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ICU COVID-19 patients with bacterial and fungal super-infections in Saudi Arabia

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Bacterial and fungal infections in COVID-19 patients were reported all over the world. A retrospective study was conducted to determine the prevalence of bacterial and fungal superinfections in COVID-19 positive patients between April 2020 and December 2021. This research included patients admitted in a specialized medical center in Saudi Arabia. A total of 2643 patients with confirmed diagnosis of COVID-19 via reverse transcriptase-polymerase chain reaction assay (RT-PCR) conducted on nasopharyngeal swab and chest computed tomography. The age forty-nine years (SD 12·5) 2087 were men (79%). Patients with positive cultures for bacteria or fungi were included in the study, and patients with negative cultures were excluded. Total number of COVID-19 patients who acquired bacterial infection was 509 (19%) and two hundred forty-six (246) of COVID-19 patients were admitted to ICU and were under mechanical ventilation (9.3%). All mechanically ventilated cases developed bacterial ventilator associated pneumonia. Multidrug resistant organisms represent 97%. Extended spectrum b-lactamase comprise 35.1%, Methicillin resistant *Staph aureus* 32.2%, vancomycin resistant Enterococci 2.1%. Clostridium difficile was 1.7%. 46 patients under mechanical ventilation acquired fungal superinfection (18.6%) with long duration of mechanical ventilation more than 14 days (p value=0.019) and under dexamethasone treatment (p value=0.027). Candida is the most common type of fungi isolated (89%). *Candida auris* isolated from three cases (6.5%), one case developed invasive mucormycosis (2%) one acquired invasive Aspergillosis (2%). 5 patients died (11%). This research tries to provide factual evidence of types of bacterial and fungal superinfection in COVID-19 cases and implement prevention and control measures of infection.

Key words: Bacteria, fungal, COVID-19, infections, PCR.

INTRODUCTION

Viral pneumonia frequently leads to bacterial and fungal infections, particularly in critically unwell individuals. They increase mortality and the requirement for intensive care. There are many ways in which human fungi illnesses are fundamentally different from other infections. Fungi are eukaryotic pathogens that resemble their host cells, which hinder the synthesis of antifungal substances (Brachman, 2003). There are more immunosuppressed patients, and many of them are quite vulnerable to fungus infections. The so-called systemic mycoses, which are fungal invasive infections, have a significant negative impact on human health. Also emphasized by the Global

Action Fund for Fungal Infections (GAFFI) is the catastrophic effect that focused fungal illnesses have on people, many of whom have healthy immune systems (GAFFI, 2018). Invasive microbial co-infections can occur during hospitalization in COVID-19 patients who have been hospitalized to intensive care units (ICU), especially for a lengthy time. These co-infections have the potential to worsen patient outcomes (Arastehfar et al., 2021). The multi-resistant pathogen, *Candida auris*, which causes infections in immunocompromised people that are resistant to all main classes of antifungal medications, has just been identified as a serious global danger to human health (Clancy and Nguyen, 2017). Invasive fungal infections are a common disease in the fields of critical care and pulmonary medicine, and they commonly present diagnostic difficulties. Among the most typical are candidemia and invasive aspergillosis. Galactomannan (GM) and polymerase chain reaction (PCR) testing in both blood and bronchoalveolar lavage (BAL), as well as other laboratory methods for Aspergillus detection, have grown in importance in recent years for the diagnosis of this infection (Ramanan et al., 2017). An infection that is becoming more prevalent in the critical care setting and one that increases mortality and resource utilization in intensive care units (ICUs) is invasive candidiasis (IC), which most frequently manifests as candidemia (Magill et al., 2014). Only invasive sample or culture can provide a conclusive diagnosis of IC, but relying on these conventional techniques runs the risk of failing to identify this potentially fatal illness in a timely manner. Imaging and laboratory tests are used to detect invasive fungal infections (IFD). Smears, cultures, serological testing, and the G test are a few other tests. These tests have some restrictions even though they can detect IFD with accuracy. While fungal smears only take a few minutes but have significant false-negative rates, fungal cultures take two days. Additionally, serological testing, especially if just one test is used, cannot differentiate between present illnesses and previous infections. The antifungal antibody or antigen is also influenced by an individual's level of immunity. Although sensitive, polymerase chain reaction (PCR) testing is unable to detect the survival and proliferation of the fungus (Verma et al., 2019).

In a study that looked at the prevalence of bacterial superinfection in COVID-19 patients, Catano-Correa et al., 2021 found that 49.6% of the patients had sixteen bacterial isolates superinfected them. *Staphylococcus aureus* and *Klebsiella* (pneumoniae and Oxytoca) were the most prevalent. The prognosis and course of COVID-19 patients' bacterial superinfection are related. The admittance to intensive care units, the use of antibiotics, and death are all increased by this circumstance (Cataño-Correa et al., 2021). According to the recommendations of the European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG), invasive fungal disease (IFD) was identified (Donnelly et al., 2020). The following are the EORTC/inclusion MSG's criteria for patients with IFD: Fever, cough, shortness of breath, and other respiratory symptoms with a recent history of IFD diagnosis, treatment, radiotherapy, chemotherapy, or hormone use; a positive sputum fungal culture and/or sputum fungal smear; a positive G test; and the detection of elevated levels of interleukin-6, procalcitonin, or both. IFD is identified and diagnosed using the G test. The idea behind this procedure is that the (1,3)-D-glucan in the fungal cell wall can activate the G factor to catalyze the coagulation cascade in the Limulus plasma. A dynamic turbidimeter is then used to identify the subsequent conversion of fibrinogen into fibrin. It could be challenging to find a particular antibody for this use because (1,3)-D-glucan is a sugar with variable levels of polymerization. GBPs are naturally occurring proteins that bind to glucan and can be found in people, animals, plants, and even microorganisms. Compared to other GBP, the clotting factor G alpha subunit (GFSub) from Limulus polyphemus was more easily detectable (Verma et al., 2019)

In order to better treat concurrent COVID-19 infections in individuals with risk factors, it was discovered in our studies that isolated bacterial and fungal infections were common among COVID-19 positive patients. In addition, multidrug resistant bacteria and fungi isolated among these risk groups were identified to control its spread.

Bacteriological, mycological smear examination and cultivation, galactomannan (GM) detection test in addition to VITEK® 2 bacterial and yeast identification cards (YST) machine systems 7.01 and 8.01 software were used to differentiate most clinically significant bacterial, yeasts and yeast-like isolated from the COVID-19 patients.

MATERIALS AND METHODS

According to the Saudi Arabian Ministry of Health's regulations for medical research, the study was approved by the research committee of the specialized Medical Center hospital. The patients approved the use of their clinical data for study when they signed an informed permission form before being admitted to the hospital.

Study type and subjects studied

The study, which began in April 2020 and ended in December2021,

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was retrospective in nature. It was carried out on patients in a specialist medical facility in Saudi Arabia, one of the main facilities for treating SAR-CoV-2 patients, who ranged in age from 18 to 80. Reverse transcriptase-polymerase chain reaction (RT-PCR) testing on nasopharyngeal swab samples and chest computed tomography (CT) scans with characteristic symptoms were used to confirm the diagnosis of COVID-19 in a total of (2643) patients.

All COVID-19 positive patients that were admitted to either the ICU with or without the need for mechanical ventilation, patients that isolated in an isolation ward and patients under home isolation without need for hospital admission were eligible for follow up to detect any signs for bacterial or fungal superinfection.

People over 60, diabetes mellitus, cardiovascular disease (coronary artery disease or chronic heart failure), lung disease (chronic obstructive pulmonary disease, emphysema, asthma), immunosuppression (prednisone > 20 mg/day for more than 14 days, methotrexate > 0.4 mg/kg/week, or biologic therapy); or poor prognostic factors like lymphopenia were among the patients who were admitted to the hospital (basal consolidation, nodules, cavitation or pleural effusion) (Cataño-Correa et al., 2021).

Determination of bacterial and fungal superinfection

The following three diagnostic criteria had to be present 48 h or more after admission (represent as new-onset, not beginning or before of admission) in order to confirm that it was a superinfection. This was the basis for the diagnosis of bacterial and fungal superinfection in patients admitted with COVID-19 (that is, the emerging infection during the course of illness):

1. Clinical criteria include deterioration of ventilatory parameters, purulent sputum, prolonged fever (higher than 38.3°C), hemodynamic instability requiring vasopressor support, and purulent sputum.
2. Leukopenia or leukocytosis worsening as well as an increase in procalcitonin or C-reactive protein constitute paraclinical criteria.
3. Radiological requirements: Deterioration of the chest radiological pattern or a pattern that is not COVID-19-specific (basal consolidation, nodules, cavitation, or pleural effusion). When these three diagnostic criteria were met, sputum, tracheal aspirate, bronchoalveolar lavage if applicable were collected to identify the mycological and bacterial etiological agent responsible for the possible superinfection.

The bronchoscopic method known as bronchoalveolar lavage (BAL) was utilized to collect microbiological samples from 246 COVID-19-critically ill patients' lower respiratory tracts. Procedures were carried out using pressure-controlled breathing and intravenous sedation. In every instance, disposable scopes were utilized, and just a small team was present at the patient's bedside. Before the procedure, all necessary tools and supplies, including saline, syringes, mucoactive medications, microbiological recipients, connectors, and bronchoscopy system were prepared outside the patient room (scope and screen). N95 or FPP3 masks, goggles, double gloves, and a plastic protective gown with a head and neck cover were all utilized as level III personal protective equipment. Sputum samples were collected from 263 patients with COVID-19 that were not mechanically ventilated and able to provide sputum samples. The same safety precautions were followed during the collection. The sputum cultures were evaluated as follows if two sputum cultures were collected within 48 h of each other and provide identical results with radiographic evidence of pneumonia. This culture was considered as a single positive culture infection and the isolated organism counted and specified. On the other hand, the culture was regarded as colonization if organism(s) with of a change in the appearance of the infiltrate. The distinction normal oral flora or yeast were growing without radiographic signs between the diagnoses of infection and colonization was made based on a review of concurrent notes from the attending infectious diseases consultants, pulmonary critical care specialists, and/or internists/hospitalists. Hospital antibiotic orders also assisted in making this distinction.

In this study, the galactomannan (GM) detection test and sample examination by smear and mycological culture for fungal diagnosis was used in addition to VITEK® 2 bacterial and yeast identification card (YST) machine systems 7.01 and 8.01 software to differentiate most clinically significant yeasts and yeast-like. Patients with negative bacterial or fungal cultures were not included in the study; only those with positive cultures were. Patient demographics, comorbidities, clinical characteristics, laboratory results, microbiological analysis, hospital stay, length of mechanical breathing if necessary, immunosuppressive use, line days outcome, and antifungal and antibiotic therapy were all recorded in the data collection.

Once the microbiological isolation was determined, automated sensitivity tests were carried out using the VITEK-2® system (bioMerieux 8.01), adhering to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) version M100. The isolation of a single or predominant bacterium was considered positive for bacterial culture (31edition). Patients with negative bacterial or fungal cultures were not included in the study; only those with positive cultures were. Patient demographics, comorbidities, clinical characteristics, laboratory results, microbiological analysis, hospital stay, length of mechanical breathing if necessary, immuno-suppressive use, line days outcome, and antifungal and antibiotic therapy were all recorded in the data collection.

Once the microbiological isolation was determined, automated sensitivity tests were carried out using the VITEK-2® system (bioMerieux 8.01), adhering to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) version M100. The isolation of a single or predominant bacterium was considered positive for bacterial culture (31edition) (Clinical Laboratory Standard Institute (CLSI), 2021)

To cultivate fungi, primary microbiological cultures were carried out on Columbia agar, chocolate agar, and thioglycolate broth (prepared culture media, Becton Dickinson, Sparks, MD, USA) (Oxoid Themo Fisher Scientific, Waltham, MA, USA). We subcultured Mucorales colonies on Sabouraud dextrose agar (Oxoid Thermo Fisher Scientific, Waltham, MA, USA). To distinguish between *C. albicans*, *C. tropicalis*, *C. krusei*, *C. glabrata* and Cryptococcus, chromagar Candida was used. Additionally, the most clinically relevant yeasts and yeast-like organisms were distinguished using the VITEK® 2 Yeast Identification Card (YST) machine systems 7.01 and 8.01 software (VITEK® 2 Systems 7.01 and 8.01 Software, 2018). Calcofluor or Blankophor were utilized to boost the sensitivity and specificity for recognizing *Aspergillus*-like features in direct microscopic inspection using the optical brightener methods for *Aspergillus* spp. conclusive confirmation by culture or non-culture approach, including; If the experiment was successful, Aspergillus morphological characteristics, including the distinctive acute angle branching septate hyphae of Aspergillus spp., were discovered under a microscope. Serum samples were used to carry out galactomannan (GM) detection (PlateliaTM Aspergillus Ag, Bio-Rad Laboratories, Munich, Germany) (PLATELIA™ ASPERGILLUS EIA). Agar plates with primary cultures were cultured for 48 h. *Aspergillus* spp. were subcultured on sabouraud-dextrose-agar (OxoidTM Thermo Fisher ScientificTM, Waltham, MA, United States of America) once growth of the species could be established. Before species identification, *Aspergillus* subcultures on sabouraud-dextrose-agar were cultured for 48 to 72 h for macroscopic and microscopic inspection. In accordance with Ministry of health of Saudi Arabia guidelines, all positive multidrug resistant bacteria strains and positive fungal infections in COVID -19 patients were sent for confirmation in the central regional laboratory.

Table 1. Prevalence of bacterial and fungal coinfections isolated from respiratory samples in ventilator admitted COVID-19 patients.

Item	Patients' numbers	Percentage	P value
Total number of positive COVID patient	2643		
Total number of patients with ventilator admitted ICU	246	9.3	p<0·0005
Total number of patients with ventilator associated bacterial infection	246	100	p<0·0001
Total number of patients with fungal superinfection	46	18.6	p<0·0005

Source: Authors

Statistical analysis

Both absolute (n) and relative percentage frequencies were used to characterize the variables. the overall prevalence of bacterial, fungal, and agent-specific superinfection were calculated. Using the Pearson Chi-square test (for nominal variables) or the Chi-square test for trend, if sociodemographic and clinical factors were related to bacterial and fungal superinfection (for ordinal variables) was determined. The prevalence ratios with a 95% confidence interval were calculated for the related sociodemographic and clinical parameters. A multivariate generalized linear model using the logarithm transformation and the binomial family was used to control confounding variables (log-binomial) (Thompson et al., 1998).

RESULTS

Between April 2020 and December 2021, 2643 patients with nasopharyngeal positive PCR COVID-19 cases [Mean age was 49.4 years (SD 12.5); 2087 (79%) were men) were enrolled. The total number of COVID-19 patients who acquired bacterial infection was 509 (19%). Two hundred and forty-six (246) patients were admitted to ICU and were under mechanical ventilation (9%). All mechanically ventilated patients acquired bacterial infections (100%) and forty-six of them acquired fungal superinfection (18.6%) (Table 1).

The 246 patients admitted to the ICU who were being ventilated mechanically and included in the statistical analysis had the following baseline characteristics: 194 (79%) of the patients were men, with a mean age of 62.4 years (12.5). Eighty-one percent of COVID-19 patients had diabetes (33), fifty percent had hypertension (123), and sixty-four percent had lymphopenia at baseline (157). The average time spent on mechanical ventilation was 27.1 days (19.8), and 41 out of 246 patients who had ventilator-associated pneumonia passed away in the intensive care unit (17%). A total of 46 patients (18.6%) had episodes of invasive fungal infections that were either confirmed or likely (pr/pb) (IFIs) (Table 2).

Out of the 246 cases admitted to the ICU and were under mechanical ventilation, forty-six (46) cases were superinfected with fungal infections (18.6%). All patients had episodes proven or probable (pr/pb) invasive fungal infections (IFIs). The patients were between 62 to 80 years. They were under dexamethasone treatment (p=0·027) and long duration of mechanical ventilation (>14 days; p=0·019). *Candida* other than *Candida auris* constituted the most common type of fungi isolate, forty-one (89%) out of the forty-six fungal superinfected cases were *Candida* other than *auris*. Three cases were *Candida auris* (6.5%), one case developed invasive *Mucormycosis* (2%) and one acquired invasive *Aspergillosis* (2%). The last five patients were died (11%) (Table 3).

Out of the 509 cases who acquired bacterial super infection in COVID-19 cases, two hundred and forty-six patients were admitted to the ICU and need mechanical ventilation (48%) while multidrug resistant organisms (MDRO) represent 97%. *Extended spectrum beta lactamases (ESBL)* comprise the most common organisms 35.1%, followed by *Methicillin resistant Staph aureus (MRSA)* 32.2%, then *Carbapenem resistant Enterobacteriaceae (CRE)* comprises 28.9% and *vancomycin resistant Enterococci (VRE)* 2.1%. The least was *Clostridium difficile (CDF*) was 1.7%. Using the VITEK-2® automated sensitivity system (bioMerieux 8.01) and adhering to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) version M100 (31 Edition), all MDRO and their drug susceptibilities were detected (Clinical Laboratory Standard Institute (CLSI), 2021) (Table 4).

263 patients out of the 509 COVID-19 cases that developed bacterial superinfection were not admitted to the ICU. 72% of the 263 infected cases, 190 cases had a genuine infection. The following were the main bacterial causal agents: *Staphylococcus* spp. (39.5%), *Escherichia coli* (21.1%), *Pseudomonas* pp. (18.4%), *Streptococcus* spp. (13.2%), *Klebsiella* spp. (7.9%). *MRSA* constitutes 26.7% of the isolated *Staph spp. and Extended spectrum beta lactamases E. coli were (27.5%) (*Table 5).

170 of the 246 COVID-19 patients admitted to the ICU were receiving Dexamethasone medication (69%) and 46 of them (27%) had a fungal infection. When patients were discharged from the intensive care unit, the mortality rate revealed that overall ICU mortality was significantly greater in patients with pulmonary Aspergilli and *Candida auris* linked with pr/pb COVID-19 than in those without (p 0.0001) (Table 6).

DISCUSSION

In China's clinical guidelines, a number of diagnostic and preventative strategies to address the issues in COVID-

Table 2. Demographic and clinical characteristics of patients with COVID-19 and bacterial-Fungal coinfections.

Item	Patients number	Percentage	P value
Age, years (n=246)	62.4 ±12		
Male	194	79	
Diabetes (n=246)	81	33	p<0·0001
Hypertension (n=246	123	50	p<0·0001
Chronic obstructive pulmonary disease (n=509)	34	13.8	p<0·005
Lymphopenia (n=503)	157	64	p<0·0001
Asthma (n=509)	14	5.7	p<0·005
LTC patients	51	20.7	p<0·005

LTCU (long terms care patients transfer to ICU).
Source: Authors

Table 3. Prevalence of fungal coinfections isolated from respiratory samples (BAL) in COVID-19 patients.

Item	Patients number	Percentage	P value
Total number of patient positive COVID	2643		
Total number of patients admitted to the ICU	246	9.3	
Total number of patients with ventilator admitted in ICU had fungal infection	46	18.6	
Invasive *aspergillosis*	1	2	p<0·0001
Candidemia	41	89	p<0·0001
Candida auris	3	6.5	p<0·0005
Invasive Mucormycosis	1	2	p<0·0001

Source: Authors.

Table 4. Prevalence of bacterial coinfections isolated from respiratory samples (BAL) in COVID-19 patients admitted to ICU

Item	Patients number	Percentage	P value
Total number of patient positive COVID-19	2643		
Total number of COVID-19 patients had bacterial infection	509	19	p<0·0005
Total number of patients with ventilator associated bacterial co-infection in COVID-19 ICU patients	246	9	p<0·005
MDRO	239	97	p<0·0001
ESBL	84	35.1	p<0·0005
MRSA	77	32.2	p<0·0005
CRE	69	28.9	p<0·0005
VRE	5	2.1	p<0·005
CDF	4	1.7	p<0·005

Source: Authors.

19 patients have been described. Secondary bacterial and fungal infections, however, have received less consideration, and a systematic diagnostic procedure is still lacking. In 2021, Garcia-Vidal and colleagues published a paper on their observations of co-infection and superinfection in COVID-19-positive hospitalized patients. 31/989 (3%) of the 989 COVID-19 patients admitted to a hospital in Barcelona, Spain had co-infections acquired in the community. *Streptococcus pneumoniae* and *Staphylococcus aureus* pneumonia made up the majority of these illnesses (Garcia-Vidal et al., 2021). 43/989 patients (4%) had a diagnosis of a hospital-acquired infection, with 25/44 (57%) of those cases occurring in critical care. Infections common to that 4% of patients included ventilator-associated pneumonia, hospital-acquired pneumonia, and bacteremia, with typical

Table 5. Prevalence of bacterial coinfections isolated from sputum samples in COVID-19 patients (Non ICU admitted).

Item	Patients number	Percentage	P value
Total number of patient positive COVID-19	2643		
Total number of COVID-19 patients had bacterial infection	509	19	p<0·0005
Total number of patients associated bacterial co-infection in COVID-19 non-ICU admitted patients	263	10	p<0·0005
True infection	190	72	P<0·005
Staphylococcus spp	75	39.5	p<0·0005
MRSA	20	26.7	p<0·0005
Streptococci spp.	25	13.2	P <0·005
Escherichia coli	40	21.1	P <0·005
ESBL (E. Coli)	11	27.5	P <0·005
Pseudomonas aeruginosa	35	18.4	P <0·005
Klebsiella spp.	15	7.9	P <0·005

Source: Authors.

Table 6. Patient characteristics, treatments, and secondary infections associated with death.

Item	Patients on ICU (n=246)	Percentage	P value
Duration of stay in ICU	32.7±24 days		
Duration of mechanical ventilator	27·1 days		
SOFA at admission	29	11.78	p<0·005
Dexamethasone	46	18.69	p<0·005
Death	11	4.47	p<0·005

SOFA: Sequential organ failure assessment.
Source: Authors.

nosocomial organisms predominating. These included Staphylococcus aureus, *Escherichia coli*, *Klebsiella* species and *Pseudomonas aeruginosa* (Garcia-Vidal et al., 2021). Coagulase-negative, the major of documented bloodstream infections (7/16; 44%) were caused by staphylococci. A co-infection with a fungus was found in 7/989 patients (0.7%). *Aspergillus fumigatus* tracheobronchitis was identified in three patients, while *Candida albicans* bloodstream infection, urinary tract infection, and intra-abdominal infection were identified in four patients.

There have also been reports of low observed rates of bacterial and fungal infection in COVID-19 patients from other nations, including the UK. In 2020, Hughes and colleagues found 51/836 COVID-19 patients (6%) admitted to two London hospitals had bacterial infections. In this cohort, secondary bacterial infection was rare. Sixty positive blood cultures were examined, and 39/60 (65%) were found to be contaminants, with coagulase-negative Staphylococci making up the majority of these (Hughes et al., 2020). Although the incidence of bacterial and fungal infection in COVID-19 patients is similar in both investigations, their limitations must be taken into account. A limited amount of microbiological sampling was carried out following the diagnosis of COVID-19, according to Garcia-Vidal and colleagues. According to Hughes and colleagues, a considerably higher percentage of patients (77% versus 27%) had blood cultures tested on them. In both investigations, only a small percentage of patients (13 and 25%, respectively) received respiratory sampling. A significant percentage of patients also got empirical antibiotic therapy. These variables may have a considerable impact on the rate of infection detection in hospitalized COVID-19 patients, resulting in underreporting of infection rates (Rawson et al., 2020).

The difference between this study and previous studies may be attributed to the substantial number of COVID-19 cases included (2643) and the extended duration of the study. In this study, the total number of patients with ventilator admitted ICU was 246 (9.3%). Unfortunately, all acquired bacterial superinfection and 18.6% acquired fungal superinfection. This may be attributed to the age of the cases and their comorbidities as fifty (50%) of them were hypertensive, 33% were diabetic, 64% had lymphopenia, 5.7% were asthmatic and 20.7% were coming from long term care centers. Sixty-nine percent were under Dexamethasone treatment. These findings are consistent with early Chinese reports that found secondary infections in up to 50% of COVID-19-dead patients but not in just 15% of COVID-19-survivors (Lu et al., 2020). In addition to the retrospective analysis of published research (n = 806 patients), only 8% of patients were reported to have bacterial and fungal coinfections, while >70% of patients received empiric antibiotic therapy (Rawson et al., 2020). In a similar vein, the comprehensive meta-analysis of 4,000 hospitalized COVID-19 patients, mostly from China, revealed that only 7% of patients had bacterial coinfections, rising to 14% in critically sick patients (Lansbury et al., 2020). This demonstrates that in COVID-19 cases, comorbidities and risk factors increase bacterial superinfection.

In the investigation, 64.8% of the total multidrug resistant organisms isolated from the ICU admitted patients were Gram negative organisms (ESBL and CRE), while 34% were resistant gram-positive organisms (MRSA, VRE). Out of all patients admitted to the ICU, the prevalence of fungal infections was 17% for *Candida* species other than *Candida* auris and 0.4% for *Aspergillus*. When Kubin et al. (2021) studied the incidence and antibiotic susceptibilities of bacterial and fungal infections in hospitalized patients with coronavirus, they discovered that gram-positive organisms accounted for 42% of bacteremias, followed by gram-negative organisms (35%) and fungi (22%). The majority of the organisms recovered from blood cultures (26%) belonged to the family Enterobacterales. But early in life, bacteremia brought on by *Staphylococci* and *Streptococci* was more common (Kubin et al., 2021). The slight difference in the percentage between the two studies may be attributed to the difference in the type of sample examined in this study. Sputum, tracheal aspirate, BAL if applicable was collected from the patients admitted to the Intensive care unit and were under invasive mechanical ventilation and BAL sample collection. The findings corroborated those of Alshrefy et al. (2022), who found a strong correlation between secondary bacterial infections and secondary fungal infections caused by invasive mechanical ventilation (p 0.001). Additionally, they stated that respiratory system infection had an incidence of 32.5% (n=102) and was the most prevalent secondary illness. Additionally, they stated that 102 (32.5%) patients, all of whom were mechanically ventilated in this study had secondary bacterial infections. The gram-negative organisms identified in this study were also the most often isolated bacterial pathogens, although their isolated organisms were as follows *Klebsiella pneumoniae* (n=17), *Pseudomonas aeruginosa* (n=34), and Acinetobacter baumannii (n=33) (Alshrefy et al., 2022) All patients in both studies were under steroids treatment. Both studies confirmed that antibiotic resistance is more prevalent with prolonged hospital stays and in patients under mechanical ventilation. This is in line with Nag and Cur (2021)'s findings, which claimed that COVID-19 patients with severe disease and those who need to spend a lot of time in intensive care units (ICUs) are more likely to get super-infected by nosocomial bacteria. Among COVID-19 patients, ventilator-associated pneumonia (VAP), bacteremia with sepsis, and urinary tract infections are the most often seen infection types (UTIs). Dexamethasone, a standard of care for COVID-19 patients who were critically ill, was also found to be substantially and independently related with superinfections, according to Sovic et al. (2022). Receiving dexamethasone was linked to superinfections (66% versus 32%, p0.0001) only patients receiving dexamethasone had invasive fungal infections [8/67 (12%) vs 0/88 (0%), p0.0001]. This is in comparison to this study; 170 out of 246 under dexamethasone treatment in the ICU (69%) all acquired bacterial infection (100%) and forty-six out them were superinfected with fungal infection (27%). Thus, there is an impact of dexamethasone therapy on the incidence of superinfections in hospitalized severely ill COVID-19 patients and more data studies are needed to establish the correlation (Søvik et al., 2022).

In this study, there were 263 COVID-19 patients who got bacterial superinfection and were not admitted to the ICU. But the true superinfection were190 cases (72%) that show bacterial culture with the same organism in two consecutive sputum cultures and in the presence of radiological findings of chest infection. Streptococcus spp. (13.2%), Staphylococcus spp. (39.5%), Escherichia coli (21.1%), Pseudomonas spp. (18.4%), and Klebsiella spp. (7.9%) were the predominant bacterial causal agents. 26.7% of the *Staph. aureus* isolates are MRSA. The findings are in line with a recent cohort study by Morovati et al. (2022) that discovered 72% of COVID-19 patients had positive sputum cultures. But he discovered that 63.2 and 8.8%, respectively, of secondary infections were caused by bacterial and fungal species respectively. *Streptococcus* spp. (21.5%), *Staphylococcus* spp. (16.7%), *Escherichia coli* (8.7%), *Pseudomonas* spp. (7.2%), Klebsiella spp. (4.7%), and Acinetobacter spp. (4.2%) were the main bacterial causal agents. Additionally, the two main fungal pathogens were *Aspergillus* spp. (2.1%) and *C. albicans* (6.7%) (Morovati et al., 2022).

CONCLUSION AND RECOMMENDATIONS

Superinfections with bacteria and fungi are very common in COVID-19 patients who require hospitalization; significantly among patients who have certain comorbidities, problems, a prolonged hospital stay, mechanical breathing, and steroid therapy. In order to identify priority clinical groups and improve care for these types of infections, which dramatically alter the development of cases with COVID-19 with the threat variables exposed in the population examined, it is crucial to use high indicator of dubitation and active surveillance.

Additionally, there is a constant need for prospective research to evaluate the effectiveness of specific biomarkers to exclude or include bacterial and fungal superinfection. If threat variables comparable to those established in this study are present, further research is required to determine whether early antifungal treatment or prophylactic is necessary. Additionally, more research is needed to define the timing of connected infections in order to distinguish between diseases tied to the population and infections linked to the healthcare system (HAI).

Additionally, academic groups and health authorities are urged to implement a workable, customized procedure for identifying bacterial and fungal illness in COVID-19 patients.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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