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Bacterial and Fungal Co-Infections in COVID-19: Mortality and Infection Control in Yazd, Iran

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ABSTRACT

Introduction: Since the emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), bacterial co-infections have become an important concern in the clinical management of coronavirus disease 2019 (COVID-19), especially in patients with severe illness, as they contribute to increased morbidity and mortality. This study aimed to investigate the epidemiological and clinical characteristics of bacterial co-infections in hospitalized patients with severe COVID-19 in Yazd Province, Iran. **Methods:** In this retrospective descriptive-analytical study, 110 adult patients with reverse transcription polymerase chain reaction-confirmed COVID-19 (RT-PCR) and positive blood cultures, hospitalized at Shahid Sadoughi Hospital between February 2020 and February 2023, were evaluated. Data were extracted from electronic health records and patient files, including demographic information, comorbidities, clinical symptoms, complications, oxygen therapy details, admission oxygen saturation, duration of hospitalization, isolated bacterial or fungal pathogens, treatments, laboratory results, and clinical outcomes. Blood culture findings were independently reviewed by two investigators, and any discrepancies were resolved through consensus or by a third reviewer to minimize bias. Statistical analysis was performed using IBM SPSS Statistics version 22.0. The normality of continuous variables was assessed using the Shapiro-Wilk test, and data were presented as mean ± standard deviation (SD) or median (interquartile range [IQR]), as appropriate. Group comparisons were conducted using t-test or Mann-Whitney U test for continuous variables, and chi-square (χ^2) or Fisher's exact tests for categorical variables. A P-value < 0.05 was considered statistically significant. **Results:** Patients had a mean age of 64.94 ± 18.86 years, of whom 52.7% were male. Fever and shortness of breath were the most common symptoms, and hypertension was the most prevalent comorbidity. *Pseudomonas aeruginosa* (37.3%) and *Klebsiella pneumoniae* (29.1%) were the most frequently isolated pathogens, with Gram-negative bacteria accounting for 74.5% of infections. The mortality rate among patients with positive blood cultures was 28.2%. **Conclusion:** Bacterial co-infections are more common in severe COVID-19 cases admitted to the intensive care unit (ICU) and are associated with prolonged hospital stays. Appropriate antibiotic stewardship and strict infection control measures are essential to prevent secondary infections and limit the spread of drug-resistant bacteria in healthcare settings.

INTRODUCTION

Since its emergence in 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread globally, affecting nearly all countries globally [1, 2]. The resulting disease, coronavirus disease 2019 (COVID-19), is characterized by symptoms such as fever, dry cough, shortness of breath, myalgia, respiratory failure, and, in some cases, multi-organ failure [3, 4].

In the context of COVID-19, co-infections—including bacterial and fungal bloodstream infections (BSIs)

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among other types—have been identified as major contributors to increased morbidity and mortality [1, 5]. Severe COVID-19 induces immune dysregulation, thereby predisposing patients to secondary infections [3]. Prior studies report variable prevalence rates, with bacterial co-infections identified in approximately half of deceased patients [6]. These co-infections are strongly associated with adverse clinical outcomes, increased pneumonia severity, and higher mortality rates [7].

Despite the growing body of research on COVID-19, there remains a lack of comprehensive data regarding the prevalence, types, and clinical implications of co-infections, particularly in severely ill patients. Many existing studies are limited by small sample sizes, narrow geographic focus, or retrospective designs that hinder the generalizability of their findings. Furthermore, there is insufficient differentiation between co-infections identified at hospital admission and secondary infections acquired during hospitalization, which can obscure the true impact of these pathogens on patient outcomes. These knowledge gaps limit the development of targeted diagnostic and therapeutic strategies.

In Iran, and specifically in Yazd Province, understanding the local epidemiology of COVID-19 and related co-infections is crucial due to regional variations in healthcare infrastructure, antibiotic stewardship practices, and resistance patterns. Yazd, a central region with a mix of urban and semi-urban populations, has a well-developed network of referral hospitals that managed a significant burden of COVID-19 patients during the 2020–2023 pandemic waves. However, region-specific data on bacterial co-infections in this setting remain scarce.

To reduce mortality among patients with COVID-19, early diagnosis and effective management of bacterial co-infections are essential. While numerous studies have explored the epidemiological and clinical features of COVID-19 itself, data on the prevalence and characteristics of co-infections, particularly under severe conditions, remain limited. Accordingly, this study aims to investigate the epidemiological and clinical characteristics of blood culture-positive bacterial and fungal co-infections in hospitalized patients with severe COVID-19. The findings may help enhance early detection and improve the management of coinfected patients.

MATERIAL AND METHODS

Participants and study design. This descriptive-analytical study was conducted at Shahid Sadoughi Hospital in Yazd, Iran. We retrospectively analyzed all consecutive adult patients (≥18 years) hospitalized with severe COVID-19 and clinically suspected bloodstream infections between February 2020 and February 2023. The inclusion criteria were: 1) Confirmed SARS-CoV-2 infection via positive reverse transcription polymerase chain reaction (RT-PCR) from nasopharyngeal swabs. 2) Positive blood culture for bacterial or fungal pathogens within the first 48 hours of hospitalization. Exclusion criteria included: 1) Outpatients or individuals not requiring hospitalization. 2) Blood cultures collected >48 hours after admission.

The study protocol was approved by the Ethics Committee of Shahid Sadoughi University of Medical Sciences (Ethics Code: IR.SSU.MEDICINE.REC.1402.254). Patient data were anonymized and handled confidentially. As this was a retrospective study, the ethics committee waived the requirement for informed consent.

Data collection and clinical assessment. Data were extracted from electronic health records and medical files, including: demographics, comorbidities, clinical symptoms, complications, oxygen therapy modality, admission oxygen saturation, hospitalization duration, isolated co-infection pathogens (bacterial/fungal), administered treatments, laboratory parameters, and clinical outcomes (*e.g.*, recovery, mortality).

In each sampling session, two blood samples were collected for aerobic and anaerobic blood cultures. Approximately 10 mL of venous blood was drawn per collection and inoculated into paired aerobic and anaerobic culture bottles. All blood culture results were independently reviewed by two investigators to minimize bias. Discrepancies were resolved through consensus or consultation with a third reviewer.

Statistical analysis. Continuous variables were compared using independent t-test or Mann-Whitney U test based on normality assessment (Shapiro-Wilk test); categorical variables were compared using chi-square (χ^2) or Fisher's exact tests. A P-value < 0.05 was considered statistically significant. All statistical analyses were conducted using IBM SPSS Statistics version 22.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Characteristics of the participants. From February 2020 to February 2023, 110 patients with severe, RT-PCR-confirmed COVID-19 and blood culture-confirmed bacterial or fungal co-infection were included in the study. All participants were RT-PCR-positive. Their mean age was 64.94 ± 18.86 years. Regarding sex distribution, 58 participants (52.7%) were men and 52 participants (47.3%) were women (Table 1). The most common clinical symptom was fever (61.8%), whereas sore throat and confusion were the least frequently reported (0.9% each). Hypertension was the most prevalent comorbidity (40.9%). The most commonly observed complication was acute respiratory distress syndrome (ARDS) (17.3%), whereas thromboembolic events were the least frequent (1.8%). The treatments received, the type of oxygen therapy administered, and the laboratory parameters of the studied patients during admission are presented in Table 1.

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Table 1. Baseline characteristics of the participants

Variables		Values
Age (in years)		64.94 ± 18.86
Sex	Male	58 (52.7)
	Female	52 (47.3)
Clinical symptoms		
Fever		68 (61.8)
Shivering		49 (44.5)
Cough		51 (46.4)
Chest pain		5 (4.5)
Myalgia		31 (28.2)
Headache		13 (11.8)
Nausea		15 (13.6)
Vomiting		17 (15.5)
Diarrhea		15 (13.6)
Shortness of breath		60 (54.5)
Sore throat		1 (0.9)
Confusion		1 (0.9)
Comorbidities		
Hypertension		45 (40.9)
Diabetes mellitus		39 (35.5)
Asthma		4 (3.6)
CKD		19 (17.3)
Cancer		12 (10.9)
Stroke		1 (0.9)
Hepatitis B		2 (1.8)
Depression		2 (1.8)
Dementia		3 (2.7)
Smoking		5 (4.5)
Drug abuse		4 (3.6)
Oxygen saturation	Admission	87.56 ± 9.64
	Final oxygen	91.95 ± 6.00
Length of hospitalization	Ward	5.58 ± 5.21
	ICU	8.92 ± 7.83
Complications		
ARDS		19 (17.3)
Cardiac		10 (9.1)
Thromboembolic		2 (1.8)
Multiple organ failure		3 (2.7)
Outcome	Discharge	79 (71.8)
	Death	31 (28.2)
Treatments*		
Actemra		3 (2.73)
Pulse methylprednisolone		22 (20)
Remdesivir		49 (44.54)
Plasmapheresis		5 (4.54)
Stem cell		1 (0.91)
Pulse methylprednisolone+ Remdesivir		14 (12.73)
Pulse methylprednisolone+ Remdesivir + Plasmapheresis		5 (4.54)
Plasmapheresis + Stem cell		2 (1.82)
Plasmapheresis + prednisone		2 (1.82)
Remdesivir + Plasmapheresis		2 (1.82)
Actemra + Plasmapheresis		1 (0.91)
Remdesivir + Stem cell + Pulse methylprednisolone		2 (1.82)
Actemra + Remdesivir		2 (1.82)
Oxygen therapy modality		
Nasal		54 (49.1)
Simple oxygen		7 (6.4)
Reservoir bag		43 (39.1)
Venturi		1 (0.9)
NIV		1 (0.9)
Intubation		4 (3.6)

Laboratory tests	Admission	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
WBC	9.79 ± 5.79	10.27 ± 4.93	11.12 ± 5.25	12.00 ± 9.33	14.87 ± 8.36	9.16 ± 1.72	9.05 ± 2.47

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LYM		1051.62 ± 874.86	1044.58 ± 638.51	910.80 ± 568.61	738.29 ± 578.42	1451.85 ± 139.60	2282.80 ± 605.44	1449.40 ± 852.20
Platelets		194.76 ± 104.33	214.23 ± 112.98	167.88 ± 81.52	115.66 ± 122.45	148.50 ± 146.95	160.51 ± 188.33	150.16 ± 183.84
ESR		54.71 ± 33.76	57.76 ± 36.04	45.40 ± 49.37	-	-	-	-
CRP**	+ 1	14 (2)	-	1 (0.1)	-	-	-	-
	+ 2	40 (5.7)	2 (0.3)	2 (0.3)	-	-	-	-
	+ 3	32 (4.6)	5 (0.7)	-	-	-	-	-
D-Dimer	< 200	13 (1.9)	-	-	-	-	-	-
	> 200	37 (5.3)	1 (0.1)	-	-	-	-	-

- The values are reported as mean ± SD or *n* (%). *: Patients received multiple treatments; percentages reflect the proportion of each therapy among all administered treatment instances (total treatment instances = 120 across 110 patients). CRP: +1: mild elevation, +2: moderate elevation, +3: severe elevation. *Abbreviations:* CKD, chronic kidney disease; ICU, intensive care unit; ARDS, acute respiratory distress syndrome; NIV, noninvasive ventilation; WBC, white blood cells; LYM, lymphocytes; ESR, erythrocyte sedimentation rate; CRP: C-reactive protein.

Co-infections. Among bacterial isolates, *P. aeruginosa* (37.3%) and *K. pneumoniae* (29.1%) were most frequent. Among fungal isolates, *Aspergillus* spp. was identified in 0.9% of cases; no mucormycosis cases were detected. No cases of mucormycosis (Mucorales infection) were detected. In total, 82 isolates (74.5% of all bacterial isolates) were Gram-negative bacteria, including *Pseudomonas* spp., *Klebsiella* spp., *Acinetobacter* spp., and *E. coli*.

Table 2. Epidemiology of the co-infections

Isolated pathogen	N (%)
Bacterial	
P. aeruginosa	41 (37.3)
K. pneumoniae	32 (29.1)
Streptococcus pneumoniae	2 (1.8)
Staphylococcus spp.	25 (22.7)
Acinetobacter	5 (4.5)
E. coli	4 (3.6)
Fungal	
Aspergillus	1 (0.9)
Mucor	0 (0.0)

Unadjusted analyses suggested associations between pathogen distribution and the absence of chest pain, chronic kidney disease (CKD), or dementia ($P = 0.01$ for each); however, given multiple comparisons without correction, these findings should be interpreted as exploratory. Given multiple comparisons, statistical significance should be interpreted cautiously; no adjustment for multiple testing was applied. Among the small subgroup of patients reporting chest pain ($n = 5$), no *P. aeruginosa* or *K. pneumoniae* isolates were detected, compared with higher frequencies in those without chest pain; however, the limited sample size precludes definitive conclusions. Similarly, in patients without CKD, these two bacteria were more frequently detected. Moreover, among patients without dementia, the occurrence of *P. aeruginosa* and *Klebsiella* was notably greater.

These associations, if validated, may have clinical implications for risk stratification and empiric antibiotic selection. Such evidence may contribute to a more accurate interpretation of clinical symptoms, support the selection of targeted antibiotics, and enhance therapeutic and management strategies for affected patients. No statistically significant associations were observed between pathogen distribution and other assessed clinical variables (data available upon request).

Table 3. Pathogen distribution stratified by presence of chest pain, chronic kidney disease, and dementia

Variables	Patients	*P. aeruginosa*	*K. pneumoniae*	*S. pneumoniae*	*Staphylococcus*	*Acinetobacter*	*E. coli*	**P*-value
Chest pain	Yes	0 (0.0)	1 (0.9)	1 (0.9)	3 (2.8)	0 (0.0)	0 (0.0)	0.01
	No	41 (37.6)	32 (28.4)	1 (0.9)	22 (20.2)	5 (4.6)	4 (3.7)	
CKD	Yes	2 (1.8)	7 (6.4)	1 (0.9)	9 (8.3)	0 (0.0)	0 (0.0)	0.01
	No	40 (35.8)	25 (22.9)	1 (0.9)	16 (14.7)	5 (4.6)	4 (3.7)	
Dementia	Yes	1 (0.9)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.9)	1 (0.9)	0.01
	No	40 (36.7)	33 (29.4)	2 (1.8)	25 (22.9)	4 (3.7)	3 (2.8)	

- The values are reported as n (%). **P*-values were calculated using χ^2 tests or Fisher's exact tests as appropriate for cell frequencies. *Abbreviations:* CKD, chronic kidney disease.

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DISCUSSION

A single-center descriptive-analytical study was carried out to investigate the clinical and epidemiological factors associated with positive blood cultures in patients with severe COVID-19. Among the isolated pathogens, *P. aeruginosa* and *K. pneumoniae* were the most frequently detected. Furthermore, the study found a statistically significant association between specific bacterial types and the absence of chest pain, chronic kidney disease (CKD), and dementia.

Other studies investigating the characteristics of hospitalized COVID-19 patients have reported varying results. Zhou *et al.* examined 191 patients, predominantly male, with a mean age of 56.0 years (range: 18–87 years) [6]. Similarly, Lv *et al*. studied 354 hospitalized patients with COVID-19, among whom 175 (49.4%) were male, with a mean age of 62 years (range: 23–90 years) [8]. Another study conducted in Saudi Arabia assessed 99 hospitalized patients, with a mean age of 44 years (range: 19–87 years), the majority of whom were male (66%) [9].

In the study by Asmarawati *et al*., patients with bacterial co-infections had a mean age of 52.45 ± 14.44 years, and 55.05% were male. Additionally, diabetes (8.3%) and hypertension (6.9%) were the most common comorbidities among patients with positive blood cultures [10]. Abad *et al*. reported co-infections in 66 patients (5.9% of their cohort), with hypertension (62.1%) and diabetes mellitus (31.8%) being the most frequent comorbidities. In that study, the most commonly reported clinical symptoms were cough (69.7%), shortness of breath (63.6%), and fever (54.5%) [11]. Similarly, Ramadan *et al*. [12] reported that fever (63.8%), cough (57.7%), and shortness of breath (40%) were the most prevalent clinical symptoms. While symptom prevalence aligns with prior reports, our cohort exhibited notably higher baseline rates of hypertension and diabetes, likely reflecting regional demographic and referral biases.

In this study, the mortality rate among severe COVID-19 patients with positive blood cultures was 28.2%. Elabbadi *et al*. investigated the rate of bacterial co-infections in 101 adult patients with severe COVID-19 pneumonia and reported that 4 out of 20 patients with bacterial co-infections died, yielding a mortality rate of 20% [13]. In contrast, Abad *et al*. [11] reported a significantly higher mortality rate of 48.5% among coinfected patients.

In the present study, patients had a mean oxygen saturation of 87.56 ± 9.64% at the time of admission. Similarly, in a study by Lardaro *et al*., a group of patients with positive blood cultures (N = 6) had a mean oxygen saturation of 86.2 ± 14.4% upon admission [14].

Several studies have reported that COVID-19 patients typically present with normal or decreased white blood cell (WBC) counts. However, a sudden increase in WBC count during hospitalization may indicate the development of a bacterial co-infection. Lymphopenia, often resulting from severe immune system dysfunction, is a common finding in COVID-19 patients [15]. Researchers attribute this immune dysregulation to a hyperinflammatory cytokine response. Elevated inflammatory markers such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) are also characteristic of COVID-19 patients with bacterial co-infections, which is consistent with the findings of the present study [16].

In this study, the average length of stay was 5.58 ± 5.21 days in the general ward and 8.92 ± 7.83 days in the ICU. These findings are directionally consistent with prior reports [17], though direct comparison is limited by differences in central tendency reporting (mean ± SD *vs*. median/IQR). For instance, Sharifpour *et al*. [18] reported a median hospital stay of 15 days (IQR: 2–39). Similarly, a 2009 study on respiratory co-infections in patients with influenza A (H1N1) virus infection showed that ICU stays were, on average, three days longer for patients with co-infections [19]. Although from a different pandemic context, this finding suggests co-infections may prolong ICU stays across respiratory viral illnesses. In another study by Zhou *et al*., patients with COVID-19 admitted to the ICU had a median length of stay of 8.0 days (IQR: 4.0–12.0) [6]. These findings suggest that the presence of a concurrent bacterial infection may contribute to prolonged ICU stays.

In our study, the most frequently isolated pathogen was *P. aeruginosa*, a Gram-negative bacterium. The total number of Gram-negative isolates—including *Pseudomonas*, *Klebsiella*, *Acinetobacter*, and *E. coli*—was 82 (74.5%). This proportion is notably higher than the 50% reported by Zhang *et al*. [20], potentially reflecting regional resistance patterns or differences in sampling protocols. Gram-negative bacteria have been reported to be responsible for more than 30% of all infections, with a predominance in ventilator-associated pneumonia (47%) and urinary tract infections (45%) [19]. In the ICU setting, Gram-negative organisms account for approximately 70% of these infections [21]. Among them, members of the *Enterobacteriaceae* family are the most prevalent and are increasing globally.

The emergence of Gram-negative bacterial infections is increasingly recognized as a complication of ICU care. Gram-negative bacterial infections, including members of the *Enterobacteriaceae* family (*e.g*., *K. pneumoniae*, *E. coli*) and non-fermenting organisms such as *P. aeruginosa* and *A. baumannii*, are increasingly recognized as complications of ICU care, with rising prevalence of extended-spectrum beta-lactamase (ESBL)– and carbapenemase-producing strains worldwide [3, 19, 22-27].

In a study by Wu *et al*., the most common pathogens identified in bloodstream infections were coagulase-

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negative *Staphylococcus*, *Enterococcus faecium*, and *Enterococcus faecalis*. In contrast, respiratory tract samples most frequently yielded *P. aeruginosa* (38%), methicillin-resistant *Staphylococcus aureus* (*MRSA*) (24%), *Enterobacter* (18.8%), and *Klebsiella* species. That study also found significantly higher in-hospital mortality rates among COVID-19 patients with bacterial co-infections and secondary infections [28].

Similarly, another study reported that the majority of bacteria isolated from intratracheal aspirate samples of COVID-19 patients were *Acinetobacter* species (41%). Other identified organisms included *P. aeruginosa* (9.1%), *Klebsiella* spp., *E. coli*, *S. aureus*, *Staphylococcus epidermidis* (4.5%), *Candida* spp. (18.2%), and *Corynebacterium* spp. (13.6%) [29].

To conclude, the rate of co-infection is increased in ICU patients with a severe form of the disease. COVID-19 patients who suffer from bacterial infections have a longer stay in the ICU. Therefore, bacterial co-infections represent a significant clinical complication for ICU-admitted COVID-19 patients. The commonly found pathogens in the present study were *Pseudomonas* and *Klebsiella*. To reduce secondary infections, in addition to appropriate antibiotic administration, strict infection control measures should be enforced during future respiratory pandemics. This helps to prevent the development and transmission of drug-resistant organisms in health care facilities.

Our research has several limitations. First, the retrospective design limits causal inference and is subject to missing data biases; a prospective multicenter cohort would provide more robust longitudinal data. Second, the time of the onset of bacterial co-infection was not determined. That is, bacterial co-infection can be community-, hospital- or ventilation-related. Furthermore, evolving diagnostic protocols and initial biosafety constraints during early pandemic waves may have delayed comprehensive microbiological workups in some centers. Currently, however, microbial culture of respiratory secretions is recommended. Moreover, a set of laboratory and radiological dynamic findings can provide more confidence in the results; we only reported bacterial infection from blood culture results. Finally, the single-center design limits generalizability, as SARS-CoV-2 prevalence and local antimicrobial resistance patterns likely vary across geographic regions. Future multicenter studies with adequate sample sizes should employ matched control groups to isolate the independent impact of blood culture positivity on ICU length of stay, complications, and pathogen-specific mortality, while adjusting for key clinical confounders.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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AI DISCLOSURE

No artificial intelligence tools were used in preparation of this manuscript.

DATA AVAILABILITY

All data generated or analyzed during this study are included in this published article. Individual-level patient data are not publicly shared to protect participant privacy but may be available from the corresponding author upon reasonable request.

AUTHORS' CONTRIBUTIONS

S.S., M.S., and F.A.M. conceptualized and designed the study. S.S., N.N., and F.A.M. acquired and analyzed the data. M.S. and N.N. interpreted the results. S.S., N.N., and F.A.M. drafted the manuscript. All authors critically reviewed and revised the manuscript, approved the final version, and agree to be accountable for all aspects of the work.

ETHICS STATEMENT

The study protocol was approved by the Ethics Committee of Shahid Sadoughi University of Medical Sciences (Approval Code: IR.SSU.MEDICINE.REC.1402.254). Given the retrospective nature of the study, the requirement for informed consent was waived by the Ethics Committee.

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