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RESEARCH ARTICLE

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Bacterial and fungal superinfections are detected at higher frequency in critically ill patients affected by SARS CoV-2 infection than negative patients and are associated to a worse outcome

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Abstract

Patients with viral infections are at higher risk to acquire bacterial and fungal superinfections associated with a worse prognosis. We explored this critical point in the setting of patients with severe COVID-19 disease. The study included 1911 patients admitted to intensive care unit (ICU) during a 2-year study period (March 2020–March 2022). Of them, 713 (37.3%) were infected with SARS-CoV-2 and 1198 were negative (62.7%). Regression analysis was performed to determine risk factors associated with the presence of bacterial and/or fungal superinfections in SARS-CoV-2 patients and to evaluate predictors of ICU mortality. Of the 713 patients with SARS-CoV-2 infection, 473 (66.3%) had respiratory and/or bloodstream bacterial and/or fungal superinfections, while of the 1198 COVID-19-negative patients, only 369 (30%) showed respiratory and/or bloodstream bacterial and/or fungal superinfections ($p < 0.0001$). Baseline characteristics of COVID-19 patients included a median age of 66 (interquartile range [IQR], 58–73), a predominance of males (72.7%), and the presence of a BMI higher than 24 (median 26; IQR, 24.5–30.4). Seventy-four percent (527, 73.9%) had one or more comorbidities and 135 (18.9%) of them had received previous antibiotic therapy. Furthermore, most of them (473, 66.3%) exhibited severe radiological pictures and needed invasive mechanical ventilation. Multivariate logistic regression analysis showed that 1 unit increment in BMI rises the risk of bacterial and/or fungal superinfections acquisition by 3% and 1-day increment in ICU stays rises the risk of bacterial and/or fungal superinfections acquisition by 11%. Furthermore, 1-day increment in mechanical ventilation rises the risk of bacterial and/or fungal superinfection acquisition by 2.7 times. Furthermore,

patients with both bacterial and fungal infections had a significantly higher mortality rate than patients without superinfections (45.8% vs. 26.2%, $p < 0.0001$). Therefore, bacterial and fungal superinfections are frequent in COVID-19 patients admitted to ICU and their presence is associated with a worse outcome. This is an important consideration for targeted therapies in critically ill SARS-CoV-2 infected patients to improve their clinical course.

KEYWORDS
bacteria, blood infections, COVID-19, fungi, respiratory infections, superinfections

1 | INTRODUCTION

Since March 2020, when the World Health Organization (WHO)[1] has declared the global pandemic of Coronavirus disease 2019 (COVID-19), SARS-CoV-2 is still having a profound impact on healthcare systems all over the world.

The clinical characteristics of COVID-19 include a large spectrum of severity ranging from asymptomatic or moderate flu-like symptoms to severe pneumonia, which may progress to respiratory failure and to acute respiratory distress syndrome and multiorgan failure.[2]

Patients with severe COVID-19 may require noninvasive or invasive mechanical ventilation and intensive care unit (ICU) admission.[3]

Superinfections are a leading cause of morbidity and mortality in ICUs because critically ill patients are extremely vulnerable, had often impaired immune responses, and are frequently subjected to invasive procedures.[4–6]

Bacterial and/or fungal superinfections are often an important complication of viral respiratory infections such as influenza with high mortality rates.[7,8]

In COVID-19 patients and especially in critically ill patients, it has also been shown that their incidence is higher.[9–12] These superinfections, mainly constituted by pneumonia (50%) and bloodstream infections (BSIs) (34%),[13] are associated with higher mortality.[14–16]

Notably, COVID-19 patients are more susceptible to developing ventilator-acquired pneumonia (VAP) than patients without COVID-19[17,18] related to the prolonged invasive mechanical ventilation required by these patients.

In addition, BSIs have a higher incidence (10.3 BSI per 1000 patients-days)[19,20] in COVID-19 patients compared to COVID-19-negative patients.

Risk factors of BSI are associated to higher SAPS II (Simplified Acute Physiology Score), longer stay in hospital before ICU admission, mechanical ventilation, previous antibiotic therapy and immunosuppressive drugs.[19,20]

In the era of COVID-19 infections, a greater probability to acquire superinfections during the hospital stay has led to higher consumption of antibiotics,[21] often unnecessary especially at hospital admission, with an increased risk of multidrug pathogens emergence.

Indeed, more information is needed about the clinical impact of healthcare-associated bacterial and fungal infections on disease severity and outcome of ICU patients to optimize the management of severe patients with SARS-CoV-2 infections.

The aims of this study were:

1. to analyze the prevalence of respiratory and/or bloodstream bacterial and fungal superinfections according to their etiology in critically ill patients admitted to ICU with and without COVID-19 pneumonia.
2. to compare the clinical characteristics of COVID-19 patients with or without superinfections;
3. to identify the independent risk factors associated with the acquisition of superinfections in ICU and ICU mortality of COVID-19 patients.

2 | MATERIALS AND METHODS

2.1 | Study design and participants

We conducted a single-center retrospective study including adult (>18 years old) patients admitted consecutively over a 2-year period from March 2020 to March 2022 to the ICUs of Spedali Civili's Hospital, Brescia, Italy.

The hospital organization during the pandemic has been extensively described elsewhere.[22] Briefly, the hospital had two ten-bed general ICUs (20 beds total), and one six-bed cardiac ICU. At the time of the pandemic, the hospital actively managed 49 COVID-19 ICU beds and 14 general ICU beds, for a total of 63 ICU beds.

All patients with and without a confirmed diagnosis of COVID-19 by an RT-PCR test on a nasopharyngeal swab constitute the total cohort of patients investigated for the prevalence of respiratory and/or bloodstream bacterial and/or fungal secondary infections acquired in ICUs. All the subsequent analyses were performed only on the subcohorts of COVID-19 patients with and without superinfections.

2.2 | Definitions

The diagnosis of infection was based on clinical symptoms and the isolation of an etiological microorganism.

The definition of COVID-19 *pneumonia* or severe COVID-19 pneumonia was done according to the WHO guidelines[21] and included clinical signs of pneumonia (fever, cough, dyspnea, fast breathing). *Severe pneumonia* was defined if a patient had a respiratory frequency ≥30 breaths/min, severe respiratory distress, or SpO2 ≤90% on room air. The *critical grade* (ICU admission reason) was defined if a patient needed mechanical ventilation due to respiratory failure, had septic shock, and/or had multiple organ dysfunctions or failure.

BSI was defined as the growth of a pathogen (bacteria or fungi) in one or more blood cultures. To consider significant a BSI caused by bacteria which are skin colonizers such as coagulase negative staphylococci, it was necessary to have their growth in two or more blood cultures drawn from different sites.

Respiratory infection was defined as a significant growth of potentially pathogenic microorganisms in a bronchoalveolar lavage, in a bronchial aspirate or in suitable sputum (>25 PMN and <10 epithelial cells × 100), associated with the clinical and radiological sign of infection, following international guidelines.[23] In this study, we did not differentiate hospital-acquired pneumonia and VAP.

An invasive fungal disease was defined as positive culture from sterile materials or histopathologic, cytopathologic or direct microscopic examination or as positive DNA amplification by PCR according to the recent guidelines.[24]

Coagulase-negative staphylococci detected in a single blood culture or *Candida* spp. present in the respiratory tract were considered contaminants and not significant infections.

Bacterial or fungal colonization was defined by isolating bacteria or fungi from respiratory samples in absence of significant laboratory and/or clinical alterations. Bacterial and fungal respiratory infection was defined with the following conditions:

1. presence of pathogen in Gram-stain preparations of samples
2. isolation of pathogen in culture
3. presence of significant laboratory and/or clinical alterations associated with infections such as fever, worsening cough and sputum production, dyspnea, leucocytosis, and increased C-reactive protein
4. pulmonary infiltrates on radiography.

Infections were classified as ICU-associated superinfections if acquired within 48 h after ICU admission.

Clinical management of ICU patients was carried out in accordance with the most recent WHO[25] clinical guidelines available at the time of patient admission (https://www.who.int/publications/i/item/WHO-2019-nCoV-Clinical-2022.2).

2.3 | Data collection

The manual health records for all the patients admitted to the ICUs with a diagnosis of severe/critical COVID-19 disease were reviewed. The most significant data were extracted and inserted into a REDCap© database[26] and finally deidentified. Data included: demographic profiles (age, sex, race, body mass index); comorbid conditions (hypertension, cancer, diabetes, primary immunodeficiencies, autoimmune diseases, and chronic heart/lung/kidney disease); clinical data (daily PaO2/FiO2 [P/F] ratio, SAPS at ICU admission, presence of mechanical ventilation and recent antibiotic therapy); baseline inflammatory markers on admission to ICUs (C-reactive protein, lymphocyte, and neutrophils counts), renal and liver function markers (creatinine, alanine aminotransferase [ALT], and aspartate aminotransferase [AST]) and lactate dehydrogenase (LDH); microbiological data on record (blood and respiratory cultures); radiological test (CT scans of the chest, chest radiographs); date of admission to ICUs; duration of ICU stay and clinical outcomes (discharge, recovery, or in-hospital death). The data collected were exported to R software for analysis.

2.4 | Microbiological assays

SARS-CoV-2 was detected by using the following PCR platforms: in first instance, Allplex™ 2019-nCoV assay, Seegene Inc, distributed by Arrow Diagnostics, Genoa, Italy and in second instance Xpert Xpress SARS-CoV-2, Cepheid Italia, Milan, Italy.

The diagnosis of bacterial and fungal infections was made following standard laboratory procedures. Samples were inoculated on blood, chocolate blood, Columbia nalidixic acid, MacConkey and Sabouraud agar plates (BioMérieux).

Blood cultures were performed by using BACT/ALERT® VIRTUO® (VIRTUO; BioMérieux) instrument. Identification of isolates was performed by Vitek® MALDI-TOF (Matrix Assisted Laser Desorption/Ionization) MS v3.0 (BioMérieux).

2.5 | Statistical analysis

Categorical variables are expressed as numbers (percentage), and continuous variables as median (interquartile range [IQR]). Categorical data were compared by using the χ^2 test or the Fisher exact test, as appropriate. The data in different groups were compared with the analysis of variance or independent *t*-test for normally distributed variables.

The presence of superinfections and ICU mortality was modeled using multivariate logistic regression. The final models were defined using a backward variable selection based on the Akaike information criterion. They started from a full model including the following variables as candidate predictors: gender, age, BMI, SAPS score, all superinfections, bacterial superinfections, fungal superinfections, bacterial and fungal superinfections, comorbidities (hypertension, cancer, diabetes, primary immunodeficiencies, autoimmune diseases, and chronic heart/lung/kidney disease), invasive mechanical ventilation, and length of ICU stay. Multivariate imputation by chained equations was used to impute the missing values.

All statistical analyses were conducted in R and figures were generated using the package ggplot2. A $p < 0.05$ was considered statistically significant.

3 | RESULTS

3.1 | Prevalence of respiratory and/or bloodstream bacterial and/or fungal superinfections and microbiological etiology

During the study period, 1911 patients [713 (37.3%) COVID-19, 1198 (62.7%) COVID-19-negative] were admitted to ICUs. Four hundred seventy-three (66.3%) COVID-19 and 369 (30%) COVID-19-negative patients showed bacterial and/or fungal superinfections ($p < 0.0001$) (Table 1). COVID-19 patients developed more bacterial superinfections (27% vs. 17.9%, $p < 0.0001$) and more bacterial and fungal superinfections (33.6% vs. 7.9%, $p < 0.0001$) (Table 1).

The etiology of the pathogens identified in both respiratory and bloodstream samples from both populations is listed in Table 2. COVID-19 patients did not differ from COVID-19-negative in respect to the total percentage of Gram-negative (54% vs. 52%, $p = 0.53$) and Gram-positive (45% vs. 37%, $p = 0.11$) bacteria.

Gram-negative bacteria such as *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii*, generally associated with a multi-resistant phenotype, had similar prevalence in both populations. COVID-19 patients had significantly higher superinfections due to coagulase-negative staphylococci (18.2% vs. 7.3%, $p < 0.0001$), *Enterococcus faecium* (6.8% vs. 1.7%, $p < 0.0001$), *Enterococcus faecalis* (5.8% vs. 1.7%, $p = 0.0009$), and *Serratia marcescens* (4% vs. 1.2%, $p = 0.02$). COVID-19-negative patients have instead significantly higher superinfections caused by *Haemophilus influenzae* (3.1% vs. 1%, $p = 0.018$) and *Streptococcus pneumoniae* (3.9% vs. 0.4, $p < 0.0001$) (Table 2).

A statistically significant increase of *fungal* superinfections due to *Aspergillus fumigatus* was detected in COVID-19 patients (49.7% vs. 8.6%, $p < 0.0001$), while the number of superinfections with both *Candida albicans* and *non-albicans* was significantly lower in COVID-19 patients (32.9% vs. 53.2%, $p < 0.0001$; 15.5% vs. 32.6%, $p < 0.0001$, respectively) (Table 2).

Other bacteria detected in COVID-19 patients were: *Aerococcus viridans*, *Chryseobacterium indologenes*, *Corynebacterium amycolatum*, *Corynebacterium propinquum*, *Corynebacterium pseudodiphteriticum*, *Corynebacterium striatum*, *Delftia acidovorans*, *Granulicatella adiacens*, *Hafnia alvei*, Pseudomonas alcaligenes, *Raoultella ornithinolytica*, and *Sphingomonas paucimobilis*.

Other fungi detected in COVID-19 patients were *Saccharomyces cervisiae* and *Pneumocystis jirovecii*.

Other bacteria detected in COVID-19-negative patients were: Aeromonas hydrophila, *C. indologenes*, *C. striatum*, *Corynebacterium* spp., *Eikenella corrodens*, *Fusobacterium nucleatum*, *H. alvei*, *Neisseria meningitidis*, *Parvimonas micra*, *Propionibacterium* spp., *P. alcaligenes*, and *Streptococcus agalactiae*.

Other fungi detected in COVID-19-negative patients were: *Mucor* spp., *Penicillium* spp., *Pneumocystis jirevocii*, *Saccharomyces cerevisiae*, and *Trichosporon asahii*.

TABLE 1 Characteristics of patients with bacterial and/or fungal superinfections according to COVID-19 disease status.

	COVID-19 patients ($n = 713$)	COVID-19-negative patients ($n = 1198$)	p Value
	Bacterial superinfections	Bacterial superinfections	
No of patients	193 (27%)	215 (17.9%)	**<0.0001**
Age, median (IQR)	62 (54-71)	62.5 (51-74)	0.22
Gender, *n* (%)			
Male	145 (75.1)	187 (86.9%)	**0.008**
	Fungal superinfections	Fungal superinfections	
No of patients	40 (5.6%)	59 (4.9%)	0.52
Age, median (IQR)	69 (62-73)	65.5 (55-76)	0.09
Gender, *n*/total (%)			
Male	31 (77.5)	35 (59.3%)	0.08
	Bacterial + fungal superinfections	Bacterial + fungal superinfections	
No of patients	240 (33.6%)	95 (7.9%)	**<0.0001**
Age, median (IQR)	68 (62-74)	69.5 (60-73.5)	0.19
Gender, *n* (%)			
Male	179 (74.5)	71 (74.7%)	1.0

Abbreviation: IQR, interquartile range.

TABLE 2 Etiology of superinfection according to COVID-19 disease status.

	COVID-19 patients (n = 713)	COVID-19-negative patients (n = 1198)	p Value[a]
Bacteria, *n* (%)			
Overall bacteria detected (respiratory and blood samples)	669 (93.8%)	409 (34%)	
Staphylococcus coagulase negative spp.	122 (18.2)	30 (7.3)	**<0.0001**
Pseudomonas aeruginosa	109 (16.2)	69 (16.8)	0.80
Staphylococcus aureus	79 (11.8)	90 (22)	**<0.0001**
Escherichia coli	61 (9.1)	52 (12.7)	0.065
Klebsiella pneumoniae	45 (6.7)	39 (9.5)	0.10
Enterococcus faecium	46 (6.8)	7 (1.7)	**<0.0001**
Enterobacter spp.	43 (6.4)	16 (3.9)	0.09
Enterococcus faecalis	39 (5.8)	7 (1.7)	**0.0009**
Serratia marcescens	27 (4.0)	5 (1.2)	**0.008**
Citrobacter spp.	19 (2.8)	8 (1.9)	0.42
Klebsiella spp.	17 (2.5)	9 (2.2)	0.83
Acinetobacter baumannii	8 (1.2)	2 (0.4)	0.33
Streptococcus spp.	12 (1.7)	5 (1.2)	0.61
Proteus spp.	9 (1.4)	5 (1.2)	1.00
Haemophilus influenzae	7 (1.0)	13 (3.1)	**0.018**
Streptococcus pneumoniae	3 (0.4)	16 (3.9)	**<0.0001**
Burkholderia spp.	2 (0.29)	1 (0.24)	1.0
Moraxella	1 (0.14)	2 (0.4)	1.0
Other	20 (2.9)	33 (8.0)	**0.0004**
Fungi, *n* (%)			
Overall fungi detected (respiratory and blood samples)	231 (32.3%)	184 (15.3%)	
Candida albicans	76 (32.9)	98 (53.2)	**<0.0001**
Candida non albicans	36 (15.5)	60 (32.6)	**<0.0001**
Aspergillus spp.	115 (49.7)	16 (8.6)	**<0.0001**
Fusarium spp.	1 (0.4)	0	–
Other	3 (1.2)	10 (5.4)	**0.02**

[a]Comparison between proportions was performed using Chi square or Fisher test, as appropriate.

3.2 | Baseline characteristics of the population admitted to ICUs with severe SARS-CoV2 infection

A total of 713 laboratory-confirmed patients with SARS-CoV-2 infection were consecutively admitted to ICU during the study period, of which 473 acquired bacterial and/or fungal superinfections. Demographic and clinical characteristics of the patients on ICU admission are listed in Tables S1 and S2 and in Table 3. The median age was 66 (IQR, 58–73), 519 were male (72.8%) and 194 female (27.2%). Most of them (527, 73.9%) had one or more comorbidities, mostly arterial hypertension (346, 48.5%), heart disease (165, 23.1%), diabetes (129, 18%) and lung disease (80, 11.2%) and the 18.9% of them (135/713) had received previous antibiotic therapy.

The majority (473/713, 66.3%) had a diagnosis of bilateral pneumonia and 474 (66.4%) required mechanical respiratory support.

COVID-19 patients with *bacterial superinfections* (vs. patients without), had a higher incidence of Caucasian race, had more arterial hypertension, and less heart disease. Furthermore, they showed a

TABLE 3 Baseline characteristics of study population with and without bacterial and fungal superinfections.

	Bacterial and fungal superinfections (*n* = 240)	No superinfection (*n* = 240)	*p* Value
Baseline characteristics			
Age, median (IQR)	68 (62–74)	65 (56–74)	**0.002**
Male, n (%)	179 (74.5)	164 (68)	0.15
Race, *n* (%)			
Caucasian	211 (87.9)	183 (76)	**0.001**
Asiatic	6 (2.5)	5 (2)	1.0
African	12 (5)	14 (5.8)	0.84
Body Mass Index (kg/m^2), median (IQR)	26.75 (24.6–30.9)	26.1 (23.9–29.2)	**0.01**
Comorbidities, *n* (%)			
Heart disease	77 (32)	55 (23)	**0.03**
Arterial hypertension	138 (57.5)	94 (39)	**<0.0001**
Diabetes	57 (23.7)	46 (19)	0.26
Chronic kidney disease	12 (5)	18 (7.5)	0.34
Lung disease	29 (12)	22 (9)	0.37
Hematological disease	5 (2)	14 (5.8)	0.058
Immunodeficiency	0	5 (2)	0.06
Dialysis	2 (0.8)	4 (1.6)	0.68
Autoimmune disease	5 (2)	11 (4.6)	0.20
Cancer	32 (13.3)	26 (10.8)	0.48
Recent antibiotic therapy, *n* (%)	37 (15.4)	53 (22)	0.16
SAPS score on admission, median (IQR)	33 (27–38)	31 (25–38)	0.27
Chest RX results, n (%)			
Negative	3 (1.2)	6 (2.5)	0.50
Lobar	7 (2.9)	7 (2.9)	1.0
Interstitial	106 (44.1)	98 (40.8)	0.51
Multifocal	25 (10.4)	15 (6.25)	0.13
Bilateral	189 (78.7)	115 (47.9)	**<0.0001**
PaO2/FiO2 ratio at admission, median (IQR)	144 (100–176)	111.5 (91.5–146.5)	**0.002**
pO2 saturation (%), median (IQR)	95 (93–96)	95 (93–97)	0.17
Interventions, *n* (%)			
Noninvasive mechanical ventilation	11 (4.5)	33 (13.7)	**0.0007**
Invasive mechanical ventilation	206 (85.8)	93 (38.7)	**<0.0001**
Length of ICU stay, days, median (IQR)	17.5 (11–25)	5 (2–10)	**<0.0001**
Outcome			
Mortality, *n* (%)	110 (45.8)	63 (26.25)	**<0.0001**
Recovery, *n* (%)	28 (11.6)	55 (22.9)	**0.001**
Discharged with therapy	63 (26.2)	99 (41.25)	**0.0007**
Lost at follow-up	4 (1.6)	6 (2.5)	0.75

Abbreviations: IQR, interquartile range; SAPS, Simplified Acute Physiology Score.

higher BMI, an increased number of radiological reports with multifocal and bilateral pulmonary involvement and a greater P/F ratio. Then, they needed more invasive ventilation and had a higher median ICU length of stay (Table S1).

COVID-19 patients with *fungal superinfections* (vs. patients without), were older, had a higher incidence of Caucasian race and had a higher BMI. Then, they had more frequently a radiological report of bilateral pulmonary involvement, needed more invasive ventilation, had a longer ICU length of stay and a higher mortality rate (Table S2).

COVID-19 patients with *bacterial and fungal* superinfections (vs. patients without) were older, had a higher incidence of Caucasian race, had a higher BMI, and had more heart disease and arterial hypertension. Furthermore, they had more frequently a radiological report of bilateral pulmonary involvement, a greater P/F ratio, a longer ICU length of stay, higher mortality and a lower recovery rate (Table 3). No statistically significant difference was detected for any laboratory parameters analyzed (leukocytes, neutrophils, PCR, AST, ALT, creatinine, and LDH) (data not shown).

3.3 | Superinfections in COVID-19 patients

Overall, 669 bacterial isolates were recovered from 473 COVID-19 patients. The first bacterial *respiratory superinfections* occurred after a median of 7 days (IQR, 4–10) after ICU admission and included 287 monomicrobial and 44 polymicrobial infections (with isolation of 2 and 3 different bacteria in 40 and 4 respiratory samples, respectively). In 90 patients, we detected second bacterial respiratory superinfections (70 monomicrobial and 20 polymicrobial infections) 7 days (IQR, 4–12) after the first superinfection.

Bacterial *blood infections* were detected after a median of 9 days (IQR, 5–17) after ICU admission and included 138 monomicrobial and 12 polymicrobial infections.

Fungal infections were detected after a median of 7 days (IQR, 4–11), and included 234 episodes. Fungemia was detected after a median of 11 days (IQR, 5–18.5) and included 31 episodes.

The detection time point and the most relevant pulmonary and blood bacterial and fungal pathogens are depicted in Figure 1. The most frequently isolated respiratory bacteria during the first 7 days after ICU admission were *Staphylococcus aureus* (45 isolates), *P. aeruginosa* (26 isolates), *Klebsiella* spp. (26 isolates), *Enterobacter* spp. (25 isolates), *Escherichia coli* (23 isolates), and *Enterococcus* spp. (21 isolates). *P. aeruginosa*, *Klebsiella* spp., *Enterococcus* spp., and *Stenotrophomonas maltophilia* were the bacteria mostly detected also after 1 month from ICU admission.

In blood, the most frequently isolated bacteria during the first 7 days after ICU admission were coagulase-negative staphylococci (30 isolates), followed by *E. coli* (12 isolates) and *Enterococcus* spp. (11 isolates). About fungi, *Aspergillus* spp. was the mold mostly detected both in respiratory and blood samples (60 and 11 isolates, respectively) after 7 days from ICU admission and remained the most frequent fungus found also after 15 and 30 days from ICU admission.

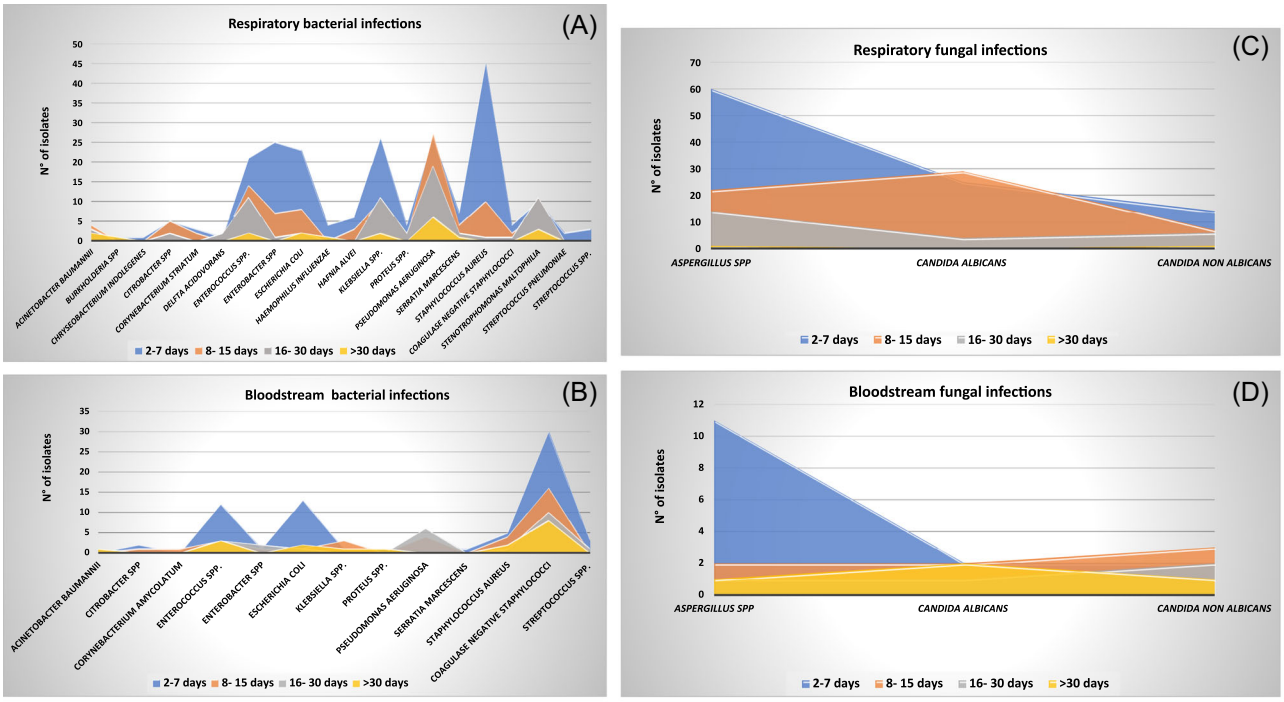

FIGURE 1 Etiology of superinfections in respiratory and blood samples according to time from ICUs admission. First detection time points of the frequently cultured respiratory (A, C) and blood (B, D) pathogens censored at 60 days. Only the first detection event of a relevant respiratory and blood pathogen in each patient is reported.

3.4 | Risk factors associated with respiratory and/or bloodstream superinfections in severe COVID-19 patients and with in-hospital mortality

COVID-19 patients are associated with an increased probability of acquiring a bacterial and/or fungal superinfection compared to negative patients referred to ICUs (OR = 4.4, 95% confidence interval [CI] = 3.63–5.39, $p < 0.0001$).

A multivariate logistic regression analysis was then performed to identify risk factors associated with bacterial and/or fungal superinfections in COVID-19 patients admitted to ICUs.

A total of three variables were identified as independent risk factors for the acquisition of superinfections: BMI (OR = 1.03, 95% CI = 1.0–1.06, $p = 0.029$), the length of ICU stay (OR = 1.11, 95% CI = 1.09–1.15, $p < 0.00001$), and invasive mechanical ventilation (OR = 2.7, 95% CI = 1.64–4.53, $p = 0.0001$). A recent antibiotic therapy, on the contrary, was associated with a reduced risk factor to acquire superinfections (OR = 0.58, 95% CI = 0.38–0.88, $p = 0.009$) (Figure 2).

Furthermore, multivariate logistic regression in patients with both bacterial and fungal superinfections showed that superinfections (OR = 2.4, 95% CI = 1.73–3.57, $p < 0.000001$), age (OR = 1.05, 95% CI = 1.03–1.07, $p < 0.00001$), invasive mechanical ventilation (OR = 2.39, 95% CI = 1.25–4.89, $p = 0.01$), and SAPS score (OR = 1.02, 95% CI = 1.01–1.04, $p = 0.0003$) were independent risk factors associated with ICU mortality. Sex female was associated instead with a lower risk of ICU mortality (OR = 0.63, 95% CI = 0.41–0.94, $p = 0.002$) (Figure 3). The multivariate logistic regression showed that no superinfections, bacterial or fungal superinfections alone were risk factors for ICU mortality (Tables S3–S5) in the population analyzed for them.

4 | DISCUSSION

Bacterial and fungal infections represent an important complication of viral diseases and can be associated with unfavorable outcomes, especially during seasonal influenza epidemics.[27] Community-acquired bacteria such as *S. pneumoniae*, *H. influenzae*, or *S. aureus* usually cause coinfections, diagnosed within the first 24–48 h of hospital admission. Multidrug-resistant organisms or hospital-acquired fungi frequently cause superinfections occurring at least 48–72 h after hospital admission.

Co-infections are quite rare in COVID-19 patients and are reported in the literature with a prevalence of about 7% in hospitalized patients.[28,29] Conversely, superinfections are diagnosed much more frequently, especially in patients with extremely severe COVID-19 infections.[9,21]

In patients with severe forms of COVID-19 and referred to intensive care, the incidence of superinfections is much higher, reaching about 45% of cases.[10,11,30–32]

In our study, we have highlighted a 4.4 times higher risk for COVID-19 patients admitted to ICUs of acquiring a bacterial and/or fungal superinfection compared to negative patients.

We found that the percentage of subjects with bacterial and/or fungal superinfections in the respiratory tract and blood was 66.3% (473/713), a finding similar to that reported in other studies.[10,11,16]

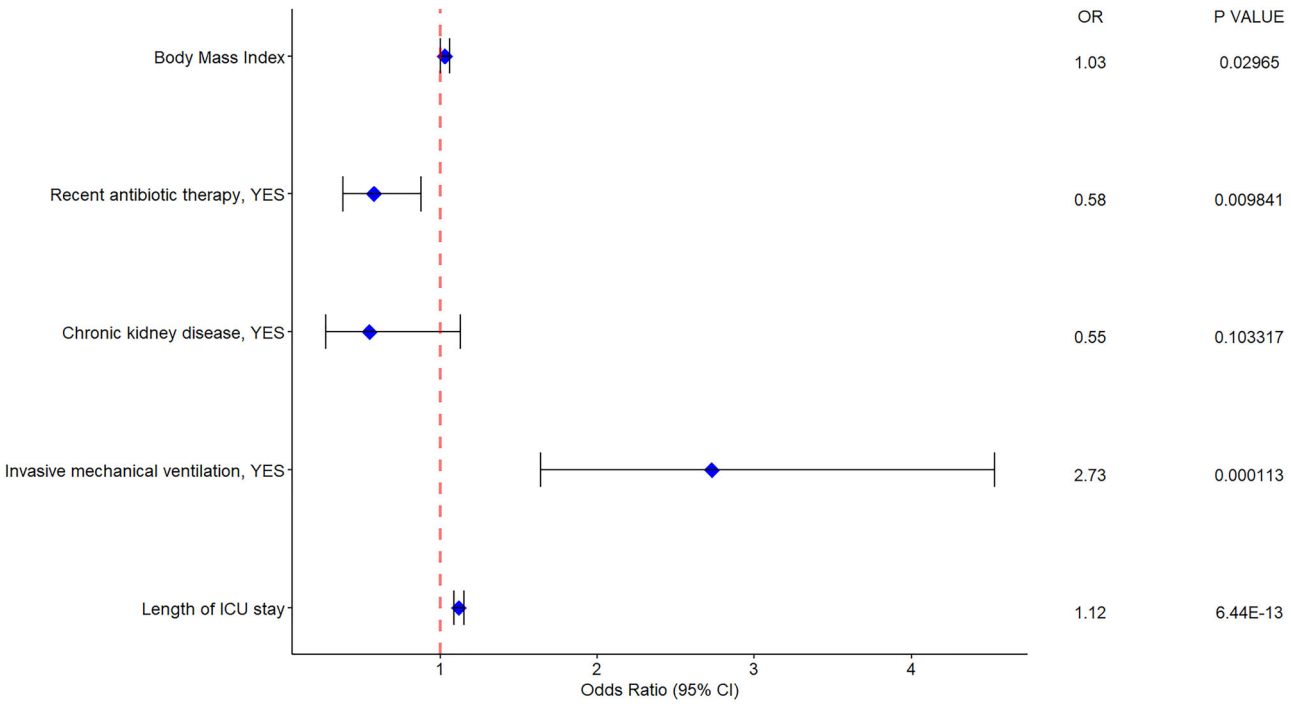

FIGURE 2 Forest plot of multivariate logistic regression on the presence of superinfections. R^2 Tjur = 0.30, $p = <2.22e{-}16$.

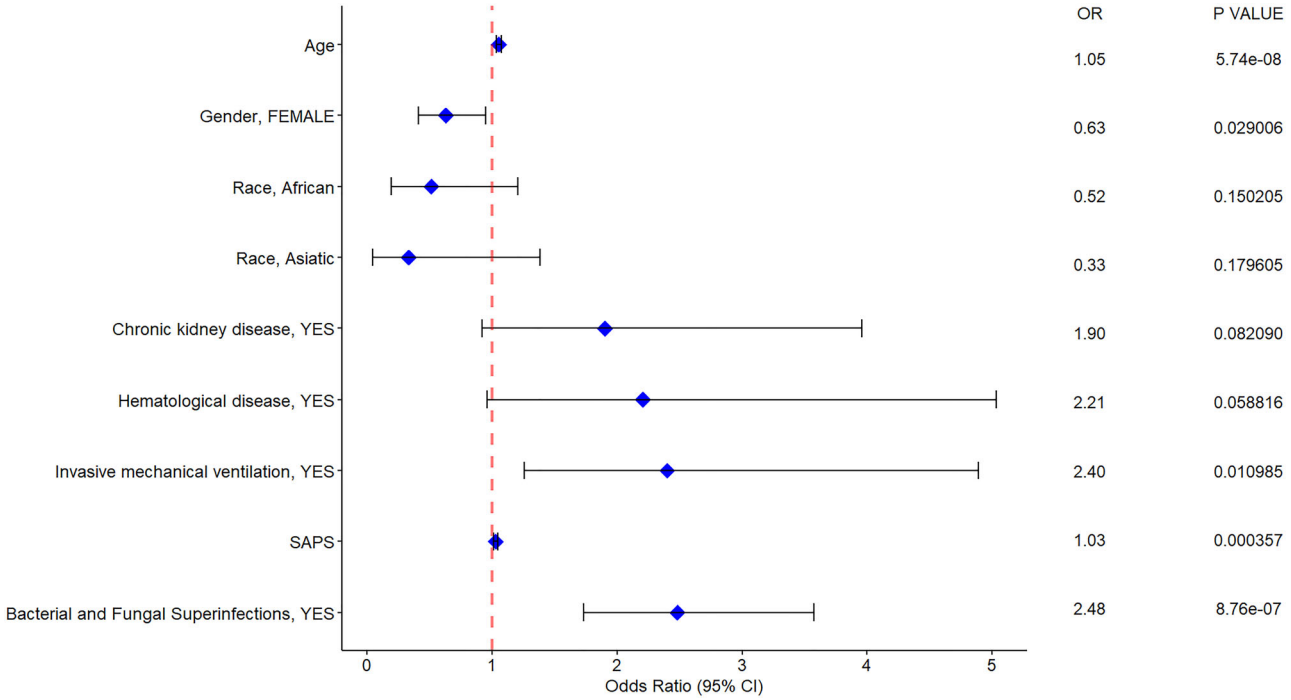

FIGURE 3 Forest plot of multivariate logistic regression on ICU mortality within the group of patients with bacterial and fungal superinfections. R^2 Tjur = 0.17, $p = 0.58$.

The percentage of bacterial superinfections alone was 27%, 5.6% for fungal infections alone and 33.6% for bacterial and fungal infections.

The higher frequency of superinfections in COVID-19 patients admitted to ICU has been associated with several risk factors. In fact, in addition to the widespread alveolar damage induced by the SARS-CoV-2 virus, patients in the ICU undergo various invasive procedures (such as insertion of endotracheal tubes or venous catheters), are generally more fragile due to the presence of more severe forms of the disease, have several pre-existing comorbidities and stay hospitalized longer. Furthermore, they are often treated with anti-inflammatory drugs and antibiotics leading to extreme vulnerability and greater susceptibility to both bacterial and fungal infections.[15,33]

We have observed a higher percentage of Gram-negative bacteria, mainly consisting of *P. aeruginosa*, *K. pneumoniae*, and *E. coli* with lower percentages of *A. baumannii*, which is instead much more frequent in the works present in the literature.[3,34] The frequencies of Gram-negative bacteria were similar in both positive and negative COVID-19 patients. Instead, our data demonstrate a higher frequency of Gram-positive bacteria in COVID-19 positive than COVID-19-negative patients have. The bacteria most represented were *S. aureus*, coagulase-negative staphylococci, *E. faecalis*, and *E. faecium*.

The high prevalence of *Enterococcus* spp. has been emphasized in other studies and was presumably related to the use of cephalosporins as initial empirical treatment.[35,36]

As already reported in the literature,[10] pulmonary superinfections from bacteria represent the most common infections. Rates of respiratory superinfections ranged from 5% to 29%[37,38]; in this study, the figure was higher (46.4%) due probably to the complexity of patients referred to ICUs.

The frequency of fungal superinfections was 39.2%, with the main involvement of *A. fumigatus* (16.1%), already identified as the most frequent fungal pathogen responsible for superinfections either in hospitalized and ICU patients.[10,15]

However, because the diagnostic criteria of invasive aspergillosis in non-neutropenic critically ill patients[39,40] are uncertain, the real prevalence of this mold in the COVID-19 patients admitted to ICU is not fully understood. The second most frequent fungal superinfections were attributed to *C. albicans* (10.6%), similar to other studies.[41,42]

BSIs represent the second most frequent complication of COVID-19 patients[43,44] and in our study, we found a rate of 21% for bacteremia and of 5.8% for fungemia.

We recovered *Aspergillus* spp. from 11 blood samples. This is a rare event and it cannot be readily associated with invasive aspergillosis. Clinical and radiological parameters are needed to establish the meaning of aspergillemia. However, all our patients with fungemia had also respiratory infections with *Aspergillus* spp. and it was reported that fungemia often might be a consequence of pulmonary aspergillosis.[45]

The bacteria mostly isolated from blood were the coagulase negative staphylococci, mostly related to catheter-associated infections (45/64, 70.3%).

Our results demonstrate that all COVID-19 patients with bacterial and/or fungal superinfections had longer ICU stay than

those without superinfections, were associated with specific comorbidities, in particular pre-existing lung diseases and arterial hypertension, and underwent mechanical ventilation procedures more often invasive with radiological findings of bilateral lung involvement.

Multivariate logistic regression analysis showed that 1 unit increment in BMI rises the risk of bacterial and/or fungal superinfections acquisition by 3% and 1-day increment in ICU stays rises the risk of bacterial and/or fungal superinfections acquisition by 11%. Furthermore, 1-day increment in mechanical ventilation rises the risk of bacterial and/or fungal superinfection acquisition by 2.7 times.

An antibiotic treatment started and continued on other wards before the admission to the ICU was instead associated with a reduced risk factor to acquire superinfections (OR = 0.58, 95% CI = 0.38–0.88, $p = 0.009$).

Patients having only fungal infections and patients having both bacterial and fungal infections had significantly higher mortality rates than patients without superinfections (35% vs. 26.25%, $p = 0.03$ and 45.8% vs. 26.25%, $p < 0.0001$, respectively), in agreement with what is reported in the literature.[46,47]

The multivariate logistic regression analysis confirmed that the presence of both bacterial and fungal infections was an independent factor associated with a higher risk for ICU mortality (OR = 2.5, $p < 0.0001$), together with age, to have a higher SAPS score and being subjected to invasive mechanical ventilation, while belonging to the female gender was a protective factor (OR = 0.6, $p = 0.02$).

The results of this study needs to be taken considering many limitations. In first instance it was conducted within a single-center so the findings might not be generalized: the frequency and microbiologic epidemiology may be different according to different geographical areas. Then the number of real cases could have been underestimated considering the complexity of differentiating viral, bacterial, and fungal pulmonary superinfections. Finally, the difficulty to distinguish between infection and colonization could have led to their overestimation.

In conclusion, our study highlights that bacterial and/or fungal secondary infections among ICU patients hospitalized with COVID-19 constitute a frequent complication and play a critical role in the worst prognosis of this category of patients, favoring the progression to severe disease and to higher mortality rate.

Taking into account the previous considerations, clinicians should estimate the risk of bacterial and fungal superinfections by combining clinical criteria, clinical course of the disease and results from microbiological assays and radiological images.

Thus, a rapid diagnosis of superinfections is compulsory because it might allow clinicians to choose a targeted therapy, which may improve the overall survival.

We found that *P. aeruginosa*, *K. pneumoniae*, *E. coli*, and *S. aureus* were frequent bacteria which cause ICU-acquired superinfections.

Our findings are important to settle the role of empiric antimicrobial therapy or stewardship strategies in patients affected by COVID-19 that were admitted to ICU and even more for those who are submitted to mechanical ventilation.

Furthermore, future studies focusing on the prevalence and etiology of superinfections in COVID-19 patients might constitute the baseline, together with this work, for recommendations of tailored empirical therapy according to the expected pathogens identified in the local settings and according to antibiotic stewardship protocols.

AUTHOR CONTRIBUTIONS

Maria Antonia De Francesco conceived and designed the study. Maria Antonia De Francesco analyzed the data and wrote the first draft of the manuscript. Silvia Corbellini, Giorgio Piccinelli, Francesca Simonetti, Valentina Carta, Lucia Mangeri, and Michela Padovani performed and analyzed microbiological tests, collected data and participated in manuscript revision. Simone Pellizzeri analyzed the data and performed statistical analyses. Simone Piva was directly involved in the patient care, collected data, analyzed the data, performed statistical analyses, and participated in manuscript revision. Daniela Vecchiati, Nicola Latronico, Liana Signorini, Benedetta Fumarola, and Francesco Castelli were directly involved in the patient care and participated in manuscript revision. Arnaldo Caruso provided study oversight and participated in manuscript revision. All the Authors approved manuscript submission.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ETHICS STATEMENT

The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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