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Retrospective Assessment of Secondary Bacterial and Fungal Infections in COVID-19 Patients at a Tertiary Care Hospital in Navi Mumbai

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ABSTRACT

Introduction: Secondary bacterial and fungal infections are a significant concern in COVID-19 patients, particularly those critically ill and requiring intensive care. This retrospective study investigated the prevalence and spectrum of secondary infections among COVID-19 patients admitted to the intensive care unit (ICU) at a tertiary care hospital in Navi Mumbai. Additionally, we explored the association between secondary infections and patient comorbidities. **Methods**: We performed a single-center, retrospective cohort study of 3234 COVID-19 patients admitted to a tertiary care hospital in Navi Mumbai, India, between August 2020 and August 2021. Microbiological data from various clinical specimens, including blood, sputum, bronchoalveolar lavage (BAL) fluid, urine, and tissue cultures, were retrospectively analyzed. Patient demographics and comorbidities were extracted from medical records. We employed descriptive statistics and Pearson's Chi-square test for data analysis to identify associations between secondary infections and patient characteristics. **Results:** Among the 3234 COVID-19 patients, 195 (6.02%) presented with clinical features suggestive of secondary infections. Microbiological analysis confirmed secondary infections in 98 patients (3.03%), with a culture positivity rate of 50.3%. Among bacterial isolates, *Klebsiella pneumoniae* was the most prevalent (43.28%), followed by *Acinetobacter baumannii* (25.37%). *Aspergillus* spp. emerged as the dominant fungal pathogen. Notably, *Escherichia coli* isolation was significantly associated with various specimen types ($P < 0.001$). However, no significant correlation was found between secondary infection rates and patient comorbidities. **Conclusion:** Gram-negative bacteria, specifically *K. pneumoniae* and *A. baumannii*, were the primary pathogens responsible for secondary infections in our cohort of critically ill COVID-19 patients admitted to the ICU. These findings underscore the importance of ongoing surveillance and monitoring of secondary infection trends, including fungal pathogens, to inform and optimize management strategies in this high-risk population.

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the etiological agent of coronavirus disease 2019 (COVID-19), primarily targets the respiratory tract, resulting in a wide range of clinical presentations, from mild upper respiratory symptoms to severe pneumonia and acute respiratory distress syndrome (ARDS) [1, 2]. This viral infection has been associated with a high risk of secondary bacterial and fungal infections, particularly in critically ill patients, which can significantly impact clinical outcomes and mortality rates.

A modeling study by Jha *et al*. (2022) estimated that the cumulative COVID-19 death toll in India by June 2021 ranged from hundreds of thousands to over 4 million [3], highlighting the significant burden of the pandemic in the country. As of January 1, 2022, India had reported a cumulative total of over 35 million COVID-19 cases, with a mortality toll exceeding 530,000 deaths as of March 11, 2023 [3]. These figures highlight the profound burden of the COVID-19 pandemic on India's healthcare system and population.

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Secondary infections (SIs) of bacterial or fungal origin are a significant complication of COVID-19, particularly in patients with pneumonia, and substantially contribute to the morbidity and mortality burden of respiratory viral infections, including COVID-19 [4, 5]. Yadav *et al*. (2021) reported a secondary infection rate of 5.4% among hospitalized COVID-19 patients [4]. Retrospective studies in China have reported secondary bacterial and fungal infection rates ranging from 3% to 15% [6], with a specific study by Chen *et al*. (2020) reporting a 5% fungal isolation rate among COVID-19 patients [7]. Consistent with these findings, a study conducted in India by Bhat *et al*. (2022) revealed that secondary infections in COVID-19 patients were predominantly bacterial (91.8%), with a notable proportion of fungal infections (23.3%) [8]. These results underscore the substantial and varied burden of secondary infections among COVID-19 patients.

Considering the substantial impact of secondary fungal infections on mortality in COVID-19 patients, prompt and accurate diagnosis is essential. The diagnostic armamentarium for fungal infections encompasses a range of modalities, including histopathology, direct microscopy, culture, serological tests such as (1,3)-β-D-glucan and galactomannan assays, and molecular diagnostics like polymerase chain reaction (PCR) [9, 10]. However, in resource-constrained settings such as India, the availability and accessibility of rapid and advanced diagnostic techniques for fungal infections are limited, which may exacerbate mortality rates [6, 11].

Various risk factors have been associated with severe COVID-19 outcomes, including male sex, diabetes mellitus, and high-dose corticosteroid therapy [12]. Additionally, several factors, including hyperglycemia, iron overload, mechanical ventilation, and corticosteroid use, have been identified as potential contributors to the development of secondary infections in COVID-19 patients [13]. The elevated risk of secondary bacterial and fungal infections, combined with the limitations of rapid diagnostic capabilities, frequently leads to the initiation of empirical antimicrobial therapy in the management of COVID-19 patients [14, 15].

This retrospective study investigated the prevalence and spectrum of secondary bacterial and fungal infections among COVID-19 patients admitted to the ICU of Terna Medical College, Navi Mumbai, India, during the transition period between the first and second waves of the pandemic. Our objectives were to determine the prevalence and types of secondary infections and to examine their correlation with patient comorbidities. While previous research has explored secondary infections in COVID-19, our study uniquely focuses on this critical transition period, addressing a knowledge gap in the literature. By examining the trends in secondary infections during this timeframe, our study aims to provide valuable insights into the evolving epidemiology of COVID-19.

MATERIAL AND METHODS

Study design and setting. This retrospective cohort study was conducted at Terna Specialty Hospital and Research Centre, Nerul, Navi Mumbai, India, from August 2020 to August 2021 (a 13-month period).

Study population. This study included patients admitted to the tertiary care hospital with a microbiologically confirmed diagnosis of COVID-19, as determined by real-time polymerase chain reaction (RT-PCR) or rapid antigen testing, and patents who were clinically suspected of having secondary bacterial or fungal infections. The study analyzed data from these patients' samples.

Inclusion and exclusion criteria. Patients were included if they developed suspected secondary infections more than 48 h after hospital admission or required invasive devices (such as mechanical ventilation or central venous catheters) during their hospital stay. Conversely, patients who tested positive for COVID-19 but were managed outpatient or those with negative COVID-19 test results were excluded from the study.

Specimen collection and processing. This study analyzed various clinical specimens, including blood, sputum, endotracheal aspirate (ETA), BAL), urine, and tissue samples. Bacterial cultures were performed by inoculating specimens onto blood agar and MacConkey agar, followed by aerobic incubation at 37°C. Significant bacterial growth was defined as moderate to heavy growth, in accordance with the Indian Council of Medical Research (ICMR) guidelines [16, 17]. Bacterial identification was performed using the Vitek 2 compact system (bioMérieux, France) with Gram-negative and Gram-positive identification cards, following the manufacturer's instructions. Fungal diagnosis was based on a combination of direct microscopic examination of clinical specimens and culture on Sabouraud dextrose agar. Culture remains the gold standard for fungal identification, but invasive fungal infections require further study with histopathology [10].

Data collection. Patient medical records were reviewed to collect data on the following comorbidities: diabetes mellitus, corticosteroid use, hyperglycemia, iron overload, and mechanical ventilation. These data were extracted and recorded for analysis.

Ethical considerations. The study was performed in accordance with the ethical standards of the Institutional Ethics Committee, which approved the study (reference number: IEC-1/012, date: July 19, 2021). This retrospective study was granted a waiver of informed consent by the Ethics Committee, as it involved the analysis of existing data and did not pose any risk to patients. Permission to use the data was obtained from the hospital authorities. Patient confidentiality was ensured through the use of de-identified data. No additional biological samples were collected or analyzed during the study. Only existing data was utilized for the purposes of

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this research.

SARS-CoV-2 detection. Respiratory tract specimens, including nasopharyngeal and oropharyngeal swabs, were collected from patients suspected of having COVID-19 and were tested for SARS-CoV-2 using RT-PCR or rapid antigen testing, according to the hospital's diagnostic algorithm.

RT-PCR. Viral RNA extraction was performed using the HiPurA® Viral RNA Purification Kit (Himedia HiGenoMB), followed by amplification using the TRUPCR® SARS-CoV-2 RT qPCR kit (3B Blackbio Biotech India Ltd). This kit targets the *E* gene and *RdRp/N* gene, and a positive result was defined as the amplification of both target genes, accompanied by a detectable fluorescent signal [18, 19].

Rapid antigen testing. SARS-CoV-2 rapid antigen testing was conducted using the STANDARD Q COVID-19 Ag test kit (SD Biosensor, India), following the manufacturer's instructions. This immune chromatographic assay detects SARS-CoV-2 antigens in nasopharyngeal or oropharyngeal swab specimens, providing a rapid diagnostic result [20].

Specimen analysis

Bacterial analysis. Specimen processing adhered to standard microbiological protocols [21]. Bacterial cultures were performed by inoculating specimens onto blood agar and MacConkey agar (ER018, EOS Lab) [10, 20, 22], followed by aerobic incubation at 37°C. Growth was considered significant if moderate to heavy. Bacterial identification was accomplished using the Vitek 2 compact system (bioMérieux, France), employing Gram-negative and Gram-positive identification cards, in accordance with the manufacturer's instructions.

Fungal analysis. Fungal diagnosis was accomplished through a combination of direct microscopic examination with KOH preparation and culture on Sabouraud dextrose agar (EOS 309 Lab). The cultures were incubated at both 37°C and room temperature (25°C) to facilitate the isolation of dimorphic fungi, as recommended by the Indian Council of Medical Research [21-23]. Morphological identification was performed using lactophenol cotton blue staining [21]. Specimens exhibiting hyphal elements in KOH preparation and positive culture growth were considered indicative of fungal infection. Yeast identification was performed using the Vitek 2 system (bioMérieux, France) with yeast identification cards, in accordance with the manufacturer's instructions.

Statistical analysis. Data were entered into Microsoft Excel and subjected to descriptive statistical analysis, including calculations of frequencies and percentages, to characterize patient demographics (age categorized as <60 years and ≥60 years), comorbidities, specimen types, and the distribution of bacterial and fungal isolates. Data from cultures with no growth or mild growth, as well as those collected within 24 h of admission, were excluded from the analysis to minimize potential biases. Pearson's chi-square test was performed using SPSS software version 20.0 to assess the statistical significance of differences in the rates of bacterial and fungal infections. A P-value ≤ 0.05 was considered statistically significant, indicating a significant difference between groups.

RESULTS

Characteristics of patients with suspected secondary infections. During the study period, a total of 3234 patients were admitted with COVID-19. Of these, 195 patients (6.02%) presented with clinical features suggestive of secondary infections, prompting the collection of specimens for microbiological culture (Table 1A). Among these suspected cases, 126 (64.62%) underwent bacterial cultures and 69 (35.38%) underwent fungal cultures. Notably, microbiologically confirmed secondary infections were identified in 98 patients (3.03% of the total admissions), yielding a culture positivity rate of 50.3% among those suspected of having secondary infections.

Table 1A. Overview of culture results

Culture type	Number of specimens	Number (%) positive	Number (%) negative
Bacterial	126 (64.62%)	67 (53.18%)	59 (46.82%)
Fungal	69 (35.38 %)	31 (44.92%)	38 (55.08%)
Total	195	98 (50.3%)	97 (49.7%)

Respiratory specimens, comprising ETA and BAL fluid, predominated (119/195, 61.02%) among the samples submitted for culture. In contrast, sputum samples were the least common (12/195, 6.2%). Meanwhile, urine, blood, nasal tissue, and sinus tissue specimens collectively accounted for 39.0% (76/195) of the samples (Table 1B).

Among the 98 positive cultures, ETA specimens predominated (51/98, 52.0%), with 64.2% (43/67) yielding bacterial isolates and 25.8% (8/31) yielding fungal isolates. BAL fluid cultures were positive in 23.5% (23/98) of cases, with 19.4% (13/67) positive for bacteria and 32.3% (10/31) positive for fungi (Table 2A). Notably, Pearson's chi-square test revealed no statistically significant difference in the rate of bacterial and fungal isolation between ETA and BAL specimens (P = 0.271), indicating similar microbiological yields from both specimen types.

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Table 1B. Distribution of specimens submitted for bacterial and fungal culture

Specimen type	No. of bacterial culture (%)	No. of fungal culture (%)	Total (%)
ETA	63 (50%)	14 (20.28%)	77 (39.49%)
BAL	16 (12.71%)	14 (20.28%)	30 (15.38%)
Sputum	12 (9.52%)	0 (0.0%)	12 (6.15%)
Blood	20 (15.87%)	0 (0.0%)	20 (10.26%)
Urine	15 (11.90)	0 (0.0%)	15 (7.7%)
Nasal Tissue	0 (0.0%)	32 (46.4%)	32 (16.41%)
Sinus Tissue	0 (0.0%)	9 (13.04%)	9 (4.61%)
Total cultures	126 (64.61%)	69 (35.38%)	195

Table 2 A. Distribution of microorganisms isolated from positive cultures

Specimen type	No. of bacterial isolates (%)	No. of fungal isolates (%)	Total (%)
ETA	43 (64.18%)	8 (25.80%)	51 (52.04%)
BAL	13 (19.4%)	10 (32.26%)	23 (23.48%)
Sputum	4 (5.97%)	0 (0.0%)	4 (4.08%)
Blood	2 (2.99%)	0 (0.0%)	2 (2.04%)
Urine	5 (7.46%)	0 (0.0%)	5 (5.1%)
Nasal Tissue	0 (0.0%)	9 (29.03%)	9 (9.18%)
Sinus Tissue	0 (0.0%)	4 (12.90%)	4 (4.08%)
Total	67 (100%)	31 (100%)	98 (100%)

Bacterial isolates and their distributions. The most prevalent bacterial isolate was *K. pneumoniae* (43.3%, 29/67), with the majority (39.5%, 17/29) recovered from endotracheal aspirate (ETA) specimens. *A. baumannii* was the second most common isolate (25.4%, 17/67), also predominantly found in ETA specimens (34.9%, 15/17). *Pseudomonas aeruginosa* was isolated from 11.9% (8/67) of specimens, with 62.5% (5/8) originating from ETA. *E. coli* was isolated from 8.9% (6/67) of specimens, with 50% (3/6) from urine samples. Notably, *E. coli* was recovered from all specimen types except sputum. Less frequent isolates included *Stenotrophomonas maltophilia* (6.0%, 4/67), *Burkholderia* spp. (3.0%, 2/67), and a single case of *Nocardia* spp. (1.5%). Statistical analysis revealed a significant association between *E. coli* isolation and specimen type ($P < 0.01$) (Table 2B).

Table 2B. Results of the bacterial culture

Bacterial species	ETA no. (%)	BAL no. (%)	Blood no. (%)	Urine no. (%)	Sputum no. (%)	Total (%)	*P*-Value	Significant at 5% level
A. baumannii	15 (34.88%)	1 (7.69%)	0 (0.0%)	0 (0.0%)	1(25%)	17 (25.37%)	0.161	Not
Burkholderia spp.	1 (2.33%)	1 (7.69%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (2.98%)	0.414	Not
E. coli	1 (2.33%)	1 (7.69%)	1 (50%)	3 (60%)	0 (0.0%)	6 (8.96%)	0.000	Yes*
K. pneumoniae	17 (39.53%)	8 (61.55%)	1(50)	2 (40%)	1 (25%)	29 (43.28%)	0.624	Not
Nocardia	0 (0.0%)	1 (7.69%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (1.5 %)	1.000	Not
P. aeruginosa	5 (11.63%)	1 (7.69%)	0 (0.0%)	0 (0.0%)	2 (50%)	8 (11.94%)	0.077	Not
S. maltophilia	4 (9.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	4 (5.97%)	1.000	Not
Total	43 (100%)	13 (100%)	2 (100%)	5 (100%)	4 (100%)	67 (100%)		

*Statistically Significant at 5% level *i.e.*, $P<0.05$.Application Pearson Chi square test and Fisher Exact test.

Fungal isolates and their distributions. Fungal isolates were predominantly recovered from BAL fluid specimens (32.3%, 10/31), with *Candida albicans* being the most common species (40%, 4/10). Nasal tissue specimens were the next most frequent source of fungal isolates (29.0%, 9/31), with *Aspergillus* spp. predominating (66.7%, 6/9). All 8 ETA specimens submitted for fungal culture yielded growth, with *C. albicans* accounting for 50% (4/8) and *Mucor* spp. and *Aspergillus* spp. each accounting for 25% (2/8). Although sinus tissue specimens were the least frequent, 75% (3/4) were positive for *Aspergillus* spp. (Table 3).

Notably, despite *Aspergillus* spp. being the most frequently isolated fungus in our study, statistical analysis did not reveal a significant association between *Aspergillus* spp. isolation and COVID-19 ($P = 0.073$). Therefore, we cannot conclude that *Aspergillus* spp. is consistently the most common fungal isolate in COVID-19 patients.

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Table 3. Distribution of fungal isolates by specimen type**

Fungal species	ETA no. (%)	BAL no. (%)	Nasal tissue no. (%)	Sinus tissue no. (%)	Total (%)	*P*-Value	Significant at 5% level
Aspergillus spp.	2 (25%)	2 (20%)	6 (66.67%)	3 (75%)	13 (41.93%)	0.073	Not
C. albicans	4 (50%)	4 (40%)	0 (0.0%)	0 (0.0%)	8 (25.81%)	1.000	Not
Candida spp.	0 (0.0%)	3 (30 %)	0 (0.0%)	0 (0.0%)	3 (9.69%)	1.000	Not
Cladosporium spp.	0 (0.0%)	0 (0.0%)	1 (11.11%)	1 (25%)	2 (6.45%)	1.000	Not
Mucor spp.	2 (25%)	1 (10%)	2 (22.22%)	0 (0.0%)	5 (16.12%)	0.675	Not
Total	8 (100%)	10 (100%)	9 (100%)	4 (100%)	31 (100%)		

**Application of Pearson Chi square test and Fisher Exact test

We evaluated the presence of comorbidities that may increase the risk of secondary infections, including age, hypertension, diabetes mellitus, catheterization, mechanical ventilation, immunosuppression (defined as the use of inhaled corticosteroids), obesity, and chronic lung disease (CLD) (Table 3). Notably, all patients with confirmed secondary infections had multiple comorbidities, highlighting the complexity of their clinical profiles (Table 5).

Table 4. Comorbidities and associated factors in patients with suspected secondary infections.

Factor	Number of patients (%)
Age (years)	
<60	61 (31.3%)
≥60	134 (68.7%)
Venous catheterization	
Yes	106 (54.4%)
No	106 (54.4%)
Urinary catheterization	
Yes	143 (73.3%)
No	52 (26.7%)
Chronic lung disease	
Yes	192 (98.5%)
No	192 (98.5%)
Diabetes mellitus	
Yes	98 (50.3%)
No	98 (50.3%)
Hypertension	
Yes	93 (47.7%)
No	93 (47.7%)
Immunosuppression (inhaled corticosteroids)	
Yes	190 (97.4%)
No	190 (97.4%)
Mechanical ventilation	
Yes	128 (65.6%)
No	128 (65.6%)
Obesity	
Yes	192 (98.5%)
No	192 (98.5%)

Among patients with secondary infections, a significant proportion (88.8%, 87/98) were aged ≥60 years, while 11.2% (11/98) were <60 years old. Notably, all three patients (100%) with chronic lung disease and COVID-19 developed secondary infections, suggesting a high risk association. Other factors associated with secondary infections included mechanical ventilation (68.4%, 67/98), hypertension (62.2%, 61/98), diabetes mellitus (53.1%, 52/98), obesity (66.7%, 2/3), and use of inhaled corticosteroids (80.0%, 4/5) (Table 5).

Table 5. Comorbidities in patients with microbiologically confirmed secondary infections.

Comorbidity	No. of patients (%)
Age ≥60 years	87 (88.8%)
Venous Catheterization	2 (2.0%)
Urinary Catheterization	5 (5.1%)
Chronic Lung Disease	3 (3.1%)
Diabetes Mellitus	52 (53.1%)
Hypertension	63 (64.3%)
Use of Inhaled Corticosteroids	4 (4.1%)
Mechanical Ventilation	45 (45.9%)
Obesity	2 (2.0%)

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DISCUSSION

Determining the accurate prevalence of secondary bacterial and fungal infections in COVID-19 patients poses a significant challenge, owing to the intricate interplay of various host, pathogen, and environmental factors [24, 25]. This retrospective study investigated the frequency and spectrum of secondary bacterial and fungal infections among COVID-19 patients requiring intensive care at a tertiary care hospital, with the goal of informing strategies for timely diagnosis, effective management, and improved patient outcomes.

Our study revealed a microbiologically confirmed secondary infection rate of 3.03% (98/3234) and a culture positivity rate of 50.3% (98/195), which is consistent with the findings of Karuna *et al*. (2022) [5]. Their study, conducted in India during the late first and peak second waves of the pandemic, reported a secondary infection rate of 3.5% [5]. The similarity in our findings suggests that secondary infection rates may have remained relatively stable across different phases of the pandemic in India. However, a study by Krithika *et al*. (2021) [13] reported a significantly higher secondary infection rate of 14.5% (83/573), with 64.8% (54/83) of these infections occurring in ICU patients. This disparity may be attributable to their study being conducted during the peak of the second wave (May-June 2021), which potentially reflects a higher burden of secondary infections during that period, possibly due to factors such as increased hospital-acquired transmission, inadequate infection control measures, or enhanced virulence of circulating pathogens.

Similarly, a multicenter study conducted by Zhang et al. (2020) [24] reported a substantially higher culture positivity rate of 57.89% among critically ill patients, suggesting that the severity of illness may be an important factor in the development of secondary infections [24]. A systematic review and meta-analysis conducted by Langford *et al*. (2020) pooled data from multiple hospitals, geographical regions, and diverse microbiological methods, reporting a secondary infection rate of 18.4% [26]. This comprehensive analysis underscores the impact of various factors, including study timeframe, patient population, and diagnostic methods, on secondary infection rates. In contrast, our study found no statistically significant difference in bacterial and fungal isolation rates, which may be attributed to the limited sample size or variations in data collection methods, highlighting the need for larger, more standardized studies to accurately determine the burden of secondary infections in COVID-19 patients.

In our study, respiratory specimens, predominantly ETA (39.5%), comprised the majority (61.0%) of samples received, differing from studies by Sonam *et al*. (2021) and Khurana *et al*. (2021), where blood and respiratory specimens were more equally distributed [27, 28]. In our study, *K. pneumoniae* (29/67, 43.3%) emerged as the most frequently isolated bacterium, followed by *A. baumannii* (17/67, 25.4%). This finding is consistent with reports by Sonam *et al*. (2021) and Khurana *et al*. (2021), who also identified *K. pneumoniae* (29.3%) and *A. baumannii* (21.1%) as the predominant pathogens in their studies [27, 28]. In agreement with our findings, Yadav *et al*. (2022) [4] also reported a predominance of Gram-negative bacteria in secondary infections among COVID-19 patients. This congruence highlights the importance of vigilant monitoring and effective management of Gram-negative bacterial infections in COVID-19 patients to prevent adverse outcomes.

Blood cultures showed the lowest positivity rate (2/20, 10.3%), with only two isolates recovered, identified as *E. coli* and *K. pneumoniae*. These bacteria are consistent with previously reported common pathogens in COVID-19 patients [13, 28]. Notably, *K. pneumoniae* and *A. baumannii* have been identified as the most frequent isolates in some studies [28]. Our blood culture positivity rate (10.3%) was significantly lower than the rates reported by Khurana *et al*. (2021) (46%) and Karuna *et al*. (2020) (56%) [5, 28]. This discrepancy may be attributed to differences in blood culture collection timing, prior antibiotic use, and patient populations studied. However, due to the retrospective nature of our study, we were unable to ascertain the specific factors contributing to this variation [29].

The pathogens identified in our study align with the World Health Organization's (WHO) priority pathogens list for research and development of new antibiotics [30], which categorizes 12 bacterial species into three priority tiers (critical, high, and medium) based on their level of antimicrobial resistance and potential to cause severe disease. Notably, while *E. coli* was not the most prevalent isolate in our study, it was recovered from all specimen types except sputum, a finding that is particularly significant given the statistically significant association observed between *E. coli* isolation and specimen type.

Our study identified *Aspergillus* spp. as the most frequently isolated fungus, followed by *Candida* spp., *Mucor* spp., and *Cladosporium* spp. These findings align with a study by Krithika *et al*. (2021) from Tamil Nadu, which also identified *Mucor* spp., *Aspergillus* spp., and *Cladosporium* spp. as common isolates [13]. Similarly, a global and multinational study on the prevalence of fungal diseases reported similar trends [31]. Low *et al*. (2011) highlighted three emerging epidemiological trends in fungal infections: an increased incidence of invasive mold infections, particularly invasive aspergillosis; a rise in infections caused by non-albicans *Candida* spp.; and the emergence of invasive mold infections caused by Zygomycetes [32]. These trends suggest potential shifts in the landscape of fungal pathogens, with *Cladosporium* spp. potentially emerging as a significant organism. However, further research is needed to confirm this observation. Although *Aspergillus* spp. was the most common fungal isolate in our study, statistical analysis did not support a definitive conclusion regarding its

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consistent predominance in COVID-19 patients. A multicenter study with a larger sample size would be necessary to establish more conclusive evidence and explore the evolving trends in fungal infections.

The presence of comorbidities and exposure to invasive procedures likely increased the susceptibility of our study population to secondary infections. Notably, secondary infections were identified after 48 h of hospitalization, suggesting a potential role for hospital-acquired infections (HAIs) [26]. Our findings are consistent with previous studies demonstrating that patients with bacterial and fungal isolates often have multiple risk factors, such as diabetes mellitus, advanced age, and corticosteroid therapy [8]. Furthermore, Bhat *et al*. (2022) reported that the combination of diabetes and hypertension was associated with increased mortality in COVID-19 patients, highlighting the potential synergistic effects of comorbidities on patient outcomes and emphasizing the need for comprehensive management of underlying health conditions to mitigate the risk of secondary infections.

Our study has several limitations. Firstly, the relatively small sample size and retrospective design may have introduced bias and limited the generalizability of our findings. Additionally, the absence of a control group precludes comparisons of secondary infection rates with the general population, hindering a comprehensive understanding of the phenomenon. To address these limitations, future studies should employ a prospective design, recruit larger sample sizes, and adopt a multicenter approach to provide a more representative and comprehensive understanding of secondary infection trends across diverse healthcare settings and patient populations.

In conclusion, our retrospective assessment reveals that *K. pneumoniae* and *A. baumannii* were the predominant bacterial pathogens responsible for secondary infections in our cohort of ICU-admitted COVID-19 patients. Notably, the isolation of fungal pathogens, including *Aspergillus* spp. and *Mucor* spp., even within our limited sample size, highlights the significance of continued surveillance for fungal infections in this vulnerable population. The presence of multiple comorbidities likely increased the susceptibility to secondary infections. Further research with larger sample sizes and comprehensive diagnostic approaches is essential to fully understand the epidemiology and clinical impact of secondary infections in COVID-19 patients, ultimately informing strategies to mitigate their occurrence and improve patient outcomes.

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COMFLICT OF INTEREST

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

REFERENCES

1. Ghanshani R, Gupta R, Gupta B, Kalra S, Khedar R, Sood S. Epidemiological study of prevalence, determinants, and outcomes of infections in medical ICU at a tertiary care hospital in India. Lung India. 2015; 32 (5): 441-8.
2. Jha P, Deshmukh Y, Tumbe C, Suraweera W, Bhowmick A, Sharma S, et al. COVID mortality in India: National survey data and health facility deaths. Science. 2022; 375 (6581): 667-71.
3. Tendulkar P, Pandey P, Panda PK, Bhadoria AS, Kulshreshtha P, Mishra M, et al. Comparative Study Between the First and Second Wave of COVID-19 Deaths in India: A Single Center Study. Cureus. 2023; 15 (4): e37472.
4. Boorgula SY, Yelamanchili S, Kottapalli P, Naga MD. An update on secondary bacterial and fungal infections and their antimicrobial resistance pattern (Amr) in COVID-19 confirmed patients. J Lab Physicians. 2022; 14 (3): 260-4.
5. Karuna T, Garg R, Kumar S, Singh G, Prasad L, KrishenPandita K, et al. Clinico-Epidemio-Microbiological Exploratory Review Among COVID-19 Patients with Secondary Infection in Central India. Infect Drug Resist. 2022; 15: 1667-76.
6. Song G, Liang G, Liu W. Fungal co-infections associated with global COVID-19 pandemic: a clinical and diagnostic perspective from china. Mycopathologia. 2020; 185 (4): 599-606.
7. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020; 395 (10223): 507-13.
8. Bhat KA, Madi D, Bhat S, Mary T, ShenoyMulki S, Kotian H. Profile of Secondary Bacterial and Fungal Infections in Hospitalized COVID-19 Patients in a Tertiary Care Centre. Infect Drug Resist. 2022; 15: 5705-14.
9. Zhu X, Ge Y, Wu T, Zhao K, Chen Y, Wu B, et al. Co-infection with respiratory pathogens among COVID-2019 cases. Virus Res. 2020; 285: 198005.
10. Kozel TR, Wickes B. Fungal diagnostics. Cold Spring HarbPerspect Med. 2014; 4 (4): a019299.
11. Chindamporn A, Chakrabarti A, Li R, Sun PL, Tan BH, Chua M, et al. Survey of laboratory practices for diagnosis of fungal infection in seven Asian countries: An Asia Fungal Working Group (Afwg) initiative. Med Mycol. 2018; 56 (4): 416–25.

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[DOI: 10.61186/JoMMID.12.1.76]

12. Roncon L, Zuin M, Rigatelli G, Zuliani G. Diabetic patients with COVID-19 infection are at higher risk of ICU admission and poor short-term outcome. J ClinVirol. 2020; 127: 104354.

13. Varshini KM, Ganesan V, Charles J. Secondary bacterial and fungal infections in COVID-19 patients. Int J Antimicrob Agents. 2021; 58: 21003526.

14. White PL, Dhillon R, Hughes H, Wise MP, Backx M. COVID-19 and fungal infection: the need for a strategic approach. Lancet Microbe. 2020; 1 (5): e196.

15. Vaughn VM, Gandhi TN, Petty LA, Patel PK, Prescott HC, Malani AN, et al. Empiric antibacterial therapy and community-onset bacterial co-infection in patients hospitalized with coronavirus disease 2019 (COVID-19): a multi-hospital cohort study. Clin Infect Dis. 2021; 72 (10): 533-41.

16. Rattani S, Farooqi J, Jabeen G, Chandio S, Kash Q, Khan A, et al. Evaluation of semi-quantitative compared to quantitative cultures of tracheal aspirates for the yield of culturable respiratory pathogens – a cross-sectional study. BMC Pulm Med. 2020; 20 (1): 284.

17. Indian Council of Medical Research. Standard Operating Procedures for Bacteriology Antimicrobial Resistance Surveillance and Research Network.2015; 01-160.

18. World Health Organization. Laboratory testing for coronavirus disease (COVID-19) in suspected human cases; WHO Interim guidance; 2020.

19. Valones MA, Guimarães RL, Brandão LA, de Souza PR, de Albuquerque Tavares Carvalho A, Crovela S. Principles and applications of polymerase chain reaction in medical diagnostic fields: a review. Braz J Microbiol. 2009; 40 (1): 1-11.

20. Kyosei Y, Yamura S, Namba M, Yoshimura T, Watabe S, Ito E. Antigen tests for COVID-19. Biophys Physicobiol. 2021; 18: 28-39.

21. Cheesbrough, M. (2005). District Laboratory Practice in Tropical Countries, Part 1 (2nd ed., pp. 105-115). Cambridge University Press.

22. Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J ClinPathol. 1966; 45 (4): 493-6.

23. Indian Council of Medical Research. (2019). Standard Operating Procedures for Fungal Identification and Detection of Antifungal Resistance Antimicrobial Resistance Surveillance and Research Network (pp. 01-114).

24. Zhang H, Zhang Y, Wu J, Li Y, Zhou X, Li X, et al. Risks and features of secondary infections in severe and critical ill COVID-19 patients. Emerg Microbes Infect. 2020; 9 (1): 1958-64.

25. Raffaelli F, Tanzarella ES, De Pascale G, Tumbarello M. Invasive Respiratory Fungal Infections in COVID-19 Critically Ill Patients. J Fungi (Basel). 2022; 8 (4): 415.

26. Langford BJ, So M, Simeonova M, Leung V, Lo J, Kan T, et al. Antimicrobial resistance in patients with COVID-19: A systematic review and meta-analysis. Lancet Microbe. 2023; 4 (3): 179-91.

27. Sonam V,Bansal N,Rao BK,Veeraraghavan B,Rodrigues C,Wattal C, et al. Secondary Infections in Hospitalized COVID-19 Patients: Indian Experience. Infect Drug Resist. 2021; 14: 1893-1903.

28. Khurana S, Singh P, Sharad N, Kiro VV, Rastogi N, Lathwal A, et al. Profile of co-infections & secondary infections in COVID-19 patients at a dedicated COVID-19 facility of a tertiary care Indian hospital: Implication on antimicrobial resistance. Indian J Med Microbiol. 2021; 39 (2): 147–53.

29. Qiao LD, Chen S, Yang Y, Zhang K, Zheng B, Guo HF, et al. Characteristics of urinary tract infection pathogens and their *in vitro* susceptibility to antimicrobial agents in China: data from a multicenter study. BMJ Open. 2013; 3 (12): 004152.

30. Asokan GV, Ramadhan T, Ahmed E, Sanad H. WHO Global Priority Pathogens List: A Bibliometric Analysis of Medline-PubMed for Knowledge Mobilization to Infection Prevention and Control Practices in Bahrain. Oman Med J. 2019; 34 (3): 184-93.

31. Bongomin F, Gago S, Oladele R, Denning D. Global and multi-national prevalence of fungal diseases—estimate precision. J Fungi (Basel). 2017; 3 (4): 57.

32. Low CY, Rotstein C. Emerging fungal infections in immunocompromised patients. F1000 Med Rep. 2011; 3: 14.

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