An Update on Secondary Bacterial and Fungal Infections and Their Antimicrobial Resistance Pattern (AMR) in COVID-19 Confirmed Patients at a Tertiary Care Hospital

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Abstract
Introduction Since coronavirus disease 2019 (COVID-19) has limited treatment options, concern has been raised over secondary infections and antimicrobial resistance (AMR) patterns. It has been observed that patients who were infected with COVID-19 were predisposed to develop secondary infections. The purpose of the study is to ascertain the prevalence of the bacterial and fungal coinfections in COVID-19 patients, and also to assess the AMR patterns in the obtained isolates.

Methods We have studied 200 clinical samples obtained from 122 COVID-19 positive patients. Pathogens were identified using Vitek 2 system. The demographic and clinical patterns were also observed.

Results A total of 122 patients developed secondary infections. Patients aged more than 40 years were majorly affected ($p$-value < 0.0001). Respiratory samples ($n = 96$) were predominant. *Klebsiella pneumoniae* ($n = 68$) was the most common pathogen isolated followed by *Acinetobacter baumannii* ($n = 54$) and an overall 6% rise in the Carbapenem resistance was observed in the isolates.

Conclusion To contain the secondary infections in COVID-19 patients, it is imperative to adhere to antimicrobial stewardship program and timely revise the empirical antibiotic policy.

Keywords ➢ antimicrobial resistance ➢ coagulase-negative staphylococcus ➢ central line associated bloodstream infections

Introduction Bacterial infections that are commonly identified in viral respiratory tract infections are the major causes of morbidity and mortality and warrant for prompt diagnosis and antibiotic treatment.1–3 The prevalence and incidence of bacterial and fungal infections in coronavirus disease 2019 (COVID-19) patients are not understood well; hence, it still remains an understudied phenomenon.4,5 Although antibiotics are ineffective for the treatment of COVID-19, they are still used for the treatment of suspected or confirmed COVID-19 cases for different reasons. One of the main reasons is the difficulty in ruling out bacterial coinfection on presentation, and also possibility of secondary bacterial and fungal infections during the course of illness. Hence, assessing the number of COVID-19 with acute respiratory bacterial coinfections and

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identifying the pathogens are critical for the treatment and it is also required to ensure antibiotic stewardship in place to avoid any injudicious usage of the antibiotics\(^3\) and to minimize adverse effects by over usage of the antibiotics\(^4\). Additionally, the bacterial coinfections in COVID-19 patients also hold a strong influence on renewing the empirical guidelines for the management of infections in COVID-19 patients. The purpose of the study is to ascertain the prevalence of the bacterial and fungal coinfections in COVID-19 patients and to assess the antimicrobial resistance (AMR) patterns in the obtained isolates.

**Materials and Methods**

*The place of study*: The study was conducted at AIG Hospitals, Gachibowli, Hyderabad, Telangana, which is a 800-bedded accredited tertiary care hospital.

*The patient identification and clinical data*: The clinical samples from April 1, 2021 till May 31, 2021 of confirmed COVID-19 patients received in the microbiology laboratory were included in the study. The confirmation of COVID-19 was done by reverse transcription polymerase chain reaction (RT-PCR) test. The required data pertaining to clinical condition, microbiological data, and clinical outcome were obtained from the patient’s medical records. Patients whose samples were not sent to the laboratory were excluded from the study.

*Collection and processing of the samples*: The samples were collected for culture: (a) one at the time of admission if bacterial infection was suspected and (b) second during the collection: (a) one at the time of admission if samples were not sent to the laboratory were excluded from the study. The required data pertaining to clinical condition, microbiological data, and clinical outcome were obtained from the patient’s medical records. Patients whose samples were not sent to the laboratory were excluded from the study.

*Antimicrobial breakpoints were interpreted according to CLSI 2021 guidelines.*

**Inclusion criteria**: All adult patients with a positive severe acute respiratory syndrome coronavirus 2-PCR result were included in the study. Cases whose positive PCR result and microbiology result occurred in the same day or preceding admission were included.

**Exclusion criteria**: Pediatric age group was excluded. Samples that were positive for skin flora were excluded (i.e., gram-positive bacilli, coagulase-negative staphylococcus (CONS), micrococcii, Kocuria spp).

*Ethical clearance*: The study was approved by Institutional Ethics Committee—AIG Hospitals on June 18, 2021.

*Statistical analysis*: The data collected from the medical records was transferred into separate study proforma. The data was entered into MS-EXCEL for further analysis after editing for completeness and consistency. The continuous variables were expressed as mean and standard deviation (SD) and categorical variables were expressed as % of frequency distribution. Mann–Whitney U test, t-test, chi-squared test, and Fisher’s exact test were used. The analysis was performed by using statistical package for social analysis (SPSS, 20th version). A p-value of less than 0.05 with two sided was considered statistically significant.

**Results**

The number of COVID-19 cases were rising from March end and peaked in the month of April, 2021. The total inpatient admissions who were positive for COVID-19 RT-PCR in the month of April–May 2021 were 2,259. In 2,259, the total number of patients admitted to intensive care unit (ICU) were 314 and non-ICU were 1,945. Out of 2,259 patients, 122 patients have developed secondary infections (males: 95, females: 27). For 122 patients, 200 samples were received. To calculate the patient’s demographics, 122 patients were considered and for analysis of the isolated organisms, 200 samples were considered. Among the 122 COVID-19 positive patients, the total ICU admissions made were 74. Of the 74 ICU admissions, males were 60 (63.15%) and females were 14 (51.85%). Among the 48 non-ICU admissions made, males were 35 (72.9%) and females were 13 (27%) (p-value = 0.827).

Two-hundred culture positive samples obtained from 122 COVID-19 positive patients were taken into study.

The mean age of the patients who required admissions to ICU was 57.1 ± 14.1 (mean ± SD) years old. The mean age of the patients who required admissions in non-ICU was 51.4 ± 15.9, and the difference was found to be statistically significant (p-value = 0.022). In 122 patients, out of 95 males who were admitted, 40% were alive, and 60% were dead. Among 27 females, 72.97% were alive and 27.03% have died.
The majority of patients who were admitted belonged to 40 years and above. In the age group of 40 years and above among the males (67/122), 50 were admitted to ICU and 17 were admitted to non-ICU, whereas in females (n = 16), 13 were admitted to ICU and 3 were admitted to non-ICU, and this was found to be statistically significant (p-value > 0.0001).

Among 122 patients, 52 (42.62%) were discharged, as of July 23, 2021. Sixty-seven (54.91%) patients have died and three are still admitted. Out of 74 (74/122) admitted to ICU, 45 (60.8%) patients have died, 26 (35.13%) are discharged, and 3 (4.05%) are still admitted. Patients who were admitted to non-ICU out of 48 (48/122), 21 (43.75%) have died, and 27 (56.25%) have discharged (p-value = 0.6653).

The average length of stay at the hospital was 10.7 days. The total number of samples positive within 48 hours of admission were 53, during the first week of stay were 42, and after 7 days were 105.

The common presenting complaints during admission were fever, shortness of breath, and cough. The major comorbidities the patients were suffering at the time of admission were diabetes mellitus type 2 and hypertension. We have found that 50 (40%) out of 122 patients were diabetic and 55 (45.08%) out of 122 were hypertensive.

Out of 200 specimens that were culture positive, the most common samples received were respiratory samples (endotracheal secretions [n = 96], sputum [n = 5], and bronchoalveolar lavage [n = 1]), followed by blood cultures (n = 70), pus(n = 10), fluids (n = 7), and urines(n = 11). Among the 190 bacteria, the most common organism isolated was *Klebsiella pneumoniae* (n = 68) followed by *Acinetobacter baumannii* (n = 54), *Enterobacteriaceae*, n = 25 (Escherichia coli [n = 13], *Proteus vulgaris* [n = 1], *Serratia marcescens* [n = 9], *Morganella morganii* [n = 1], *Enterobacter cloacae* [n = 1]), Pseudomonas aeruginosa (n = 8), Enterooccus faecalis (n = 4), Staphylococcus aureus (n = 2), Coagulase negative Staphylococcus [n = 6] (Staphylococcus epidermidis [n = 1], Staphylococcus hominis [n = 2], Staphylococcus haemolyticus [n = 3]), nonfermenters, n = 18 (Burkholderia cepacia [n = 2], Elizabethkingia meningoseptica [n = 8], Sphingomonas paucimobilis [n = 4], Stenotrophomonas maltophilia [n = 4]).

Among 13 fungal isolates, Candida tropicalis (n = 4), Candida auris (n = 3), Mucor (n = 3), Aspergillus niger (n = 1), Aspergillus fumigatus (n = 1), Aspergillus flavus (n = 1).

The susceptibility percentage of different antibiotics for various organisms isolated is shown in Table 1.

As the number of organisms for gram-positive cocci and Candida were very low, the susceptibility pattern was not analyzed as it would not be representative.

### Discussion

From our study, it has been observed that males were more affected than female patients. Even the number of ICU admissions were more among males than females. Although the age group of 40 years and above as majorly affected, the mean age for ICU admissions was 57, whereas for non-ICU the mean age was 51, which was statistically significant (*Table 2*). Out of 122 patients, death rate was higher in males than females. Our findings were in agreement with study conducted by Parohan et al. This could be attributed to the age-related B cell and T cell function defects and also excess production of cytokines type 2, which could cause prolonged proinflammatory responses and also defect in controlling the viral replication and thus leading to a poor outcome. Among the 122 patients studied, 52 (42.62%) patients were discharged, 67 (54.9%) have died, and 3 are still admitted in the hospital (*Table 2*). The overall death rate was higher in ICU admissions that non-ICU admissions.

### Table 1 Susceptibility percentage for various antibiotics observed

<table>
<thead>
<tr>
<th></th>
<th>Klebsiella pneumoniae (n = 68)</th>
<th>Acinetobacter baumannii (n = 54)</th>
<th>Enterobacteriaceae (n = 25)</th>
<th>Nonfermenters (n = 18)</th>
<th>Pseudomonas (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxycillin-clavulanate</td>
<td>18.2</td>
<td>16.8</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Piperacillin-tazobactam</td>
<td>19.6</td>
<td>3.84</td>
<td>43.75</td>
<td>–</td>
<td>87.5</td>
</tr>
<tr>
<td>Cefoperazone-sulbactam</td>
<td>23.8</td>
<td>5.55</td>
<td>58.33</td>
<td>–</td>
<td>87.5</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>11.11</td>
<td>0.03</td>
<td>35.29</td>
<td>–</td>
<td>87.5</td>
</tr>
<tr>
<td>Cefepime</td>
<td>21.21</td>
<td>5.55</td>
<td>33.33</td>
<td>–</td>
<td>87.5</td>
</tr>
<tr>
<td>Imipenem</td>
<td>27.11</td>
<td>5.66</td>
<td>47.05</td>
<td>–</td>
<td>87.5</td>
</tr>
<tr>
<td>Meropenem</td>
<td>23.5</td>
<td>5.76</td>
<td>56</td>
<td>–</td>
<td>87.5</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>7.69</td>
<td>–</td>
<td>16</td>
<td>–</td>
<td>87.5</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>6.15</td>
<td>5.88</td>
<td>18.75</td>
<td>46.66</td>
<td>87.5</td>
</tr>
<tr>
<td>Amikacin</td>
<td>33.8</td>
<td>11.11</td>
<td>40</td>
<td>–</td>
<td>100</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>56</td>
<td>66.66</td>
<td>84</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Colistin</td>
<td>75.7</td>
<td>98.14</td>
<td>75</td>
<td>–</td>
<td>100</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>23</td>
<td>–</td>
<td>47.82</td>
<td>85.71</td>
<td>–</td>
</tr>
</tbody>
</table>
The higher mortality rate can be due to the patients who are elderly, immunosuppressed, critically ill, requiring mechanical ventilation, who are admitted with severe disease and also presenting comorbidities such as diabetes mellitus and hypertension at the time of admission.\textsuperscript{6,8,9}

The number of samples, which were positive in 48 hours of admission, were 53, followed by 42 positive samples during first week of admission and 105 after 1 week of admission. It is well established that the viral respiratory tract infections are prone to increased risk of bacterial coinfections and bacterial infections are major cause of mortality.\textsuperscript{10} Out of 200 samples collected, respiratory samples were majorly followed by blood culture samples. This trend of samples was also seen in the study conducted by Nori et al.\textsuperscript{11} and Zhang et al.\textsuperscript{12}

We observed that Klebsiella pneumoniae and Acinetobacter baumannii were the most common organisms isolated followed by other Enterobacteriaceae (Escherichia coli, Proteus mirabilis, Serratia marcescens, Morganella morganii, Enterobacter cloacae), Pseudomonas aeruginosa, Enterococcus species, Streptococcus species, Staphylococcus aureus, and nonfermenters (Elizabethkingia meningoseptica, Sphingomonas paucimobilis, Burkholderia cepacia, Stenotrophomonas maltophilia) (►Fig. 1). Klebsiella pneumoniae and Acinetobacter baumannii were also the common pathogens found in the studies conducted by Chen et al.\textsuperscript{13} and Khurana et al.\textsuperscript{14}

Unlike now, during non-COVID-19 times in early 2020, (January–May 2020) when COVID-19 admissions were not done at our hospital, we have observed that the samples that were most commonly received at microbiology laboratory were blood and urine, whereas after COVID-19 admissions, the common samples received were respiratory secretions. This can be because COVID-19 virus has more affinity to the respiratory tract.

During early 2020, Klebsiella pneumoniae was the most common organism isolated, followed by Escherichia coli, Enterococcus faecium, Enterococcus faecalis, Pseudomonas aeruginosa, and Acinetobacter baumannii. It has also been observed that when compared with previous antibiotic resistance pattern, there has been increase in the carbapenem resistance during first week of admission and 105 after 1 week of admission. It is well established that the viral respiratory tract infections are prone to increased risk of bacterial coinfections and bacterial infections are major cause of mortality.\textsuperscript{10} Out of 200 samples collected, respiratory samples were majorly followed by blood culture samples. This trend of samples was also seen in the study conducted by Nori et al.\textsuperscript{11} and Zhang et al.\textsuperscript{12}

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### Table 2: Demographics, infections, timing of outcome, and culture results

<table>
<thead>
<tr>
<th></th>
<th>n = 122 Distinct patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, years, median (IQR)</strong></td>
<td>58</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>95</td>
</tr>
<tr>
<td>Females</td>
<td>27</td>
</tr>
<tr>
<td><strong>Infection</strong></td>
<td></td>
</tr>
<tr>
<td>Respiratory</td>
<td>66</td>
</tr>
<tr>
<td>Bloodstream</td>
<td>40</td>
</tr>
<tr>
<td>Others</td>
<td>16</td>
</tr>
<tr>
<td><strong>Outcomes</strong></td>
<td></td>
</tr>
<tr>
<td>Average length of stay</td>
<td>10.7 days</td>
</tr>
<tr>
<td>Discharged</td>
<td>52</td>
</tr>
<tr>
<td>Deceased</td>
<td>67</td>
</tr>
<tr>
<td>Still admitted to hospital</td>
<td>3</td>
</tr>
</tbody>
</table>

**Abbreviation:** IQR, interquartile range.

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![Fig. 1 Bacteria and fungus profile.](image-url)
There is nearly a 6% rise in the carbapenem resistance in the present isolates in comparison with the previous ones. We have also observed that there is 5% increase in the environmental isolates (Elizabethkingia meningoseptica, Burkholderia cepacia, Sphingomonas paucimobilis, Stenotrophomonas maltophilia). The reason for increase in the environmental isolates including Acinetobacter baumannii is because most of the environmental isolates exhibit intrinsic resistance for high-end antibiotics such as colistin and carbapenems. There were no specific guidelines or validated treatment for COVID-19 and one could not understand what the exact attributes for clinical deterioration of patients were, whether due to the virus or any other unascertainable cause for superimposed secondary infections due to bacteria and fungus. Thus, it led to the usage of invasive devices to salvage the patient, administration of high-end antibiotics, steroids, and other agents (e.g., remdesivir, chloroquine, ivermectin, lopinavir) to curb the bug. This resulted in increase in the AMR, certainly paving a way for the environmental organisms to survive, colonize, and to become an opportunistic pathogen.

The CONS, which were isolated from bloodstream, related to central line infections, were reported as they were considered significant in view of central line-associated bloodstream infections.

Conclusion

We have found an overall secondary infectivity rate in COVID-19 patients at our hospital to be 5.4%. As very limited information is known about the predisposition for the development of secondary infections in COVID-19 infected patients and with the third wave of COVID-19 looking imminent, the need of the hour is to strengthen the ongoing infection control practices, ensuring strict adherence to bundle checklists, and advocacy for implementation of antimicrobial stewardship programs. These practices could have a significant impact for updating the empirical antibiotic therapy guidelines for the treatment of COVID-19 patients.

Conflict of Interest

None.

References