Evaluation of Bacterial Co-infections of the Respiratory Tract in COVID-19 Patients

Gurung K¹, Pant P², Pokhrel S³, Neupane DR¹, Gurung S³

ABSTRACT

Introduction: The coronavirus disease 2019 (COVID-19) pandemic is caused by severe acute respiratory syndrome coronavirus 2. The co-evolution of viral and bacterial respiratory pathogens has created an environment in which a viral infection allows concurrent or secondary bacterial co-infections. Aims: This study was carried out to isolate the different respiratory bacterial pathogens from COVID patients and evaluate their antibiotic sensitivity pattern. Methods: Hospital based descriptive cross sectional study was conducted over a period of between 2nd Jestha to 5th Shrawan 2078 (16th May to 20th July 2021) in Nepalgunj Medical college, Kohalpur during the second wave of COVID-19 in Nepal. The sputum was inoculated onto different culture plates such as Blood Agar (BA) and MacConkey Agar (MA) and incubated at 37 °C. After overnight growth and in Gram staining, different Gram’s positive and negative bacteria were observed. Antibiotic sensitivity test was performed by Kirby-Bauer Disk Diffusion method. Results: Out of 100 sputum samples, 44% positive bacterial growths, majority isolates were Klebsiella spp and Staphylococcus aureus. Overall, the pathogens were mostly sensitive to meropenem followed by colistin, piperacilin/tazobactam respectively. Conclusion: Bacterial co-infection is common in hospitalized patients with SARS-CoV-2.

Keywords: Antibiotic sensitivity tests, Bacterial co-infections, COVID-19

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INTRODUCTION

A novel strain of corona virus, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was identified as the causative agent of viral pneumonia in December 2019 in Wuhan, China. The disease was later named corona virus disease 2019 (COVID-19). SARS-CoV-2 is an enveloped positive-sense single-stranded RNA virus. COVID-19 has resulted in the largest mobilization of public health resources. At the time of writing this report, the virus had infected 226 million people resulting in over 4.6 million deaths globally.

Respiratory viral infections have been well known to predispose patients to co-infections and lead to increased disease severity and mortality as was observed in the 1918 influenza outbreak, where most mortalities were due to bacterial infection. The co-evolution of viral and bacterial respiratory pathogens allows concurrent or secondary bacterial co-infections, leading to increased morbidity and mortality. At the beginning of the pandemic, with the panic of facing many existing medicines were repurposed to treat the virus. This included widespread use of antibiotics in treatment. In developing countries, where there is high burden of multidrug-resistant organisms in ICU settings, super-infections in COVID-19 patients can pose a biggest challenge leading to increase mortality. Effective antibiotic stewardship limits the unnecessary use of antimicrobials and is “need of the hour.” Super-infections are well documented in influenza and other respiratory viral illnesses. The incidence of bacterial co-infection in COVID-19 ranges from 3% to 30%.

A study showed that predominant co-infection was hospital acquired Gram negative organisms. Hence the study was conducted to assess the association of bacterial co-infection
METHODS

This study was a hospital-based descriptive cross-sectional study. It was conducted over a period of between 2nd Jestha to 5th Shrawan 2078 (16th May to 20th July 2021) in Nepalgunj Medical College, Kohalpur during the second wave of COVID-19 in Nepal. About 100 COVID patients proved by reverse transcription polymerase chain reaction (RT-PCR) were enrolled in this study. The informed consent was obtained from all the subjects and their relatives. Sputum samples were collected in leak-proof containers, tightly sealed and transported in leak-proof zip plastic bags. Patient name, hospital ID number, date, and time of collection were written on the sample container for proper and details of patients were recorded. The sputum samples were transferred to the microbiology laboratory and processed immediately. Samples were processed following the standard protocol of bio-safety.

The sputum was inoculated onto different culture plates such as Blood Agar (BA) and MacConkey Agar (MA) and incubated for overnight at 37°C. After overnight, growth was observed, smear was prepared from bacterial colony and simultaneously transfer to peptone water. The smear was fixed by heat and stained by Gram’s Method. The smears were examined under light microscope than using oil immersion lens. Gram stain was used to observe for presence of different Gram’s positive and Gram’s negative bacteria.

For Gram’s positive cocci, catalase, coagulase tests were done and, Gram’s negative bacilli different biochemical tests; indole, triple sugar iron agar, citrate urease and oxidase were performed. Finally, antibiotic sensitivity test was performed by Kirby-Bauer Disk Diffusion method.

RESULTS

The total numbers of cases were 100. Their age ranged from 17-83 years. The male to female ratio was 2.3:1. There were 14 patients’ falls under 0-30 age group, 64 patients in the 31-60 age group followed by 22 patients in the 61-90 age groups as shown in Table I.

<table>
<thead>
<tr>
<th>Age</th>
<th>Gender</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-30</td>
<td>Male</td>
<td>6 (42%)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>8 (58%)</td>
</tr>
<tr>
<td>31-60</td>
<td>Male</td>
<td>46 (72%)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>18 (28%)</td>
</tr>
<tr>
<td>61-90</td>
<td>Male</td>
<td>18 (82%)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>4 (18%)</td>
</tr>
<tr>
<td>Total</td>
<td>Male</td>
<td>70 (70%)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>30 (30%)</td>
</tr>
</tbody>
</table>

Table I: Age and sex wise distribution of the study subjects

Out of 100 patients, bacterial pathogens were isolated from 44 (44%) samples. Of the 44 positive bacterial growths, majority isolates were Klebsiella spp. (63%) followed by Staphylococcus aureus (31%), Pseudomonas aeruginosa (18%), Acinetobacter spp. (4%) and Enterobacter spp (4%). More information about isolation of potential respiratory pathogens is presented in Table II.

<table>
<thead>
<tr>
<th>Respiratory pathogens</th>
<th>Total Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acinetobacter spp.</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>Enterobacter spp.</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>Klebsiella spp.</td>
<td>28 (63%)</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>8 (18%)</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>14 (31%)</td>
</tr>
<tr>
<td>Total</td>
<td>44</td>
</tr>
</tbody>
</table>

Table II: Characteristics of potential respiratory pathogens \(n = 44\) in 100 patients hospitalized with COVID-19

In this study, there were 16 antibiotics were used for sensitivity test. They were amikacin (AK), amoxicillin/clavulanate (AMC), ampicillin/salbactam (A/S), azithromycin (AZM), clindamycin (CD), cefixime (CFM), colisitin (CL), cotrimoxazole (COT), ceftriaxone (CTR), doxycycline (DO), levofloxacin (LE), moxifloxacin (MO), meropenem (MRP), ofloxacin (OF), piperacilin/tazobactam (PIT), tobramycin (TOB). Klebsiella spp. was the major isolate, which was most sensitive to meropenem (67%) and colistin (67%) followed by piperacilin/tazobactam (56%), similarly sensitive to amoxicillin/clavulanate, amikacin, doxycycline, tobramycin 56%, 50%, 44% and 44% respectively. Overall, the pathogens were mostly sensitive to meropenem followed by colistin, piperacilin/tazobactam respectively. Indeed, Table III shows that 44% of COVID-infected patients (44/100) were treated with broad-spectrum antibiotics who had different pathogens isolated from sputum samples.
Table III: Percentage of antibiotic sensitivity to different pathogens

**DISCUSSION**

This study was done to isolate of potential respiratory pathogens and evaluate the current antibiotic susceptibility pattern. Overall males (70%) were higher than females (30%). Although several different species can cause respiratory infection, most infections in all populations are caused by the Gram negative and this current study showed higher percentage of encountered pathogens are Gram negative bacteria. The study done by Singh V also suggested higher number of co-infected pathogens are Gram negative bacteria. A study done by Cheng LS has reported that organisms isolated from 42% of patients who underwent testing for respiratory infections. The isolation percentage of pathogens compared to this current almost similar. The current study suggested that common pathogens are Klesbiella spp, Staphylococcus aureus, Pseudomonas aeruginosa, Acinetobacter and Enterobacter, which is agreed with study done by Fattorini L. An article also suggested the similar type of respiratory pathogens which is covered in this current study. This current study also suggested that hospitalization and intubation are more important than COVID-19-specific effects in conferring susceptibility to specific pathogens.

The widespread use of antibiotics for the treatment of COVID-19 patients and the potential consequences for antimicrobial resistance are a growing concern. Antibiotics use may be warranted in managing COVID-19 patients with suspected bacterial co-infections and severe/critically ill patients with increased risks of developing bacterial co-infections due to long hospital stays or immunosuppression. The current study showed several group of antibiotic are becoming resistance to pathogens such as quinolone, cephalosporin, penicillin.

**LIMITATIONS**

The current study was only focused on COVID-19 patients, not in non- COVID-19 patients. The comparison between COVID-19 and non- COVID-19 patients could give better idea of isolation of microorganisms and sensitivity to antibiotics.

**CONCLUSION**

Bacterial co-infection is common in hospitalized patients with SARS-CoV-2, with Klesbiella spp having a major one and usage of antibiotics was high. The study suggests that meropenem followed by colistin, piperacilin/tazobactam has relatively better susceptibility to pathogens that can be chosen for the empirical treatment.

**REFERENCES**


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