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Secondary bacterial and fungal infections in critically ill COVID-19 patients: Impact on antimicrobial resistance

Eman E. Hegazy^{*1}, Ibrahim.S. Ibrahim², Marwa Gamal Bahey¹

1-Department of Microbiology and Immunology, Faculty of Medicine, Tanta University.

2 Chest Department, Faculty of Medicine, Tanta University.

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Background: The primary burden among severely ill COVID-19 cases allocated to ICUs is secondary bacterial and fungal infections. Antimicrobial resistance is aggravated more likely by empiric overusing of antimicrobials. This study aimed to assess the microbiological profile of fungal and bacterial superinfections in laboratory confirmed COVID-19 cases and their antimicrobial susceptibility pattern. **Methods:** Various clinical samples were obtained from 117 critically ill COVID-19 patients in the clinical suspicion of secondary infections for assessing the pathogens accountable for the superinfections and their antimicrobial susceptibility pattern according to standard microbiological procedures. **Results:** Among 117 COVID-19 patients allocated to ICU, 68 (58%) had secondary infections. The most prevalent infection was of the lower respiratory tract. Most infections were bacterial 85.8%. Gram-negative isolates were the most predominant strains, accounting for 71.7%. among them, *Klebsiella pneumoniae* 43.4 % and *Acinetobacter baumannii* 20.7% were the most predominant. Majority of the bacterial strains were multidrug-resistant, all gram-negative strains showed one hundred percent resistance rate to cephalosporins, amoxicillin, and amoxicillin-clavulanic. The lowest resistance was observed for tigecycline. All gram-positive strains were susceptible to linezolid and vancomycin. Additionally, all candida isolates were susceptible to the tested antifungals. **Conclusions:** In hospitalized severely ill COVID-19 patients, secondary infections are most frequently caused by Gram-negative pathogens exhibiting high rate of antibiotic resistance and are associated with poor outcomes. Strict adherence to infection control measures as well as regular microbiological surveillance are required.

Introduction

Globally, the novel corona virus disease (COVID-19) outbreak brought on by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) which are positive-sense enveloped RNA viruses, having spike-like projections on their surface, introducing them a crown's appearance [1,2].

Age, immune system health, and the patient's underlying condition all affect the disease's symptoms, which can range from being asymptomatic to causing acute respiratory failure

that necessitates admission to an intensive care unit (ICU) with an extreme mortality rate [3,4]. Critically ill COVID-19 patients require prolonged hospitalization especially in ICUs which predisposes them to unfavorable outcomes such as secondary infections (superinfections) [5].

Bacterial and fungal super-infections are frequent issues of viral infections making diagnosis, treatment, and prognosis more difficult with poor outcome in COVID-19 patients [6]. Super-infection

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* Corresponding author: Eman Elsayed Ali Hegazy

E-mail address: eman.hegazi@med.tanta.edu.eg.

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can be associated with virus-induced respiratory system damage, a reduction in muco-ciliary clearance, and immune system damage [7]. The virus has the capacity of deteriorating lymphocytes, particularly T cells, B cells, and Natural Killer cells, causing the immune system to deteriorate throughout the disease, so the main reason for super-infection is a reduction in the host's immune function and lymphocytes [8].

SARS-CoV-2 can also indirectly cause an abnormal cytokine storm, which is characterized by an elevation in serum concentrations of several pro-inflammatory cytokines in COVID-19 cases particularly in those with serious disease [9]. Immuno-suppressants such as dexamethasone and tocilizumab have been prescribed to reverse COVID-19 cytokine storm, that contribute to high risk of secondary fungal and bacterial infections [10,11].

Overlapping between clinical characteristics, radiological results and laboratory factors make distinguishing intense COVID-19 infection from secondary bacterial or fungal infection difficult so, clinicians often use broad spectrum antibiotics [12,13]. This elevates issues about the antimicrobial resistance (AMR) emergence [14].

Currently, World Health Organization does not suggest routine antibiotic medication in COVID-19 even if the disease is moderate with no clinical suspicion of bacterial infection [15]. So, the use of antibiotics should be guided by culture-based approaches to limit antimicrobial resistance and treatment costs [16].

Few studies were published in Egypt on fungal and bacterial super-infections' incidence in COVID-19 cases with the susceptibility patterns of the causative pathogens. Therefore, the aim of this study was assessment of the microbiological profile of bacterial and fungal super-infections in laboratory affirmed COVID-19 patients admitted to the isolation ICUs of Tanta University hospital and El-menshawey general Hospital and evaluation their antimicrobial susceptibility pattern in order to guide appropriate empirical antimicrobial use and reduce unnecessary antibiotic prescriptions.

Materials and methods

Study design and Study subjects

This cross-sectional study conducted on 117 COVID-19 patients admitted to isolation ICUs of Tanta University hospital and El-menshawey general Hospital as tertiary hospitals between October 2021 and May 2022. All research subjects provided their documented informed consent. Each specimen was assigned a code number in order to assure patient privacy and data confidentiality. The Tanta University Faculty of Medicine's Research Ethics Committee gave the study protocol their seal of approval (approval code: 35344/3/22). The Declaration of Helsinki's guidelines were followed in conducting the study. This study included adult patients who were admitted to the ICU for at least 48 hours and were found to be SARS-CoV-2 positive by Real-Time Polymerase Chain Reaction (RT-PCR). The study excluded patients who were admitted for less than 48 hours and children. Complete blood counts, coagulation profiles, d-dimer, ferritin levels, and serum inflammatory markers like Interleukin-6 (IL6), C-reactive protein (CRP), and procalcitonin (PCT) were all checked on all patients

Data collection

Demographic data including age, sex, underlying comorbidities, smoking status, length of ICU stay, previous antibiotic treatment, anti-inflammatory treatments, clinical and outcome data were reviewed from patients' medical records.

Detection of secondary infections and Microbiological Examination

Secondary infections, also known as superinfections, were caused by bacterial/ and or fungal infections that developed during ICU stays, and the signs and symptoms appeared more than 48 hours after admission, implying that they were not existing at the time of COVID-19 presentation. [17]. On clinical suspicion of a secondary infection in cases who were admitted to isolation hospitals, three various clinical specimens from each COVID-19 patient were obtained in the microbiology laboratory. The existence of the following diagnostic requirements, which had to be conveyed 48 hours or more after admission, was used to make the diagnosis of secondary fungal and/or bacterial infections. Purulent sputum, a long-lasting fever (above 38.3 C), hemodynamic instability, chest radiological pattern deterioration and elevated inflammatory markers such as pro-calcitonin (PCT),

white blood cell count, and/or CRP, to exclude community-acquired infections. The protocol specified that microbiological specimens such as blood, respiratory samples such as sputum or endotracheal aspirates, and urine specimens were collected if there was a suspicion of bacterial superinfection. Empirical antibiotic treatment was then started right away in line with local epidemiological data. The adequate antibiotic regimen was then modified according to culture's findings.

Samples collection and processing

Throughout the course of the study, 351 clinical specimens—involving blood, urine, and respiratory specimens collected. Utilizing the suggested personal protective equipment and strict adherence to the Centers for Disease Control and Prevention's (CDC) guidelines was applied during specimens processing. The collected samples were quickly transferred to the microbiology laboratory, where pathogen identification was conducted in accordance with accepted microbiological practices [18]. According to the rules for managing biomedical waste, all samples were disposed.

Processing of blood sample

Ten milliliters of venous blood were aseptically drawn and inoculated into blood culture bottles. The inoculated broth was kept in an aerobic environment at 37°C overnight and monitored for indications of microbial growth. Gram staining, sub-culturing onto MacConkey agar, blood agar plate, and chocolate agar (Oxoid UK), and aerobic incubation at 37°C for 24 hours. After 7 days had passed with no visible growths, the blood culture bottles were declared to be negative [18]. Skin microbiota-positive specimens were not included in the research.

Urine sample

Freshly voided midstream urine samples were inoculated into Cysteine Lactose Electrolyte Deficient Medium (Oxoid UK) using 0.001mL calibrated loop. At 37°C, for 24 hours. Significant bacteriuria was considered and further processed from catheterized and non-catheterized individuals that yield growth $\geq 10^2$ CFU/mL and $\geq 10^5$ CFU/mL, respectively [18].

Respiratory samples

The patients were taught to wash their mouths with water before having approximately 2mL of purulent sputum in a sterile wide-mouth container. To determine whether the sputum was suitable for culture, it was smeared and examined, specimens that had more than 25 polymorphonuclear

leukocytes and fewer than 10 epithelial cells and endotracheal aspirate were inoculated on blood, MacConkey, and chocolate agar (Oxoid UK) and the incubation period was 24 hours at 37°C [18].

Identification of bacterial isolates

Gram stain as well as standard biochemical tests, (eg, catalase, coagulase, oxidase, mannitol salt agar, DNase, bile esculin, triple sugar iron agar, citrate, urease, etc.) were performed for pathogen identification [18]. Moreover, species identification was confirmed by automated Vitek 2 Compact system (bioMerieux, France), regarding to manufacturer's instructions.

Identification of fungal isolates

Direct smears of lactophenol-cotton blue were investigated under the microscope. After that, the specimens were grown on Sabouraud dextrose agar (Oxoid UK) in the presence of chloramphenicol and with or without cycloheximide. The plates were incubated at room temperature and 37 °C [18]. *Candida* species were distinguished using automated Vitek 2 Compact (bioMerieux, France), according to manufacturer's instructions.

Antimicrobial susceptibility testing

The antibiotic susceptibility test was performed for all bacterial strains utilizing the standardized Kirby–Bauer disk diffusion procedure, in consistent with the Clinical & Laboratory Standards Institute. The utilized antibiotics (Oxoid UK) were cefepime (30 µg), ceftriaxone (30 µg), ceftazidime (30 µg), cefoxitin (30 µg), amikacin (30 µg), meropenem (10 µg), imipenem (10 µg), gentamicin (10 µg), tetracycline (30 µg), azithromycin (15 µg), levofloxacin (5 µg), ciprofloxacin (5 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg), penicillin (10 U), erythromycin (15 µg), linezolid (30 µg), piperacillin/tazobactam (100/10 µg), and vancomycin (µg). Antifungal susceptibility test was conducted by automated Vitek 2 Compact (bioMerieux, France) utilizing AST-YST cards. Antimicrobial breakpoints were interpreted as per CLSI 2021[19].

Statistics

The present study was statistically presented and analyzed using the mean, standard deviation, unpaired student t-test for comparisons between two groups of quantitative data, and chi-square test for comparisons between groups of qualitative data (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.). Significant

level: >0.05 Non significant <0.05* significant <0.001* High significant

Regarding to the study outcome, a contrast between COVID-19 patients who established secondary infections and those who did not is demonstrated in **table (1)**. Among 117 patients enrolled in this study, 68 (58 %) patients had secondary infections either blood stream, respiratory or urinary tract infections. In addition, contrasted to COVID-19 without super-infections, patients with secondary infections had ICU stays that were significantly longer. Total leucocyte count, CRP, and PCT values were all noticeably higher in patients who established super-infections. Patients with secondary infections a mortality rate of 58.8%, contrasted to 10.2% for those without super-infections. Additionally, patients with concurrent secondary infections died in hospitals at a rate that was considerably higher ($p<0.001$) than that of patients without.

As demonstrated in **table (2)**, 351 clinical samples in total were gathered for microbiological culture, 254 (72.4%) of those specimens revealed no growth. While, 97 samples (27.6%) tested positive for bacterial or fungal growth. In terms of COVID-19 patients who were hospitalized, 68 out of 117 (58%) experienced episodes of super-infections. 90.7 % of cases had mono-microbial infections while 9.3 % had poly-microbial infections. Noticeably, some patients experienced more one episode of super-infection. There were 97 episodes in overall.

Regarding the secondary infection's etiology, an overall of 106 pathogens were isolated from all cultures. The etiology of the secondary infection, is reported in **table (3)**. 46.2% isolated from respiratory sample culture, 28.3% isolated from blood culture, and 25.5% isolated from urine culture. Bacterial infection represents 85.8% (91/106) and fungal infection accounting for 14.2%. Gram-negative bacteria were the most frequent isolate, responsible for 71.7%. The most frequent

Results

isolates were *Klebsiella pneumoniae* (*K. pneumoniae*) 43.4 % and *Acinetobacter baumannii* (*A. baumannii*) 20.7%. Gram positive isolate and fungi responsible for 14.2 for each. *Staphylococcus aureus* (*S. aureus*) and *candida albicans* with percentage 11.3% and 6.6% consecutively. Blood stream infections, lower respiratory tract infections, and urinary tract infections all had different distributions of the bacteria that caused the secondary infections. Among the microorganisms cultured on blood cultures, *k. pneumoniae* with a percentage of 40%, *A. baumannii* 36.7% followed by *S. aureus* 10% were the most prominent. In respiratory tract cultures, *K. pneumoniae* consists of the majority with a percentage of 38.8%, accompanied by candida 20.4% and *A. baumannii* with a percentage of 18.4%. In urine culture, significant growth was 25.5% with *E. coli* and *S. aureus* consists of the majority with a percentage of 22.2% each, accompanied by *Proteus mirabilis* with a percentage of 14.8%

As per the antibiogram's findings, as shown in **tables (4,5)** most bacterial isolates were multidrug-resistant. All gram-negative isolates showed one hundred percentage resistance rate to cephalosporins, amoxicillin, and amoxicillin clavulanic. Resistance to meropenem was present in 81.8 % of *K. pneumoniae* isolates and 80 % of *A. baumannii* isolates, respectively. Meropenem resistance was present in 87.5 % of isolated strains of *Pseudomonas aeruginosa*. The drugs tigecycline and amikacin showed the least resistance. In the meantime, vancomycin and linezolid were effective against all Gram-positive isolates.

Regarding the antifungal susceptibility testing, all candida isolates were susceptible to the tested antifungals (Amphotericin B, micafungin, flucytosine, fluconazole, voriconazole, and caspofungin).

Table 1. Demographic and clinical characteristics of hospitalized COVID-19 patients with and without secondary infections.

	COVID-19 With secondary infection (N=68)	COVID-19 Without secondary infection (N=49)	Tests	
			t/X ²	P-value
Age (years) Mean ± SD	53.50±10.24	36.33±14.59	7.487	<0.001*
Gender				
Female	32(47.1%)	15(30.6%)	3.205	0.073
Male	36(52.9%)	34(69.4%)		
Comorbidity				
No comorbidity	0(0.0%)	16(32.7%)	54.842	<0.001*
Asthma	2(2.9%)	9(18.4%)		
Diabetes mellitus	23(33.8%)	4(8.2%)		
Hypertension	2(2.9%)	8(16.3%)		
Liver disease	17(25.0%)	9(18.4%)		
COPD	10(14.7%)	1(2.0%)		
Ischemic heart disease	11(16.2%)	2(4.1%)		
Renal disease	3(4.4%)	0(0.0%)		
Length of ICU stay (days)	9.56±3.27	2.80±1.91	12.968	<0.001*
Inflammatory markers				
WBC (10³ /µl)	11.44±2.47	6.70±1.23	12.374	<0.001*
CRP (mg/L)	127.47±55.80	71.35±28.85	6.442	<0.001*
PCT (ng/ml)	0.88±0.29	0.49±0.17	8.302	<0.001*
Outcome				
Died	40(58.8%)	5(10.2%)	28.442	<0.001*
Survived	28(41.2%)	44(89.8%)		

Table 2. Microbial culture results of the collected samples from COVID-19 patients with suspected secondary infection.

Total number of samples from COVID patients with suspected infection (N=351)	
No growth	N=254 (72.4%)
Positive growth	N= 97 (27.6%)
Monomicrobial	Polymicrobial
N= 88 (90.7%)	N=9 (9.3%)
	<i>Candida & Staph aureus</i> N= 2
	<i>Candida & E. coli</i> N =1
	<i>Candida & Klebsiella pneumonia</i> N = 2
	<i>Pseudomonas & Klebsiella pneumonia</i> N =2
	<i>Acinetobacter baumannii & Staph aureus</i> N =2

Table 3. Distribution of isolated pathogens according to site of specimen .

	Blood	Respiratory	Urine	Total
Gram negative	25 (83.3%)	36(73.5%)	15(55.6%)	76(71.7%)
<i>Klebsiella pneumoniae</i>	12(40%)	19(38.8%)	2(7.4%)	33(43.4%)
<i>Acinetobacter baumannii</i>	11(36.7%)	9(18.4%)	2(7.4%)	22(20.7%)
<i>E. coli</i>	1(3.3%)	1(2%)	6(22.2%)	8(7.5%)
<i>Proteus mirabilis</i>	0(0%)	1(2%)	4(14.8%)	5(4.7%)
<i>Pseudomonas aeruginosa</i>	1 (3.3%)	6 (12.2%)	1(3.7%)	8(7.5%)
Gram positive	4(13.3%)	3(6.1%)	8(29.6%)	15(14.2%)
<i>Staphylococcus aureus</i>	3(10%)	3(6.1%)	6(22.2%)	12(11.3%)
Enterococci	1(3.3%)	0 (0%)	2(7.4%)	3(2.8%)
Fungi	1(3.3%)	10 (20.4%)	4(14.8%)	15(14.2%)
<i>Candida albicans</i>	0	4(8.2%)	3(11.1%)	7(6.6%)
<i>Candida tropicalis</i>	0	3(6.1%)	1(3.7%)	4(3.8%)
<i>C. glabrata</i>	0	2(4.1%)	0(0)	2(1.9%)
<i>C. parapsilosis</i>	1(3.3%)	1(2%)	0(0)	2(1.9%)
Total n %	30(28.3%)	49 (46.2%)	27 (25.5%)	106 (100%)

Table 4. Antimicrobial resistance pattern of Gram-negative isolates.

Antimicrobials	<i>K. pneumoniae</i> N=33 (%)	<i>A.baumannii</i> N= 22(%)	<i>P.aeruginosa</i> N=8 (%)	<i>E coli</i> N=8(%)	<i>P.mirabilis</i> N=5 (%)
Amikacin	17(51.5)	5 (25)	4 (50)	2 (25)	0(0)
Gentamicin	20(64.5)	10 (50)	3 (37.5)	4 (50)	2 (40)
Amoxicillin	3(100)	22 (100)	8 (100)	8(100)	5(100)
Amoxicillin/clavulanic acid	33(100)	22(100)	8 (100)	8(100)	5 (100)
Ciprofloxacin	16(48.5)	20(100)	7 (87.5)	8 (100)	5(100)
Levofloxacin	12(36.4)	16 (80)	6 (75)	5 (50)	2 (40)
Cefepime	33(100)	22(100)	8 (100)	8(100)	5(100)
Ceftriaxone	33(100)	22(100)	8(100)	8(100)	5(100)
Cefoxitin	33(100)	22(100)	8(100)	8(100)	5(100)
Ceftazidime	33 (100)	22(100)	8(100)	8 (100)	5(100)
Meropenem	26(81.8)	16 (80)	7 (87.5)	5 (62.5)	3 (60)
Trimethoprim/Sulphamethoxazole	30 (91)	18 (90)	6(75)	5(62.5)	5 (100)
Tigecycline	4(12.1)	5 (22.7)	1 (12.5)	0 (0)	0 (0)
Aztreonam	22 (66.7)	18 (82)	6 (75)	5 (62.5)	1 (20)
Piperacillin +Tazobactam	30(91)	19 (86.4)	6 (75)	2 (25)	0(0)

Table 5. Antimicrobial resistance pattern of Gram-positive strains.

Antimicrobials	<i>Staphylococcus aureus</i> (N=12)	Enterococci (N=3)
Penicillin G	12 (100)	3(100)
Ciprofloxacin	10 (83.3)	0 (0)
Levofloxacin	8 (66.7)	0(0)
Gentamicin	7 (58.3)	3 (100)
Erythromycin	12 (100)	1 (33.3)
Clindamycin	5(41.7)	0 (0)
Cefoxitin	12 (100)	3 (100)
Ceftriaxone	12 (100)	3 (100)
Tetracycline	3 (25)	0 (0)
Teicoplanin	0(0)	0 (0)
Linezolid	2(16.7)	0(0)
Vancomycin	0(0)	0(0)

Discussion

There is currently little information available on secondary infections in severely ill COVID-19. Admission to ICU raises the risk of health-care-associated infections and the multidrug-resistant organisms' spread [20]. The objective of this study was assessment of the microbiology of fungal and bacterial super-infections in laboratory confirmed COVID-19 individuals, and their antimicrobial susceptibility pattern.

In the present study, we found that sixty-eight (58%) of severely ill COVID-19 cases had secondary infections that demonstrating a high incidence of nosocomial infections among COVID-19 individuals, this was more or less similar to that reported by **Alqahtani et al.** [21]. As per the recent studies, secondary infections were detected in 40.7% and 50% of COVID-19 patients respectively [22,23].

Moreover, Higher rate of secondary infections (87.9%) was reported by **Florida et al.** involving 138 of 157 patients enrolled in the study [24]. In **Sharifipour et al.** study, all COVID-19 patients in the ICU100% were positive for bacterial infections [25]. The high infection rate that was detected in the previous studies may be attributed to the utilization of immune-modulators, such as anti-IL-6 (tocilizumab) which, increased the risk of developing super-infections [10].

In contrary to our result, lower rate of secondary bacterial infections (11.9%), (13%), and (21.9%) respectively were reported in several studies [5,26, 27].

Regarding the patient outcome, in the current study, the length of ICU stay in cases with secondary infection was significantly longer than in patients without super-infection ($p = 0.0001$). Our outcomes are in line with numerous studies that reported that secondary infection extend the ICU stay's length [21,28,29].

In the present study, patients with secondary infections had a mortality rate of 58.8%, contrasted to patients without super-infections, who had a mortality rate of 10.2%. Inpatient mortality was significantly greater among patients with concurrent secondary infection ($p < 0.001$) than it was among those without. Our findings were in concordance with several studies [21,26,30].

In line with earlier studies [31,32], we found that males were more frequently infected than females in the present study. Sex hormones, which are essential for both innate and adaptive immunity, may explain why females typically have stronger immune systems than males and are less susceptible to viral infections [33].

In the current study, an overall of 351 clinical specimens gathered for microbiological culture, of which 72.4% specimens were culture-negative. 27.6% specimens were positive for fungal

or bacterial growth. **Vijay et al.** reported that, 11.89 % of the specimens gathered for microbiological culture were positive for bacterial or fungal infection [30]. In contrast, **Khurana et al.** noted that 40% the samples which were received for microbiological culture revealed no growth while 60% were culture positive [5].

In the current study, bacterial infection represents 85.8% and fungal infection accounting for 14.2% which were inconsistent with **Pourajam et al.** who noted that out of a total collected specimens 19.2% and 1.6% specimens were positive for bacterial and fungal growth, consecutively [26]. Furthermore, **Lansbury et al.** demonstrated that COVID-19 cases had a low rate of fungal infections, which is in line with our findings [34].

In the existing study, regarding to the site of SBIs, the most popular sites of infection in COVID-19 cases were respiratory sites which was similar to the other studies [24,26,35]. Moreover, **Nori et al.** noted that 60% of SARS-CoV2 individuals had positive respiratory cultures, (54%) had positive blood cultures [36]. In contrast to our study, **Puzniak et al.** noted that for SARS-CoV-2-positive patients, respiratory specimens were the most popular specimen generating microbial growth, accompanied by urine [37].

In the present study, according to the etiology of the secondary infections, Gram-negative bacteria were the most frequent isolate, accounting for 71.7% which were in line with a prior study in Egypt [32]. Moreover, our findings are in concordance with a several studies that demonstrated that gram-negative bacteria were the most popular organisms derived from COVID-19 patients with bacterial super-infections [21,27,30,35].

In our study, *K. pneumoniae* and *A. baumannii* were the SBIs' predominant pathogens in COVID-19 patients, our findings were in accordance with several studies [21,26,35]. In contrary to our findings, **Mahmoudi** reported that *Klebsiella* species and methicillin-sensitive *Staphylococcus aureus* were the most predominant bacteria isolated from COVID-19 with SBIs [38]. Moreover, **Florida et al.** reported that enterococci were the most frequently recovered pathogens in blood stream infections, followed by enterobacterales, and *A. baumannii* [24].

Numerous factors can be used to explain the various results. The existence of bacterial/fungal infections in cases with coronavirus infection

appears to be significantly influenced by antimicrobial policy, geography, surveillance for healthcare-correlated infections, and antimicrobial resistance [34].

The strict infection control measures' application is particularly important as most of the identified pathogens are significant etiologies of nosocomial infections in hospital settings [32]. The prevalence of Gram-negative pathogens may be owing to invasive device-associated infections through hospitalization in these patients [30]. This can also be associated with the use of azithromycin in the COVID-19 treatment regimen, which is mainly effective against Gram-positive bacteria [39]. A growing problem that has an impact on global health is antimicrobial resistance, which results from the antibiotics' misuse or overuse as well as alteration in antimicrobial use throughout pandemics. Antibiotic resistance may soon rise as a result of the broad-spectrum empirical antimicrobials' prescription to COVID-19 patients. To justify the use of antibiotics, strict antimicrobial stewardship programs are therefore necessary [26].

In our study, the majority of the isolated bacteria had a multidrug-resistant (MDR) pattern. Consequently, in SARS-CoV-2 cases where secondary bacterial infections are present, antibiotics must be administered based on antimicrobial sensitivity reports. Furthermore, these results emphasize the strict adherence to infection control measures' importance and the role of antimicrobial stewardship throughout a pandemic to decrease the complications, mortality, and nosocomial spread of these MDR organisms [40,41].

According to current study, carbapenem resistant *K. pneumoniae* and carbapenem resistant *A. baumannii* were the predominant in ICUs. This observation was consistent to the results reported by **Sharifipour et al.** [42]. The bacterial isolates from COVID-19 cases with secondary bacterial infections were found to be highly resistant to frequent antibiotics, according to **Mahmoudi** But amikacin was effective against all strains. Strains of *S. aureus* were vancomycin-susceptible [38].

In the current study, all candida species were susceptible to the antifungals tested. This finding was in agreement with **Kordylewski et al.** who reported that Micafungin and azole drugs were effective against all *Candida* species isolates [43]. Moreover, **Salehi et al.** found that the majority of the *Candida* isolates were susceptible to all tested

antifungal drugs [44]. However, **Khalil et al.** reported that candida spp. isolates were highly susceptible to amphotericin and nystatin. Fluconazole, on the other hand, demonstrated decreased activity against a variety of candida strains [45].

All study subjects obtained two or more empiric antibiotic therapies as soon as they were admitted to the intensive care unit, the majority of which were broad-spectrum antibiotics like levofloxacin, azithromycin, ceftriaxone, clarithromycin, imipenem, meropenem, and linezolid, which can prevent the growth of non-MDR pathogens in culture specimens. In cytokine storm, Anti-inflammatory and Immune-modulators were prescribed.

Conclusion

Bacterial infections are common and are correlated with poor findings in seriously ill COVID-19 cases. secondary infections are most frequently caused by Gram-negative bacterial pathogens exhibiting high rate of antibiotic resistance and are associated with poor outcomes. Our results emphasize the importance of microbiological surveillance on a regular basis as well as strict infection control measures are required.

Limitations

In the treatment of COVID-19, steroids and other immune-modulatory agents such as tocilizumab are prescribed, which may predispose to secondary infections. No control to compare between the incidence of infections in other ICUs in comparison with COVID-19 ICUs. which is required to truly validate differences in antimicrobial resistance and clinical findings. Despite these drawbacks, we described the pathogens that cause these secondary infections and their antimicrobial profiles, which highlights the urgent need to rationalize antibiotic prescriptions.

Conflict of interest

We declare that we have no conflict of interest.

Financial disclosures: nothing to declare.

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