Bacterial coinfections in COVID: Prevalence, antibiotic sensitivity patterns and clinical outcomes from a tertiary institute of Northern India

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ABSTRACT

Purpose: Bacterial coinfections are a leading cause of morbidity and mortality during viral infections including corona virus disease (COVID-19). The COVID-19 pandemic has highlighted the need to comprehend the complex connection between bacterial and viral infections. During the current pandemic, systematic testing of the COVID-19 patients having bacterial coinfections is essential to choose the correct antibiotics for treatment and prevent the spread of antimicrobial resistance (AMR). This study was planned to study the prevalence, demographic parameters, comorbidities, antibiotic sensitivity patterns, and outcomes in hospitalized COVID-19 patients with bacterial coinfections. Material and Methods: The COVID-19 patients having bacterial coinfections were selected for the study and analyzed for the prevalence, antibiotic sensitivities, comorbidities, and clinical outcomes. The bacterial isolates were identified and the antibiotic susceptibility testing was performed according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Results: Of the total 1,019 COVID-19 patients screened, 5.2% (n = 53) demonstrated clinical signs of bacterial coinfection. *Escherichia coli* were the most common isolate followed by *Pseudomonas aeruginosa* and *Klebsiella spp.* among the gram-negative bacterial infections. Coagulase-negative *Staphylococcus* species (CONS) and *Staphylococcus aureus* were most common among the gram-positive bacterial infections. The antibiotic sensitivity profiling revealed that colistin (99%), imipenem (78%), and fosfomycin (95%) were the most effective drugs against the gram-negative isolates while vancomycin (100%), teicoplanin (99%), and doxycycline (71%) were most potent against the gram-positive isolates. The analysis of the clinical parameters and outcomes revealed that among the COVID-19 patients with bacterial coinfections, the mortality rate was higher (39%) than the control group (17%) (P-value < 0.001). Conclusion: This study reveals the significantly increased rates of bacterial coinfections among COVID-19 patients which may lead to an increase in mortality. This study will guide the physicians at the primary level on the rational and correct usage of antibiotics in such COVID cases. Hence, systematic testing of COVID-19 patients with bacterial coinfections is the need of the hour to decrease the mortality rate and limit the spread of AMR.

Keywords: Antibiotics, bacterial, coinfections, COVID-19, resistance

Introduction

The humans have witnessed the emergence of four severe viral outbreaks in the last two decades: the 2002 Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) epidemic, the 2009 influenza A, the H1N1 pandemic, the 2012 Middle East Respiratory Syndrome (MERS) outbreak, and most recently, the COVID-19 pandemic. The SARS-CoV-2 belongs to the family: Coronaviridae, order: Nidovirales. Corona represents crown-like spikes on the outer surface of the virus, hence the name “Coronavirus.”
Material and Methods

Place and duration of study

The study was conducted at a tertiary care hospital in North India between March 2020 and August 2020 (6 months) during the COVID pandemic.

Patient selection

During six months, a total of 50 hospitalized COVID-positive patients were included in the study. The inclusion criteria were the presence of bacterial coinfection as determined by the presence of characteristic clinical features and positive blood, respiratory, urine, or pus/aspiration cultures. The institutional ethics committee's (IEC) permission was taken before the study.

Molecular testing for SARS-CoV-2

Two swabs: oropharyngeal and deep-nasal were collected from the suspected patients and transported in the viral transport media (VTM). Viral RNA extraction was done using the Qiagen viral RNA kit (QIAamp, USA) according to the manufacturer's instructions. Real-time polymerase chain reaction (PCR) was performed by DIAGSsure™ n CoV-19 Detection Assay (Multiplex, TaqMan-based) kit manufactured by GCC BIOTECH.[12]

Bacterial culture and processing: All the specimens (blood, respiratory, urine or pus/aspiration) were cultured on both MacConkey and blood agar plates according to the standard microbiological techniques. Further, the colonies were isolated and subcultures were done accordingly.

A. Identification:

1. Conventional method using biochemical tests:

The bacterial isolates were first identified using routine staining and biochemical tests as are being followed in our laboratory.[13]

2. Automated methods: The identity of the bacteria was confirmed by MALDI TOF MS and Vitek 2 system (Biomerieux, France), automated identification and susceptibility testing system.[14]

B. Antibiotic susceptibility testing was done by Kirby–Bauer's disk diffusion method on Muller Hinton agar and interpreted based on the Clinical and Laboratory Standards Institute (CLSI) guidelines.[15]

Patient follow-up

The organism profiles, sensitivity, and other study parameters were kept in the computer database along with the particulars of the patients. A follow-up was planned in the suspected cases for any repeat culture, outcomes, and associated comorbidities.

Statistical analysis

The results were analyzed using the SPSS version 22 software (SPSS Inc., Chicago, IL, USA). The frequencies were shown with 95% confidence intervals (95% CI). The Chi-square and Mann–Whitney U tests were used to analyze the statistically

It is an enveloped virus 65–125 nm in diameter and contains a single-stranded positive-sense RNA (size ranging from 26 to 32 kb). It has emerged as a global pandemic affecting millions of people worldwide and is proving to be a greater danger than MERS and SARS coronaviruses.[2]

A plethora of symptoms has been described in the past few months, clearly indicating that COVID-19 is a complex disease, which in no way consists only of a respiratory infection. It presents with a variety of unspecific symptoms so that the differential diagnosis encompasses a wide range of infections. The most common symptoms cluster of COVID-19 encompasses: respiratory: cough, sputum, shortness of breath, fever; musculoskeletal: myalgia, joint pain, headache, fatigue; enteric: abdominal pain, vomiting, diarrhea; and mucocutaneous (less commonly).[3] Lung infections may progress in a few cases to ARDS (acute respiratory distress syndrome), shock, and death. Cytokine storm, immune dysregulation, and various viral evasion mechanisms in the presence of various comorbid conditions have contributed to fatal outcomes in the COVID-19 patients.[4]

Superinfection with bacterial pathogens has been identified in various viral respiratory illnesses in the past and contributed to significant rates of morbidity and mortality.[5–7] Mortality has been reported to be as high as 20–30% in such cases of superinfection.[3] The existence of bacterial co-pathogens in such respiratory patients leads to an increased hospital stay, greater chances of acquiring nosocomial infections, and the overall increase in the cost of hospital stay.[9]

SARS-CoV-2 is a new virus with still limited knowledge about its pathogenesis and clinical manifestations. Hence, there are several areas of knowledge gaps regarding this novel coronavirus. One such area of potential research and studies is about the coexistence, prevalence, and incidence of bacterial pathogens in SARS-CoV-2.[10,11] Various antivirals and immunomodulatory agents are being tried in COVID-19-hospitalized patients and several others are in the experimental phase and under trials. The antibiotics are of no use in such patients but are routinely prescribed due to the potential risk of secondary bacterial infections.[16] Various pieces of literature and studies have advocated the use of antibiotics in patients suffering from respiratory symptoms of COVID-19. However, such irrational and unguided antibiotic use ultimately leads to the emergence of antimicrobial resistance (AMR).[11]

Hence, there is a dire need to study bacterial coinfections in COVID-19 patients and understand the exact incidence and prevalence of such co-pathogens for the proper and rational use of antibiotics. This study was planned to study the prevalence, demographic parameters, risk factors, antibiotic sensitivity patterns, and outcomes in hospitalized COVID-19 patients with bacterial coinfections.
significant variables. The statistically significant values were considered as $P$ value $<0.05$.

**Results**

A total of 1,019 patients tested positive for COVID-19 in a span of 6 months and were hospitalized at our COVID hospital. Approximately, 67% of these cases were asymptomatic and only 5.2% (53) of the patients showed clinical signs of bacterial coinfection. The mean age of the patients was 68 years and the range was 8–75 years. The males comprised most of the cases: 61% (32/53) compared to females. Various comorbidities in the patients were also studied and hypertension (61%) and diabetes mellitus (58%) emerged as the most prevalent medical conditions. Chronic obstructive pulmonary disease (COPD)/asthma (27%), chronic kidney disease (CKD) (23%), and chronic liver diseases (CLD) (19%) were the other major comorbidities among the COVID patients. Among the various samples collected from the COVID patients suffering from coinfections, blood was received from most of the cases (37%). Urine (31%), respiratory specimens (28%), and pus/aspirated fluid (4%) contributed to the other bacteriology samples. Bacteremia was seen in a majority of the COVID-positive patients, followed by respiratory and urinary symptoms.

*Escherichia coli* was the common isolate (21%), followed by *Pseudomonas aeruginosa* (13.2%), and *Klebsiella spp.* (11.3%) among the gram-negative organisms. *Burkholderia cepacia*, *Stenotrophomonas maltophila*, *Morganella morganii*, and *Proteus mirabilis* were isolated from one patient each. On the other hand, coagulase-negative *Staphylococcus* species (CONS) (24.5%) and *Staphylococcus aureus* (17%) formed most of the isolates in gram-positive organisms. Methicillin-resistant *Staphylococcus aureus* (MRSA) was seen in four, MRCONS in seven, *Enterococcus* spp. in five, and *Streptococcus* spp. in one case.

The antibiotic sensitivity testing revealed colistin (99%), imipenem (78%), and fosfomycin (95%) (in urinary isolates only) as the most effective drugs against gram-negative isolates, whereas vancomycin (100%), teicoplanin (99%), and doxycycline (71%) emerged as the most potent ones for the gram-positive ones. Detailed antibiotic sensitivity patterns for both gram-positive and gram-negative isolates are summarized in Figures 1 and 2.

The two groups of COVID-positive patients with and without bacterial coinfections were compared in different clinical parameters and outcomes [Table 1]. The patients with bacterial coinfections belonged to the older age group as compared to the other group ($P$-value $= 0.007$). No statistical significance was noted in terms of gender and other comorbidities like hypertension, diabetes, CKD, CLD, COPD/asthma on comparing between the two groups. However, on comparing the clinical outcomes, a significant difference was noted in terms of in-patient mortality, use of ventilators, and vasopressors ($P$-value $<0.001$). The patients with bacterial coinfections experienced a mortality rate of 39% as compared to the non-infection group (17%). Ventilators (35%) and vasopressive drugs (32%) were used more frequently in these patients.

**Discussion**

SARS-CoV-2 is a newly emerging virus that has led to a global pandemic in a span of only a few months. The immunology, pathogenesis, clinical features, and implications of COVID-19 on the health care settings are still to be fully understood. There is a lack of clinical research and data on bacterial infections in these COVID patients. Our study reported a bacterial coinfection rate of 5.2% which is in agreement with other similar studies done in various parts of the world. A case series from Washington reported the bacterial coinfection rate as 4.8%, while some studies from China also reported a rate of 5–9%. A meta-analysis done by Lansbury *et al.* reported a bacterial coinfection rate of around 6.8% in hospitalized COVID-19 cases.

This study stressed the prevalence of bacterial coinfections to be higher among the elderly age group (>65 years). Several studies have emphasized the point of enhanced pathogenesis of COVID-19 in the elderly age group. A lot of factors like decreased mucociliary clearance, ciliary ultrastructural anomalies, and immunosenescence play a key role in this. “Inflamm-aging” or increased release of inflammatory mediators and cytokines leading to a cytokine surge is involved in tissue damage and multiorgan failure in such patients. A higher proportion of comorbidities like diabetes and hypertension was seen in the COVID cases in our study. This can be supported by the fact that diabetes mellitus itself downregulates the immune system by decreasing the effective T-cell and neutrophil response. It causes decreased phagocytosis, ineffective chemotaxis, and decreased killing of the invading microbes by the neutrophils and macrophages leading to increased susceptibility to secondary bacterial infection. Our study, however, failed to show any statistical significance for the association of various comorbidities to the increase in the bacterial coinfection rates.
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The past literature search revealed no other similar studies in the COVID‑19 patients which reported high rates of bacteremia and concurrent urinary tract infection (UTI) in these groups of patients. Ours is the first such study revealing bacteremia (37%) and UTIs (31%) as the most common coinfections in the COVID‑positive patients. Other isolates were also isolated from respiratory (28%) and pus/aspirate (4%) samples in the current study. *Escherichia coli, Klebsiella, and Pseudomonas* were the predominant gram‑negative pathogens. One similar study in the COVID patients reported UTI in almost half of the patients (57%).[17] It also reported *Escherichia coli* and *Enterobacter cloacae* as the most isolated pathogens. Another study by Lansbury et al.[21] reported respiratory pathogens, such as *Mycoplasma pneumoniae, Pseudomonas aeruginosa, and Haemophilus influenza*, as the most isolated ones. The increased prevalence of gram-negative as well as gram-positive pathogens in the current study can be attributed to the immune dysregulation and gut dysbiosis in the COVID-positive patients. The inflammatory mediators disrupt the intestinal permeability leading to the leakage of the gut microbes and associated metabolites into circulation. The leaked microbes and products via circulation migrate to organs including the lungs and produce bacteremia, UTIs, and various other infections.[28] The high rate of diabetes in our group of patients also predisposes to an increase in secondary bacterial infections including UTIs.[29] Immune dysregulation in the SARS-CoV-2 infection is characterized by lymphopenia, increased neutrophil–lymphocyte ratio, decreased NK-cells and CD8 + T-cell activity, decreased regulatory T-cells, and increased CD4+ to CD8+ ratio. The failure to eliminate the virus due to inappropriate interferon (IFN) response and decreased number and function of CD8+ and NK-cells further leads to virus-induced tissue damage and makes the body prone to secondary bacterial infections.[29]

Colistin, fosfomycin, and vancomycin proved to be some very effective drugs in treating bacterial infections in COVID-positive cases. The administration of antibiotics in these coinfection subgroups is vital to combat the ongoing bacterial infection in the form of blood stream, urinary and respiratory infections as well as to avoid the increased chances of acquiring secondary bacterial infections in such comorbid patients. There was no significant history of any antibiotic or antimicrobial usage in these groups of patients. Providing the correct and narrowed antibiotic coverage through proper antibiotic sensitivity testing will look after the ill effects caused by a broader empiric treatment in such cases. The antibiotic stewardship programs will lead the path toward righteous treatment in the COVID infection group and will prevent the after-effects of long-term treatment.

| Table 1: Comparison of comorbidities and clinical outcomes in the two groups of COVID-positive patients (with and without bacterial coinfections) |
|-----------------|-------------------------------------------------|---------------------------------|-----------------|
| Clinical parameters | Cases with bacterial coinfection (n=53,%) | Cases without bacterial coinfection (n=966,%) | P |
| Age (mean, range in years) | 68 (8-75) | 59 (3-71) | 0.007 |
| Gender | | | |
| Males | 32 (61) | 609 (63) | 0.019 |
| Females | 21 (39) | 357 (37) | |
| Comorbidities | | | |
| Hypertension | 32 (61) | 628 (65) | 0.622 |
| Diabetes mellitus | 31 (58) | 608 (63) | 0.588 |
| COPD/asthma | 14 (27) | 280 (29) | 0.712 |
| CKD | 12 (23) | 174 (18) | 0.421 |
| CLD | 10 (19) | 203 (21) | 0.399 |
| Heart diseases | 5 (10) | 125 (13) | 0.512 |
| Malignancies (Organ/hematological) | 7 (14) | 87 (9) | 0.098 |
| Outcomes | | | |
| In-patient mortality | 21 (39) | 164 (17) | <0.001 |
| Use of ventilators | 18 (35) | 203 (21) | <0.001 |
| Use of vasopressive drugs | 17 (32) | 135 (14) | <0.001 |
| Intubation | 19 (37) | 251 (29) | 0.120 |

Figure 2: Antibiotic sensitivity pattern in gram-positive isolates (X-axis: percentage of antibiotic sensitivities, Y-axis: type of organisms)

Among the COVID cases, as such, no role of increase in bacterial coinfection rates per se has been described so far in these subgroups of patients.

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Discerning the clinical outcomes of our study, we noted a much higher rate of in-patient mortality in the bacterial coinfection COVID group (39%) as compared to the no infection group (17%). A similar study by Goncalves et al. reported a mortality rate of 50% in the COVID-19 patients with a concomitant bacterial infection. While, a study from Wuhan, China, also reported similar mortality rates in these groups of patients. The increase in the mortality rates may also be attributed to the older population involved with bacterial coinfections along with the presence of other comorbidities. The older population has a weakened immune system due to immunosenescence, and hence, succumb to the infections more easily than the younger ones. These people, therefore, more likely require interventions in the form of intubation, mechanical ventilation, and the use of vasopressor drugs to improve the outcome and increase their life expectancy. This fact was strongly suggested in our study where we noticed statistical significance in these COVID cases with bacterial coinfection for the use of ventilatory support, intubation, and use of vasopressors as compared to the no infection group.

**Conclusion**

A lot of aspects from pathogenesis to prevention still need to be explored in the current pandemic situation for a better understanding of its prevention and control. Bacterial coinfections not necessarily but certainly may increase the mortality rates in COVID-positive patients. This study will prove to be a useful and informative guide for primary care physicians in identifying the high-risk patients with bacterial coinfections and proper prescription of rational antibiotics in such COVID cases. The antibiotic prescription, as well as usage in this current situation, must be properly guided through proper culture reports, sensitivity testing, and stringent antibiotic stewardship programs.

**Ethics approval**

Informed consent was obtained from all the patients and their legal guardians (in the case of minors) regarding the publication of images and clinical information in the journal. They were informed of the confidentiality of the data, however, anonymity was not guaranteed.

**Author’s contribution**

S.S., A.P performed literature search, data analysis, and first draft of the manuscript and figures. SnS, SSP, AG performed laboratory work and investigations. CS, AG and UG contributed with the final draft of the manuscript and editing.

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**Conflicts of interest**

There are no conflicts of interest.

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