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Pattern of Microbial Growth with Antibiotic Susceptibility in Sputum Sample of Hospitalized COVID-19 Patients in a Tertiary Care Hospital of Bangladesh

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Abstract

Background: Bacterial co-infections are common complications of many viral respiratory tract diseases, such as influenza, and the same is presumed to be true in Corona Virus Disease-19 (COVID-19). Co-pathogens of other viral diseases are predominantly bacteria, followed by fungus. These co-infections play an important role in the progression of COVID-19 infections by escalating the severity and mortality rate. Antibiotics are therapeutic agents to combat these microbial infections. But their irrational use can promote antimicrobial resistance (AMR). During the COVID-19 pandemic, empiric antibiotic therapy was observed throughout the globe without any prior knowledge of bacterial co-infection patterns and their antimicrobial susceptibility.

Materials and Methods: This was a prospective study conducted in the microbiology diagnostic laboratory of Sylhet Women's Medical College and Hospital in collaboration with the COVID-19 isolation unit of this institute. The study period was from June 2021 to November 2021 (six months). A total of 535 RT-PCR-positive confirmed COVID-19 cases were enrolled in the study. Sputum samples were collected from them for microbiological analysis. The culture and sensitivity patterns of the isolated microbes from the samples were evaluated through standard microbiological procedures.

Results: Out of 535 samples, 150 samples were culture positive (28%). Male to female ratio among culture-positive patients was 1.3:1. Patients in the 60 to 69 years age group showed the highest culture-positivity rates (22.67%). About 24.5% of the isolates were pathogenic bacteria. Among them, *Klebsiella pneumoniae* had the highest positivity rates (38.7%) followed by *Staphylococcus aureus* (30.0%), *Streptococcus* species (11.3%), and *Pseudomonas* spp (7.3%). Growth of fungus and normal flora was found in 10.7% and 2% of the samples respectively. The top sensitive antimicrobials for *Klebsiella pneumoniae* were amikacin (89.7%), colistin (91.4%), amoxicillin and clavulanic acid (72.4%), meropenem (94.8%), imipenem (89.7%), piperacillin and tazobactam (91.4%) levofloxacin (79.3%) and ciprofloxacin (77.6%). Most of the organisms are resistant to azithromycin, cefixime, cefuroxime, ceftazidime, linezolid, ceftriaxone, vancomycin, and doxycycline.

Conclusion: Regular microbiological evaluation should be done for COVID-19 pneumonia infection in order to develop an effective therapeutic guideline.

Keywords: *Sputum; Microbial Culture Growth; COVID-19; Antibiotic Sensitivity; Klebsiella pneumoniae*

Introduction

Corona or SARS-COV-2 (Severe Acute Respiratory Syndrome COVID-2) virus is an enveloped single-stranded positive polarity RNA virus belonging to the family of coronaviridae. The virus responsible for coronavirus disease was first recorded in December 2019 in Wuhan, China. Afterward, in March the world health organization (WHO) announced it was a pandemic [1]. SARS-COV-2 is a beta strain of the coronavirus family that causes severe acute respiratory syndrome. The prominent symptoms of COVID-19 infections are fever, cough, tiredness, and dyspnea [2,3]. Co-infection of SARS-CoV-2 with microbial pathogens is a crucial factor in the development of COVID-19, making the diagnosis, management, and prognosis difficult [4].

Many hospitalized patients have been affected dangerously as a fatality of the disease, and COVID-19 cases have dramatically risen, with 445 million confirmed cases and 6 million deaths globally [3,4,6]. A few days after the initial SARS-CoV-2 infection, critically ill patients often develop respiratory tract distortion or pulmonary dysbiosis, which can further develop into a secondary bacterial or fungal infection. Lung pathology shows viral destruction, bacterial superinfection, immune-mediated endothelium, and micro thrombosis [2,6,7]. Bacterial infections were spread through contact with hospitalized patients, healthcare workers, and hospital equipment, either directly or indirectly [8,9]. Antimicrobial resistance (AMR) emerges and spreads as a result of increased antibiotic use, posing a serious worldwide health concern [12]. The widespread use of empirical medication in hospitalized COVID-19 patients has resulted in significant AMR among bacterial isolates [5,16]. The World Health Organization (WHO) does not suggest routine antibiotic treatment for COVID-19, even if the condition is modest and there is no clinical suspicion of bacterial infection [14]. The COVID-19 pandemic exemplifies the potential long-term effects of AMR, which are less severe but no less relevant because their measurements and outcomes are comparable [4]. High AMR rates are another growing concern around the world, and efforts are being undertaken to address this issue [15]. The incidence of antibiotic resistance differs from region to region because of the effects of antibiotic use [13,14]. AMR is a severe problem in high-income countries. Each year, 2.8 million antibiotic-resistant infections are recorded in the United States, with over 35,000 deaths [18]. The overuse of antibiotics is most likely due to a fear of bacterial co-infections of the respiratory system [15]. One of the most essential recommendations for addressing this issue is to optimize antibiotic use by ensuring that the proper antibiotic is administered at the appropriate dose, for the appropriate period, and in a manner that assures the best outcome while minimizing side effects and AMR [12]. In the absence of specialized therapy, current antimicrobials are used to treat critically ill COVID-19 patients. Hospital-acquired infections have been associated with *Acinetobacter* species, *Enterobacter* species, *Enterococcus* species, *Escherichia coli, Klebsiella pneumoniae*, *Pseudomonas* species, and *Staphylococcus* species [20]. According to the Infectious Disease Society of America [21], the major causes of death from infection in critical care units include *Escherichia coli, Klebsiella*, multidrug-resistant *Pseudomonas*, and carbapenem-resistant *Acinetobacter* species [22]. Bacterial infection of the lower respiratory tract is one of the primary causes of death in COVID-19, causing more damage to the respiratory system, a loss in pulmonary function, and severe hypoxia, all of which have a domino effect on the body [16]. Antimicrobials have numerous potential functions in the therapy of COVID-19 [23]. Given medication dosing for respiratory tract infections, some SARS-CoV-2 infected patients who report to the hospital have a clinical profile similar to that of atypical bacterial pneumonia [23-26]. About one-fifth of SARS-CoV-2 patients had coinfection, and a majority of the patients were not admitted to the intensive care unit (ICU), despite the fact that intensive care unit patients had a higher frequency of superinfections (41%) [27]. The majority of the early studies came from China, indicating the requirement for larger study numbers, a broader geographic spread, and longer patient follow-ups [28-30]. Lower respiratory tract bacterial infection affects 25% to 30% of ICU patients, with proportionally high mortality (22 - 71%) and morbidity [31-34]. Several studies on the outbreak of bacterial co-infections with COVID-19 patients have revealed highly heterogeneous distributions (more than 50% differences) that can be attributed to clinical and epidemiological characteristics of each geographic location, as well as diagnostic methods and criteria used [35-37]. Iranian researcher Mustafa Awad found that sputum culture was the most effective way to diagnose bacterial co-infection in the lower respiratory tract [16].

Therefore, a significant public health problem is prolonged antibiotic therapy for secondary infections in COVID-19 users [38]. It is critical for clinicians treating ICU patients to have access to frequently updated hospital antibiograms to rationally administer antibiot-

ics [39]. This can ensure that effective antibiotic medications are delivered for new strains of the same bacterial species based on locally accepted parameters, ensuring pathogenic bacteria eradication success [11]. To combat antimicrobial resistance and enhance the quality of care for patients with illnesses by maximizing clinical outcomes while avoiding toxicity, the guideline justifies the prudent use of antibiotics in hospitals [40]. Culture and sensitivity tests assist us in determining which organisms are susceptible to specific drugs [41]. However, the epidemiological value of sputum cultures as a tool in providing information about the microbiological profile and antibiotic sensitivity pattern in different geographical areas of the world cannot be underestimated. The current observational study was carried out in a tertiary care hospital in Bangladesh that aimed to evaluate the prevalence of common microbial growth with associated antibiotic sensitivity patterns in positive sputum cultures among hospitalized COVID-19 patients.

Materials and Methods

Study setting and duration

This prospective observational study was conducted in the microbiology diagnostic laboratory of Sylhet Women's Medical College and Hospital in collaboration with the COVID-19 isolation unit of this institute. The study period was from June 2021 to November 2021 (six months). A total of five hundred and thirty-five (535) RT-PCR-positive COVID-19 patients were enrolled in the study. Sputum samples were collected from them for microbiological analysis.

Patient selection

Inclusion criteria:

1. Sample from RT-PCR positive cases.
2. Patients aged 18 or above.

Exclusion criteria:

1. Patients on antibiotics therapy within the previous 72 hours.
2. Patients with an active or previous history of pulmonary tuberculosis.
3. Previously hospitalized COVID-19 patients.

Study techniques

Samples were collected from COVID patients by maintaining the standard guidelines for COVID-19 infections and transferred to the microbiology lab for microbiological analysis. Initially, Gram staining was done before inoculating on the culture plates. The samples were placed in the biosafety cabinet and subsequently inoculated on MacConkey, blood, and chocolate agar. After 24 hours of incubation at 37°C, the culture plates were observed for bacterial growth and positive samples were subjected to further laboratory tests. Negative culture samples were reported as negative if no growth was detected after 48 hours. The growth patterns were noted. For bacteriological identification, colonies were gram stained and observed under a microscope for detailed features. The bacteria were identified by colonial morphology, gram's staining results, and biochemical profiling as relevant. The culture and sensitivity patterns of the isolated microbes from the samples were evaluated through standard microbiological procedures.

Antibiotic susceptibility testing

Isolated organisms were tested for antibiotic susceptibility as per the clinical and laboratory standard institute recommendations (CLSI). The antibiotic sensitivity test was performed on Mueller-Hinton agar media by standard Kirby-Bauer disc diffusion method. The agar plates were incubated for 24 hours at 37°C and then observed for the zone of inhibition around the disc and the organisms were classified as sensitive or resistant. The following antibiotic discs were used: amoxicillin and clavulanic acid, amikacin, azithromycin, cefaclor, cefixime, cefuroxime, ceftazidime, linezolid, ceftriaxone, vancomycin, gentamicin, doxycycline, ciprofloxacin, colistin, meropenem, imipenem, piperacillin, and tazobactam.

Ethical approval

Informed written consent was taken from every patient before final enrollment and data collection. Approval of the protocol was obtained from the Institution of Ethical Committee of the Institution.

Statistical analysis

Results were expressed in frequency, and percentages and analyzed for statistical significance by Chi-Square test. We used the significance level as 5% level and a p-value < 0.05 was considered statistically significant. For entering and analyzing the data we used Statistical Package for Social Science (SPSS) version 26.

Results

In this observational study, the common microbial growth with their antibiotic sensitivity patterns in the sputum sample of COVID-19 patients was observed over a period of six months. Among 535 enrolled patients, 150 (28%%) were positive, 385 (72%) sputum tests were negative (Figure 1), and of them 87 (58%) patients were male and 63 (42%) were female. The proportion of males to females was 1.3:1 (Figure 2). Based on the results of the sputum test, this study found that a maximum of 38.7% of the culture growth was *Klebsiella pneumonia* followed by *Staphylococcus aureus* 30%. *Streptococcus pneumoniae* 11.3%. Fungus (*Candida* spp.) were found at around 11%, *Pseudomonas* spp. and Normal flora was only 7% and 2% of growth respectively (Figure 3 and table 1). The majority of study subjects belonged to the age group of 60 - 69 years 34 (22.7%) and the second highest was found in the 50 - 59 years 31 (20.7%). The result also revealed that the maximum culture growth of *Klebsiella pneumoniae* was observed in the 50 - 59 years age group and the lowest culture growth was found in the age group of 20 - 29 years (Figure 4 and table 2). These findings show that older people are more vulnerable to bacterial co-infections. In this study, there were 18 antibiotics used for the sensitivity test. Amoxicillin, clavulanic acid, amikacin, azithromycin, cefaclor, cefixime, ceftriaxone, ciprofloxacin, colistin, doxycycline, gentamicin, imipenem, levofloxacin, linezolid, meropenem, piperacillin and tazobactam, and vancomycin. The study showed the highest culture positivity rate for *Klebsiella pneumoniae* which is a gram-negative organism followed by *Staphylococcus aureus* which is a gram-positive organism. Maximum of the bacterial pathogens were highly sensitive to amoxicillin and clavulanic acid, amikacin, cefaclor, ciprofloxacin, piperacillin and tazobactam, meropenem, gentamicin, imipenem, colistin and resistant to azithromycin, cefixime, cefuroxime, ceftazidime, linezolid, ceftriaxone, and vancomycin, doxycycline.

Figure 1 presents the culture's positive status. 150 (28%%) COVID-19 patient sputum tests were positive, and 385 (72%) of the COVID-19 patient's sputum tests were negative.

Figure 2 represents the gender-based status of the COVID-19 patient, among 150 of culture positive sputum87 (58%) of the patients were males and 63 (42%) of the patients were females.

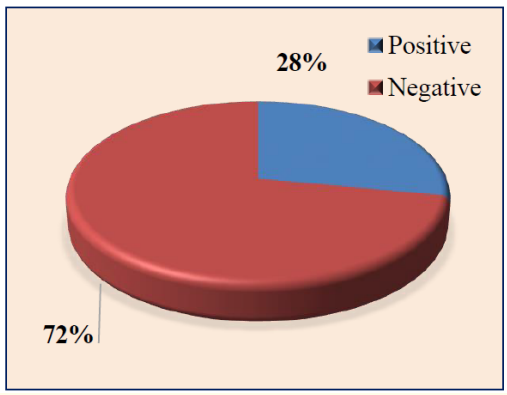

Figure 1: *Culture-positive rate of COVID-19 patients.*

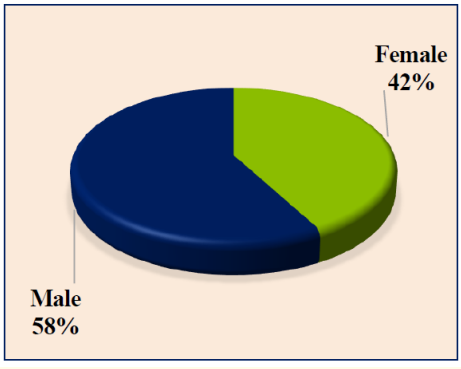

Figure 2: *Gender-based sputum test status of the COVID-19 patient.*

Figure 3 shows the percentage of culture growth of the sputum positive COVID-19 patients. Based on the reports of the sputum test, this study found that a maximum of 38.7% of the culture growth was *Klebsiella pneumonia* followed by *Staphylococcus aureus* 30%. *Streptococcus pneumoniae* 11.3%. Fungus (*Candida* spp.) were found at around 11%, *Pseudomonas* spp. and Normal flora was only 7% and 2% of growth respectively (See below table 1).

SL no	Culture Name	Frequency	Percent(c/o)
1	*Klebsiella pneumoniae*	58	38.7
2	*Staphylococcus aureus*	45	30.0
3	*Streptococcus pneumoniae*	17	11.3
4	*Pseudomonas species*	11	7.3
5	Fungus (candida spp)	16	10.7
6	Normal Flora	3	2.0
	Total	150	100

Table 1: *Culture growth of (common pathogen) sputum sample of the COVID-19 patient.*

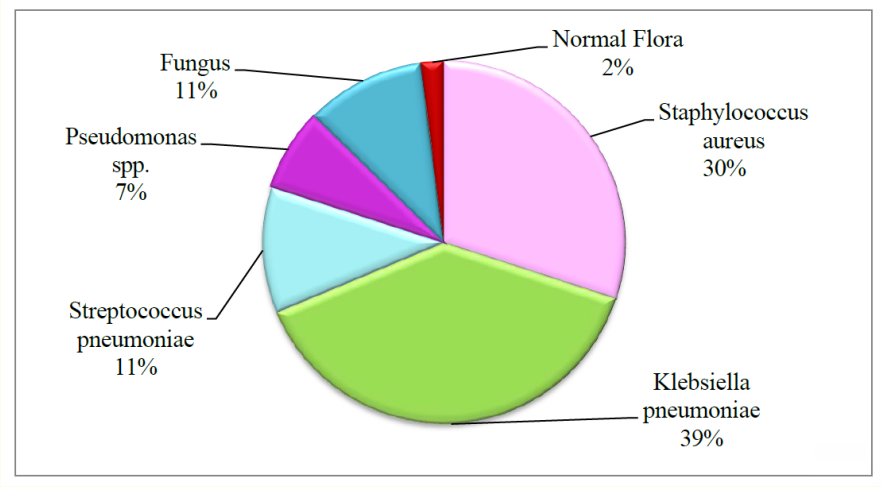

Figure 3: *Culture growth of the sputum positive COVID-19 patient.*

Figure 4 illustrate the age distribution and sputum culture-positive isolates of COVID-19 patients. The graph shows the majority of study subjects belonged to the age group of 60 - 69 years 34 (22.7%) and the second highest was found in the 50 - 59 years 31 (20.7%). The result also revealed that the maximum culture growth of *Klebsiella pneumoniae* was observed in the 50 - 59 years age group and the lowest culture growth was found in the age group of 20 - 29 years (Figure 4 and table 2). These findings show that older people are more vulnerable to bacterial co-infections. Similarly, "30-39" age group was 22 (14.7%), "40 - 49" age group was 20 (13.3%), "70 - 79" age group was 19 (12.7%), and "80 and above" age group was 13 (8.7%) of the growth (See below table 2).

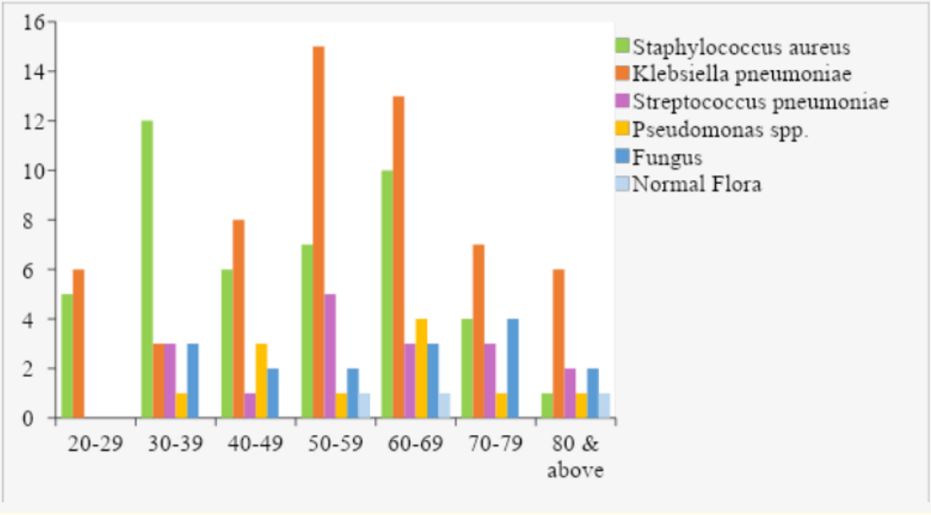

Figure 4: *Age distribution and sputum culture positive isolates of COVID-19 patients.*

		Culture growth						
		Staphylococcus aureus	***Klebsiella pneumoniae***	***Streptococcus pneumoniae***	***Pseudomonas* spp.**	**Fungus**	**Normal Flora**	**Total**
Age group	20-29	5 (45.5)	6 (54.5)	-	-	-	-	11 (7.3)
	30-39	12 (54.5)	3 (13.6)	3 (13.6)	1 (15.0)	3 (13.6)	-	22 (14.7)
	40-49	6 (30.0)	8 (40.0)	1 (5.0)	3 (15.0)	2 (10.0)	-	20 (13.3)
	50-59	7 (22.6)	15 (48.4)	5 (16.1)	1 (3.2)	2 (6.5)	1 (3.2)	31 (20.7)
	60-69	10 (29.4)	13 (38.2)	3 (8.8)	4 (11.8)	3 (8.8)	1 (2.9)	34 (22.7)
	70-79	4 (21.1)	7 (36.8)	3 (15.8)	1 (5.3)	4 (21.1)	-	19 (12.7)
	80 and above	1 (7.7)	6 (46.2)	2 (15.4)	1 (7.7)	2 (15.4)	1 (7.7)	13 (8.7)
	Total	45 (30.0)	58 (38.7)	17 (11.3)	11 (7.3)	16 (10.7)	3 (2.0)	150

Table 2: *Age distribution and sputum culture positive isolates of COVID-19 patients.*

In this study, there were 18 antibiotics used for the sensitivity test. Amoxicillin, clavulanic acid, amikacin, azithromycin, cefaclor, cefixime, ceftriaxone, ciprofloxacin, colistin, doxycycline, gentamicin, imipenem, levofloxacin, linezolid, meropenem, piperacillin and tazobactam, and vancomycin.

Table 3 presents *Klebsiella pneumoniae* antibiotic sensitivity patterns. This study reveals that amoxicillin and clavulanic acid (72.4%), amikacin (89.7%), ciprofloxacin (77.6%), colistin (91.4%), gentamicin (74.1%), imipenem (89.7%), levofloxacin (79.3%), meropenem (94.8%), and piperacillin and tazobactam (91.4%) were found to be sensitive and that was statistically significant for 5% level of significance. The antibiotics that were resistant to *Klebsiella pneumoniae* were azithromycin 84.5%, cefaclor 91.4%, cefixime 98.3%, ceftriaxone 79.3%, cefuroxime 93.1%, doxycycline (63.8%), ceftazidime 93.1%, linezolid 98.3%, and vancomycin 89.7% all of which were statistically significant at the 5% level of significance.

Isolate	**Effect**		**p-value**
	Sensitive	**Resistant**	
	N (%)	**N (%)**	
Amoxicillin and Clavulanic Acid	42 (72.4)	16 (27.6)	<0.05
Amikacin	52 (89.7)	6 (10.3)	<0.05
Azithromycin	9 (15.5)	49 (84.5)	<0.05
Cefaclor	5 (8.6)	53 (91.4)	<0.05
Cefixime	1 (1.7)	57 (98.3)	<0.05
Ceftriaxone	12 (20.7)	46 (79.3)	<0.05
Cefuroxime	4 (6.9)	54 (93.1)	<0.05
Ceftazidime	4 (6.9)	54 (93.1)	<0.05
Ciprofloxacin	45 (77.6)	13 (22.4)	<0.05
Colistin	53 (91.4)	5 (8.6)	<0.05
Doxycycline	21 (36.2)	37 (63.8)	<0.05
Gentamicin	43 (74.1)	15 (25.9)	<0.05
Imipenem	52 (89.7)	6 (10.3)	<0.05
Levofloxacin	46 (79.3)	12 (20.7)	<0.05
Linezolid	1 (1.7)	57 (98.3)	<0.05
Meropenem	55 (94.8)	3 (5.2)	<0.05
Piperacillin and Tazobactam	53 (91.4)	5 (8.6)	<0.05
Vancomycin	6 (10.3)	52 (89.7)	<0.05

Table 3: *Sensitivity patterns of Klebsiella pneumoniae.*

Table 4 this study found that amoxicillin and clavulanic acid (86.7%), amikacin (86.7%), ciprofloxacin (80.0%), doxycycline (68.9%), gentamicin (82.2%), imipenem (95.6%), levofloxacin (68.9%), meropenem (91.1%), piperacillin and tazobactam (86.7%) were also found to be sensitive and it's statistically significant for 5% level of significance. The resistant antibiotics to this organism were azithromycin 80%, cefaclor 75.6%, cefixime 93.3%, ceftriaxone 66.7%, and ceftazidime 93.3%, which is statistically significant at the 5% level of significance.

Isolates	Effect		p-value
	Sensitive	Resistant	
	N (%)	N (%)	
Amoxicillin and Clavulanic Acid	3 (86.7)	6 (13.3)	<0.05
Amikacin	39 (86.7)	6 (13.3)	<0.05
Azithromycin	9 (20.0)	36 (80.0)	<0.05
Cefaclor	11 (24.4)	34 (75.6)	<0.05
Cefixime	3 (6.7)	42 (93.3)	<0.05
Ceftriaxone	15 (33.3)	30 (66.7)	<0.05
Cefuroxime	17 (37.8)	28 (62.2)	0.101
Ceftazidime	3 (6.7)	42 (93.3)	<0.05
Ciprofloxacin	36 (80.0)	9 (20.0)	<0.05
Colistin	28 (62.2)	17 (37.8)	0.101
Doxycycline	31 (68.9)	14 (31.1)	<0.05
Gentamicin	37 (82.2)	8 (17.8)	<0.05
Imipenem	43 (95.6)	2 (4.4)	<0.05
Levofloxacin	31 (68.9)	14 (31.1)	<0.05
Linezolid	20 (44.4)	25 (55.6)	0.456
Meropenem	41 (91.1)	4 (8.9)	<0.05
Piperacillin and Tazobactam	36 (86.7)	6 (13.3)	<0.05
Vancomycin	24 (53.3)	21 (46.7)	0.655

Table 4: *Sensitivity patterns of Staphylococcus aureus.*

Table 5 present the antibiotic sensitivity pattern of *Streptococcus pneumoniae*. This study found that amoxicillin and clavulanic acid (76.5%), amikacin (88.2%), colistin (82.4%), gentamicin (82.4%), imipenem (94.1%), meropenem (94.1%), and piperacillin and tazobactam (94.1%) were found to be sensitive and that was statistically significant for 5% level of significance. *Streptococcus pneumoniae* was reported to have an 82.4% resistance to azithromycin, 82.4% to cefaclor, 100% to cefixime, 76.5% to cefuroxime, and 100% to ceftazidime, which were statistically significant at the 5% level of significance.

Table 6 present the antibiotic sensitivity pattern of *Pseudomonas* spp. This study shows that amikacin (81.8%), ciprofloxacin (81.8%), colistin (81.8%), gentamicin (90.9%), imipenem (81.2%), meropenem (81.8%), and piperacillin and tazobactam (81.8%) were found to be sensitive to *Pseudomonas* species and that was statistically significant for 5% level of significance. The completely resistant antibiotics for this organism were cefaclor, cefixime, cefuroxime, and linezolid, ceftriaxone, ceftazidime, followed by vancomycin with 90.9%, and doxycycline with 81.8% of resistance, all of which were statistically significant at the 5% level of significance.

Isolates	Effect		p-value
	Sensitive	Resistant	
	N (%)	N (%)	
Amoxicillin and Clavulanic Acid	13 (76.5)	4 (23.5)	<0.05
Amikacin	15 (88.2)	2(11.8)	<0.05
Azithromycin	3 (17.6)	14 (82.4)	<0.05
Cefaclor	3 (17.6)	14 (82.4)	<0.05
Cefixime	-	17 (100.0)	--
Ceftriaxone	6 (35.3)	11 (64.7)	0.225
Cefuroxime	4 (23.5)	13 (76.5)	<0.05
Ceftazidime	-	17 (100.0)	--
Ciprofloxacin	10 (58.8)	7 (41.2)	0.467
Colistin	14 (82.4)	3 (17.6)	<0.05
Doxycycline	8 (47.1)	9 (52.9)	0.808
Gentamicin	14 (82.4)	3 (17.6)	<0.05
Imipenem	16 (94.1)	1 (5.9)	<0.05
Levofloxacin	11 (64.7)	6 (35.3)	0.225
Linezolid	5 (29.4)	12 (70.6)	0.090
Meropenem	16 (94.1)	1 (5.9)	<0.05
Piperacillin and Tazobactam	16 (94.1)	1 (5.9)	<0.05
Vancomycin	8 (47.1)	9 (52.9)	0.808

Table 5: *Sensitivity patterns of Streptococcus pneumoniae.*

Isolates	Effect		p-value
	Sensitive	Resistant	
	N (%)	N (%)	
Amoxicillin and Clavulanic Acid	4 (36.4)	7 (63.6)	0.366
Amikacin	9 (81.8)	2 (18.2)	<0.05
Azithromycin	3 (27.3)	8 (72.7)	0.132
Cefaclor	-	11 (100.0)	--
Cefixime	-	11 (100.0)	--
Ceftriaxone	1 (9.1)	10 (90.9)	<0.05
Cefuroxime	-	11 (100.0)	--
Ceftazidime	1 (9.1)	10 (90.9)	<0.05
Ciprofloxacin	9 (81.8)	2 (18.2)	<0.05
Colistin	9 (81.8)	2 (18.2)	<0.05
Doxycycline	2 (18.2)	9 (81.8)	<0.05
Gentamicin	10 (90.9)	1 (9.1)	<0.05
Imipenem	9 (81.2)	2 (18.2)	<0.05
Levofloxacin	11 (100.0)	--	--
Linezolid	--	11 (100.0)	--
Meropenem	9 (81.8)	2 (18.2)	<0.05
Piperacillin and Tazobactam	9 (81.8)	2 (18.2)	<0.05
Vancomycin	1 (9.1)	10 (90.9)	<0.05

Table 6: *Sensitivity patterns of Pseudomonas spp.*

Discussion

SARS-CoV-2 infected patients frequently experienced co-infections with a variety of bacteria and fungi, which have a significant effect on the severity and fatality rates of COVID-19 [6]. The current study showed the common microbial growth with antibiotic sensitivity patterns of the isolated pathogens from sputum samples of the COVID-19 patients. *Klebsiella pneumoniae* was the predominant microorganism isolated (38.7%) from these samples. This study found that the patients aged between 60 to 69, had a significantly higher percentage of bacterial culture growth of 43.4%, while in Iraq, a 43% of infection rate was found in the patients aged between 56 to 80 years [16]. A study from Lahore, Pakistan found for COVID-19 patients, the sputum test positive rate was 3.12%, and among the respondent patients 56% were males and 44% were females [6]. A study from Egypt found that 55.4% of the males and 44.6% of the female's sputum tested positive for [42]. Another study in Nepal stated that males were 70% and females were 30% positive in the sputum test [43]. In India, pathogenic bacterial co-infection was 39.5% of COVID-19 patients, where 57.9% of the patients were male and 42.1% of the patients were female [41]. This study found the pathogenic bacterial co-infection rate as 24.5% while 58% of the positive cases were males and 42% of the positive cases were females. The male-to-female ratio was 1.3:1. Another study from the mid-Atlantic United States, for COVID-19 pneumonia patients based on sputum reports found that *Staphylococcus aureus* (33.3%) (7.5%) were the most growth culture, and 16.3% were normal flora [44]. A study from the Hospital Nacional Hipólito Unanue examined that the most frequently found bacteria were *Staphylococcus aureus* (11.83%), *Streptococcus agalactiae* (10.75%), *Klebsiella pneumonia* (8.6%), and *Streptococcus pneumoniae* was found 1.08% [45]. This study found that the growth of *Klebsiella pneumoniae* (38.7%) was the dominant growth culture, others were *Staphylococcus aureus* (30%), *Streptococcus pneumoniae* (11.3%), Fungus (10.7%), *Pseudomonas* spp. (7%), and normal flora (2%) of sputum-positive culture growth. Cheng L.S. discovered isolated microorganisms in 42% of the COVID-19 patients who underwent testing for respiratory illnesses [46]. In India, *Klebsiella pneumoniae* was the most commonly found organism among COVID-19 patients [41]. The current investigation confirms previous studies by Fatorini L. [47] and Gurung K. [43] that *Klebsiella pneumoniae, Staphylococcus aureus*, and *Pseudomonas aeruginosa* are frequent infections. This study examined the most commonly associated pathogens were *Klebsiella pneumoniae, Staphylococcus aureus, Streptococcus pneumoniae*, and *Pseudomonas* spp. Gurung K. examined that *Klebsiella* spp. was the primary confirmed isolate from the sputum test of COVID-19 patients and most sensitive to meropenem (67%) and colistin (67%) followed by piperacillin/tazobactam (56%), similarly sensitive to amoxicillin/clavulanate, amikacin, doxycycline, tobramycin 56%, 50%, 44%, and 44% respectively [43]. In another study in India, Vagdalkar found that microorganisms were found highly sensitive to amikacin, ciprofloxacin, imipenem, piperacillin-tazobactam, nitrofurantoin, and chloramphenicol, and found highly resistant to cefepime, ampicillin, ceftazidime, and cotrimoxazole [41]. These studies correlate with the present study that most of these microorganisms were found highly sensitive to amoxicillin and clavulanic acid, amikacin, cefaclor, piperacillin and tazobactam, meropenem, gentamicin, imipenem colistin and resistance to azithromycin, cefixime, cefuroxime, ceftazidime, linezolid, ceftriaxone, and vancomycin and demonstrated statistically significant.

Conclusion

The severity and mortality rates of COVID-19 have been demonstrated to be significantly influenced by co-infections with a range of bacteria in SARS-CoV-2-infected patients. In hospitalized SARS-CoV-2 patients, antibiotic usage was high which indicates that more proper use of antibacterial agents is necessary for the diagnosis of secondary bacterial infections (SBIs). This study found that bacterial co-infection with *Klebsiella pneumoniae* was common and Most of the bacterial pathogens were highly sensitive to amoxicillin and clavulanic acid, amikacin, azithromycin, cefaclor, piperacillin, and tazobactam, meropenem, gentamicin, imipenem and highly resistant to azithromycin, cefaclor, cefixime, cefuroxime, ceftazidime, linezolid, ceftriaxone, and vancomycin. Overuse of antimicrobials increases the risk of multi-resistant nosocomial secondary infections, which are associated with unfavorable clinical outcomes. The results of the present study indicate the necessity of ongoing bacterial co-infection surveillance as well as the rational use of antibiotics to avert antimicrobial resistance rates and to develop infection control strategies for local and worldwide pandemic control. Therefore, the practice of empirical antibiotic coverage in COVID-19 patients must be carefully evaluated.

Limitations of the Study

One of the disadvantages of the study is the small sample size in a single study center. More studies utilizing large-scale and multi-institutional methods should be carried out to understand more about the co-infections and patterns of antibiotic resistance in COVID-19 patients. Additionally, other co-morbidities of these COVID-19 patients were not categorized. All RT PCR Positive patients who presented with respiratory infections and sputum culture positive were evaluated in this study.

Conflict of Interest

It is a self-funded study, and the authors declare no conflict of interest.

Author's Contribution

Concept, Design of the study and manuscript editing, Data analysis, Data collection, Critical review of the manuscript**.**

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