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ORIGINAL PAPER

Evaluation of Bacterial Co-Infections and Antibiotic Resistance in Positive COVID-19 Patients

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ABSTRACT

Aim: *Due to the fact that patients with COVID--19 can have a bacterial co-infection, physicians should be careful when prescribing antibiotics, with rather considering the sensitivity and resistance of these drugs than various bacteria. Therefore, the main purpose of the present study was to evaluate bacterial co-infections and antibiotic resistance in positive COVID-19 patients.*

Method: *This descriptive cross-sectional study was performed on 450 hospitalized COVID-19 patients who were selected by simple random sampling. Blood culture (BC) and endotracheal aspirate (ETA) were performed for all COVID-19 patients participating in the study. Antibacterial susceptibility was assessed using the standard Kirby-Bauer disk diffusion method on Mueller Hinton agar for all isolated strains in accordance with the Institute of Clinical and Laboratory Standards guidelines. Finally, susceptibility of all identified bacteria to 10 types of antibiotics was assessed.*

Results: *Based on the results of endotracheal aspirate (ETA) culture, we found that 79 (17.5%) patients had COVID-19 and bacterial co-infection. Among COVID-19 patients with bacterial co-infection,* Klebsiella species *had the highest frequency (21.6%), followed by Methicillin-sensitive* Staphylococcus aureus *(MSSA) (19%),* Escherichia coli *(17.7%), Methicillin-resistant* Staphylococcus aureus *(MRSA) (15.2%),* Enterobacter *species (13.9%) and* Pseudomonas aeruginosa *(12.6%), respectively. Based on the results of the present study, it was found that the level of antibiotic resistance for different bacteria varied from 0-100%.*

Conclusion: *The results of the present study indicate that patients with COVID-19 are susceptible to bacterial co-infection, which leads to the conclusion that excessive use of antibiotics is an important factor in the development of antimicrobial resistance. Therefore, caution is needed in prescribing different antibiotics*

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to patients with COVID-19. In addition, considering the SARS-CoV-2 co-infection with other pathogens, it is necessary to use an optimal treatment method for this purpose.

Keywords: COVID-19, co-infections, bacteria, antibiotics resistance.

INTRODUCTION

Coronavirus (CoV) is a ribonucleic acid (RNA) virus isolated from the family Coronaviridae and belonging to the order Nidovirales, which commonly causes respiratory and gastrointestinal infections. Complications may be mild to more severe, such as viral pneumonia with systemic dysfunction (1). In the last decade, CoV has been responsible for two major epidemics, acute respiratory syndrome (SARC) and Middle East respiratory syndrome (MERS), which killed 8,098 (mortality rate 10.5%) and 2,519 (mortality rate 34.4%) people (1, 2). Later on, Coronavirus 2019 (COVID-19) was first detected in December 2019 and spread from Wuhan, Hubei Province, China (2). The virus was initially named nCoV-2019 and subsequently renamed SARS-CoV-2, and eventually the associated disease was renamed COVID-19 (3).

Because the disease can cause serious problems in the human respiratory system, some patients need hospitalization and severe cases require intensive care with mechanical ventilation support (4, 5).

Bacterial co-infections are often found in viral infections of the respiratory tract such as the flu and they represent a major cause of death. Therefore, timely diagnosis and antibacterial treatment for such infections is essential (6-8). The frequency, incidence and characteristics of bacterial co-infections in COVID-19 patients are not known, so this is a large knowledge gap in critical situations like these ones (9-12). Although antibiotics are ineffective in treating COVID-19, physicians prescribe them to patients with suspected or confirmed COVID-19 for various reasons (13).

COVID-19 patients with severe bacterial infections during this pandemic received several instructions for the use of experimental antibiotic therapy (13). It is difficult to prevent bacterial co-infection when COVID-19 occurs, but there is also a high risk of secondary bacterial infection during the disease. For these reasons, there are concerns about the potential overuse of antibiotics and subsequent harmful consequences of bacterial resistance. Due to the increase in mortality among patients with severe bacterial infection during the influenza pandemic, various guidelines have been developed to support the use of experimental antibiotics for patients with severe COVID-19 (13).

Although COVID-19 mortality has occurred mainly in the elderly with severe underlying diseases (14), nosocomial pneumonia (NP) is a major risk factor for patients in intensive care units (ICU). In addition, NP may pose a greater risk to patients' health, especially when intubated. The presence of nosocomial infections (NIs) is commonly described as infections that occur during hospitalization within 48 to 72 hours of hospitalization and are spread mainly through contact, personal devices, and tools (15). The most common bacterial pathogens causing Nis include *Staphylococcus*, *Enterococcus*, *Klebsiella pneumoniae*, *Enterobacter*, *Escherichia coli*, *Acinetobacter* and *Pseudomonas* (16).

Due to the fact that patients with COVID-19 can have a bacterial co-infection, physicians should be careful when prescribing antibiotics, rather considering the sensitivity and resistance of these drugs than various bacteria (11). Therefore, the main purpose of the present study was to evaluate bacterial co-infections and antibiotic resistance in positive COVID-19 patients as a case study in India. ❑

METHOD

This descriptive cross-sectional study was performed on 450 patients admitted to an Indian hospital from April 25, 2020 to December 31, 2020, who were selected by simple random sampling. Participation eligibility was based on meeting the criteria in the questionnaire completed by each patient.

Study plan

Initial laboratory evaluations for study participants included complete blood count (CBC), erythrocyte sedimentation rate (ESR), arterial blood gas (ABG), lactate dehydrogenase (LDH), and C reactive protein (CRP). All patients were positive for SARS-CoV-2 in vitro swab samples using quantitative RT-PCR (qRT-PCR). Clinical laboratory findings were evaluated for all COVID-19 patients and finally all obtained information was recorded in a data collection form.

Isolation and identification of bacteria

Blood culture (BC) and endotracheal aspirate (ETA) were performed for all COVID-19 patients in the study. To identify and separate the bacteria from each other, swabs and blood were cultured on blood agar and McConkey agar plates and then incubated at 37 °C for 24 hours. Standard microbiological methods were used to identify isolated bacteria (17).

Evaluation of bacterial susceptibility to antibiotics

Antibacterial susceptibility was assessed using the standard Kirby-Bauer disk diffusion method on Müller-Hinton agar (Merk Co., Germany) for all isolated strains in accordance with guidelines of the Institute of Clinical and Laboratory Standards (CLSI; 2019, M100-S29). To test for the aforementioned discs from Gentamicin (10 micrograms), Vancomycin (30 mg), Trimethoprim/sulfamethoxazole (25 mg), Amikacin (30 mg), Tobramycin (10 mg), Cephalothin (30 mg), Norfloxacin (50 mg), and Ceftizoxime (30 mg) were used (18-20). ❑

RESULTS

The identified bacterial species isolated from COVID-19 patients as well as the abundance of each of the identified species are shown in Table 1. Among the 79 (17.5%) patients with COVID-19 and bacterial co-infection, the presence of *Klebsiella* spp., Methicillin-sensitive *Staphylococcus aureus* (MSSA), Methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli*, *Pseudomonas aeruginosa* and *Enterobacter* species was reveled in 10 (12.7%), 9 (11.4%), 5 (6.3%), 9 (11.4%), 5 (6.3%) and 6 (7.6%) subjects, respectively, according to the results of the endotracheal aspirate (ETA) culture, and in 7 (8.9%), 6 (7.6%), 7 (8.9%), 5 (6.3%), 5 (6.3%) and

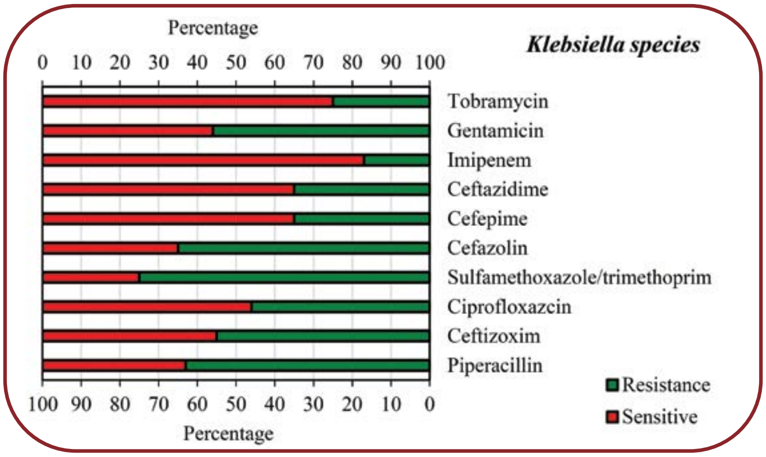

FIGURE 1. Antimicrobial susceptibility patterns of *Klebsiella* species isolated from COVID-19 patients

Bacterial species	Endotracheal aspirate culture		Blood culture		Total	
	Frequency					
	N (Person)	F (%)	N (Person)	F (%)	N (Person)	F (%)
Enterobacter species	6	7.6	5	6.3	11	13.9
Pseudomonas aeruginosa	5	6.3	5	6.3	10	12.6
Escherichia coli	9	11.4	5	6.3	14	17.7
Methicillin-resistant *Staphylococcus aureus* (MRSA)	5	6.3	7	8.9	12	15.2
Methicillin-sensitive *Staphylococcus aureus* (MSSA)	9	11.4	6	7.6	15	19
Klebsiella species	10	12.7	7	8.9	17	21.6
Total	44	55.7	35	44.3	79	100

TABLE 1. The frequency of bacterial species isolated from positive COVID-19 patients

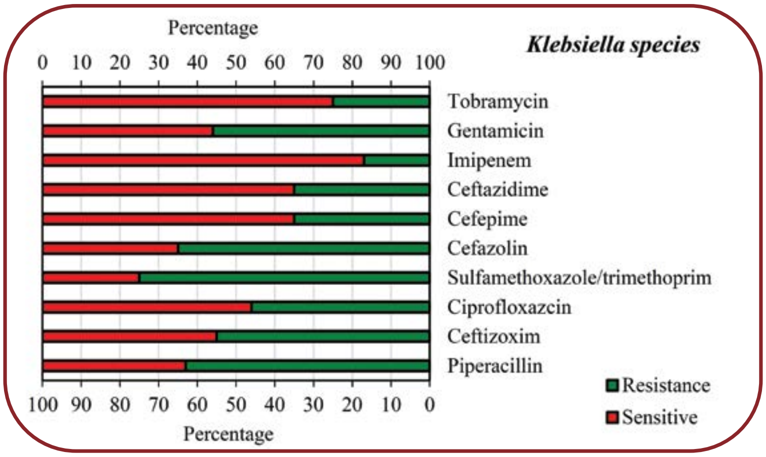

FIGURE 2. Antimicrobial susceptibility patterns of *Escherichia coli* isolated from COVID-19 patients

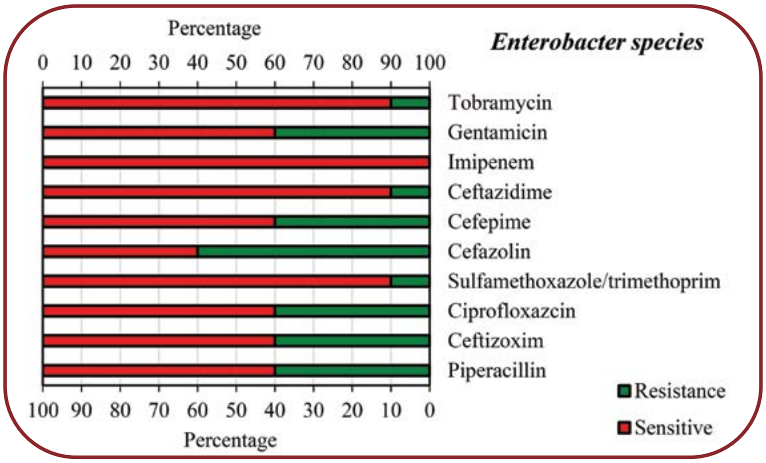

FIGURE 3. Antimicrobial susceptibility patterns of *Enterobacter* species isolated from COVID-19 patients

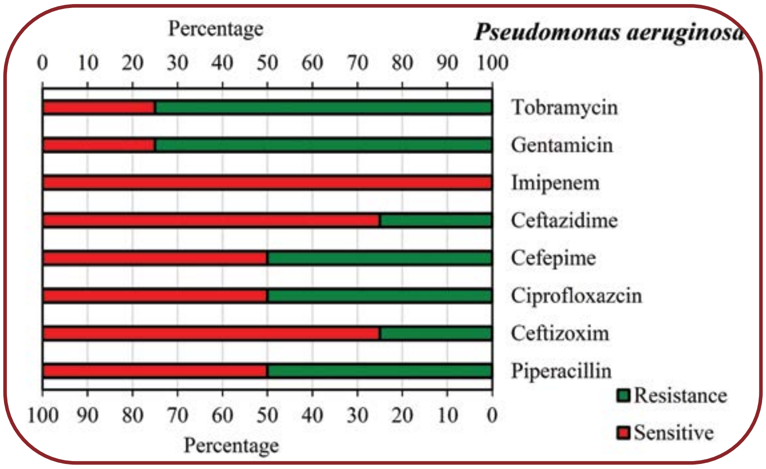

FIGURE 4. Antimicrobial susceptibility patterns of *Enterobacter* species isolated from COVID-19 patients

5 (6.3%) patients, respectively, according to results based on blood culture. Antimicrobial susceptibility patterns of various bacteria isolated from COVID-19 patients were shown in Figures 1 to 6. ❑

DISCUSSION

COVID-19, an unusually prevalent viral pneumonia, is considered a new concern and threat to public health worldwide. Based on the results of some previous studies, it has been determined that 2019-nCoV or SARS-CoV-2 originated from an animal source and later, by crossing the species barrier, eventually caused the infection of humans (20-24). The main purpose of the present study was to evaluate the burden of concurrent infections in 450 patients with COVID-19 in an Indian hospital. A total of 75 (17.5%) patients had secondary bacterial infection among the evaluated patients with COVID-19. The results of the present study showed that the most commonly identified bacteria isolated from endotracheal aspirate culture and blood culture included *Klebsiella* species, Methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli*, Methicillin-sensitive *Staphylococcus aureus* (MSSA), *Enterobacter* and *Pseudomonas aeruginosa*. Among people with COVID-19 who had a bacterial co-infection, *Klebsiella* species was the most frequent one (21.6%), followed by MSSA (19%), *Escherichia coli* (17.7%), MRSA (15.2%), *Enterobacter* species (13.9%) and *Pseudomonas aeruginosa* (12.6%), respectively.

According to the results of previous studies, hospitalization for any reason can increase the risk of secondary infections related to health care as well as the risk of transmission of multidrug-resistant diseases. In this regard, various microorganisms can increase the use of antibiotics (25, 26). A recent study in ICU across 88 countries found that although only 54% of patients had suspected or confirmed COVID-19, 70% of them had received at least one antibiotic to either treat or prevent bacterial infection (27). In Norton's study conducted at Montefiore Medical Center (New York), 71% of more than 5,800 COVID-19 patients who had been hospitalized from March to May 2020 received at least one dose of antibiotics (28). Results of studies carried on by Norton (28) and Getahun *et al* (27) showed that about 72% of hospitalized COVID-19 patients received various antibiotics, while only 8% of them had severe bacterial or fungal infections.

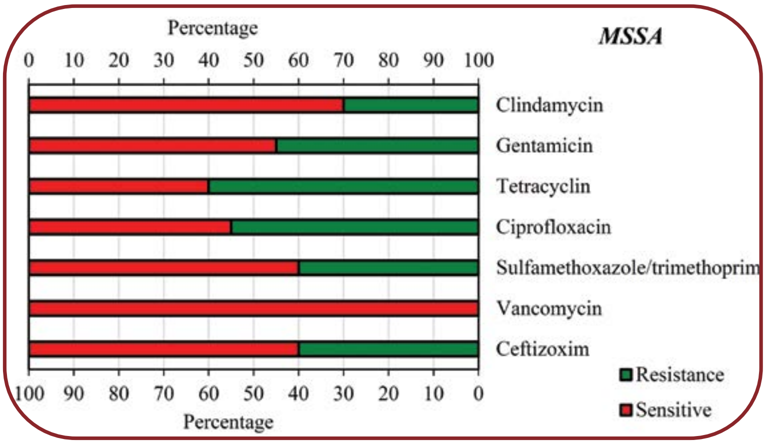

FIGURE 5. Antimicrobial susceptibility patterns of Methicillin-sensitive *Staphylococcus aureus* (MSSA) isolated from COVID-19 patients

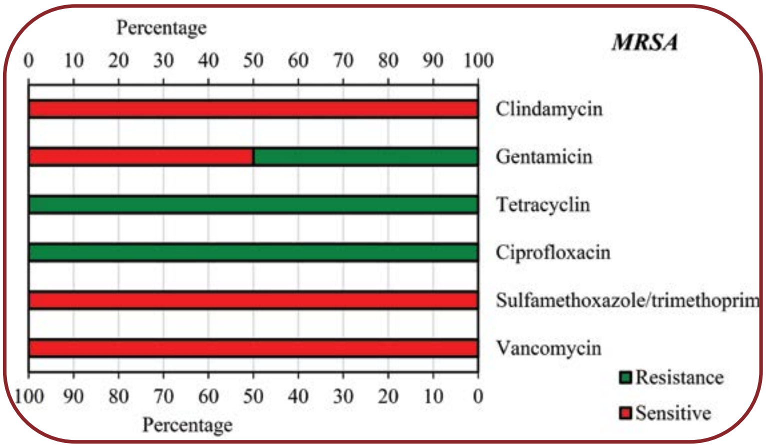

FIGURE 6. Antimicrobial susceptibility patterns of Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from COVID-19 patients

The results of the present study in terms of the amount and type of bacterial co-infection were consistent with those reported by previous studies, but not also with some others. Sharifipour *et al* (2020) found that COVID-19 patients admitted to ICU had a secondary infection with *Acinetobacter baumannii* and two strains of *Staphylococcus aureus* (21). Mahmoudi (2020) also showed that 12.5% of 340 COVID-19 patients had a secondary bacterial infection, most commonly with *Klebsiella* spp, MRSA, *E. coli*, MSSA, *Enterobacter* and *Pseudomonas aeruginosa* spp (20). In terms of bacterial species, the results of Mahmoudi's study were very similar to ours, while the amount of secondary bacterial infection in the present study was higher than that reported in the above-mentioned study (17.5% *vs.* 12.5%). In this regard, the results obtained by Yang *et al* in their research (2020) showed that 13.5% of patients with COVID-19 had nosocomial infections; among them, one (2%) patient had a pulmonary and blood infection with *K. pneumoniae*. Other microorganisms identified from respiratory secretions of five (10%) patients with secondary infections included *Aspergillus flavus*, *Aspergillus fumigatus*, extended-spectrum β-lactamase (ESBL)-positive *K. pneumoniae*, ESBL-positive *P. aeruginosa*, and SBL-negative *Serratia marcescens* (29). Xavier Lescure *et al* (2020) reported that patients with evaluated COVID-19 had two pathogens, including antibiotic-susceptible *A. baumannii* and *A. flavus* (30).

Chen *et al* (2019) reported that some COVID-19 patients, especially with severe forms, had a bacterial co-infection. The results of their study revealed that the usual bacterial cultures of patients with secondary infections identified *A. baumannii* and *K. pneumoniae*, with the first one being more resistant to antibiotics than the latter (31). Based on the findings of Xing *et al* (2020), *Mycoplasma pneumoniae* and *Legionella pneumophila* were the most commonly identified respiratory pathogens in COVID-19 patients, with prevalence rates of 23.3% and 20%, respectively (32). Based on a systematic review performed by Lansbury *et al* (2020), about 7% of hospitalized COVID-19 patients had a bacterial co-infection, and the most commonly reported strains included *M. pneumoniae*, *P. aeruginosa* and *Haemophilus influenza* (24). In the UK, Toombs *et al* (2020) reported that two COVID-19 patients had co-infection with *Staphylococcus pneumoniae* (33). The results of all the above-mentioned studies (20-33) support the hypothesis of secondary infection in COVID-19 patients.

According to data from studies on the use of antibiotics in the treatment of COVID-19 patients, an average of 70% of patients receive antibiotics. However, given that the improper use or overuse of antibiotics is an important factor in the development of antimicrobial resistance, physicians should be very careful when prescribing various antibiotics to COVID-19 patients. Therefore, many efforts have been already made against antimicrobial resistance to reduce inappropriate or excessive use of antibiotics (34). In addition, during the COVID-19 pandemic, soaps and antimicrobial disinfectants have been increasingly used by hospital staff. If these products are based on chlorhexidine digluconate or quaternary ammonium compounds, this can also lead to antibiotic resistance (20).

The present study showed that COVID-19 patients with secondary bacterial infections were highly resistant to some common antibiotics for isolated bacteria. According to our findings, *Klebsiella* species showed the highest resistance to Sulfamethoxazole/trimethoprim (75%), Cefazolin (65%), Piperacillin (63%), Gentamicin (56%) and Ceftizoxim (55%), respectively, while other antibiotics showed less resistance (<50%) to this bacterium. Regarding *Escherichia coli*, the highest resistance was seen towards Ciprofloxazcin (65%), Cefazolin (54%), Ceftazidime (54%) and Ceftizoxim (54%), while for other antibiotics, the resistance level was less than 50%. *Enterobacter* species had a high resistance (60%) only to Cefazolin, 10-40% resistance to other antibiotics and no resistance to Imipenem. *Pseudomonas aeruginosa* showed 75% resistance to Gentamicin and Tobramycin, 50% to Cefepime, Ciprofloxazcin and Piperacillin, 25% to Ceftizoxim and Ceftazidime, but no resistance to Imipenem. MSSA showed a high resistance to Tetracyclin (60%) and Ciprofloxacin (55%), moderate resistance to other antibiotics (30-45%) and no resistance to Vancomycin, while MRSA showed the highest resistance (100%) to Ciprofloxacin and Tetracyclin, 50% resistance to Gentamicin and no resistance to Vancomycin, Sulfamethoxazole/Trimethoprim, and Clindamycin.

The study of Mahmoudi (2020) found that enterobacteriaceae isolates from COVID-19 patients had the highest resistance to Cotrimoxazole (74%), Piperacillin (67.5%), Ceftazidime (47.5%) and Cefepime (42.5%). In addition, all isolates were susceptible to Amikacin (100%). *S. aureus* isolates were susceptible to Vancomycin (100%) and the rates of resistance to Oxacillin, Erythromycin and Clindamycin were higher than 90%. *P. aeruginosa* was susceptible (90%) to Imipenem (20).

Our results together with those reported by Mahmoudi (20) show that the excessive use of antibiotics without following medical instructions as well as the use of various antiseptics, disinfectants and detergents can increase not only antibiotic resistance for different bacteria, but also the health-related risks of COVID-19 patients, the length of hospital stay as well as hospital staff's workload.

Study limitations

One of the main limitations of the present study was the low number of samples taken for the culture and identification of bacteria. Other limitations include the fact that the study was conducted in a single hospital setting and did not review previous records of bacterial infection. ❑

CONCLUSION

The results of the present study show that hospitalization for any reason, especially COVID-19, can increase the risk of secondary bacterial infections related to health care as well as that of transmission of multidrug-resistant diseases. Also, our findings enable us to conclude that excessive use of antibiotics is an important factor in the development of antimicrobial resistance. Therefore, caution is needed when prescribing antibiotics to COVID-19 patients. In addition, considering the co-infection of SARS-CoV-2 and other pathogens requires the use of an optimal treatment approach. ❑

Conflicts of interest: none declared.
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