpretation. Another problem was the lack of full access to all samples, and their corresponding records were not available in some cities of North Khorasan Province. As noted, antibiotic resistance profiles of the identified bacterial pathogens were not assessed, which is an important area for future research. Moreover, the impact of coinfections on specific subgroups, such as immunocompromised individuals or patients with underlying comorbidities, warrants further investigation. Future research should prioritize prospective studies with standardized protocols to better understand the incidence, risk factors, and prognostic implications of coinfections. Long-term follow-up studies would also provide insights into the recovery trajectories and potential long-term consequences of coinfections among COVID-19 survivors.

Conclusion

This study highlights the significant impact of bacterial and viral coinfections on the clinical course and outcomes of SARS-CoV-2 infection. Coinfections contribute to increased disease severity, complicate diagnosis, and pose challenges for effective management. Our results suggest that active screening for co-infections, particularly upon hospital admission, could improve patient stratification and clinical management. Furthermore, promoting influenza vaccination and reinforcing robust infection-control practices in healthcare settings, especially against nosocomial pathogens such as *A. baumannii*, are crucial public health measures. A multidisciplinary approach involving clinicians, microbiologists, and public health experts is crucial to optimize diagnostic strategies, guide appropriate treatment decisions, and implement effective infection-control measures. Addressing the complexity of coinfections can ultimately improve patient outcomes and reduce the overall burden of the COVID-19 pandemic.

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Competing Interests

The authors declare no conflict of interests.

Ethical Approval

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