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Frequency Of Bacterial Co-Infections Isolated from Covid-19 Positive Patients From Tertiary Care Hospital Of Karachi

Fouzia Zeeshan Khan[1], Ambreen Fatima[2], Hareem Gohar[3], Mehwish Sajjad[4], Mona Siddique[5], Saima Naseem[6]

Abstract

Objective: This study aims to determine the frequency of bacterial co-infections in COVID-19-positive patients.
Methodology: A prospective cross-sectional study was conducted in the Department of Microbiology, Pakistan, from November 15, 2021, to April 15, 2022. Blood and respiratory tract samples were collected, including sputum, bronchial lavage, and tracheal aspirate. Clinical specimens were inoculated onto a Sheep blood agar plate, Chocolate agar plate (aerobic with 5% CO2), and MacConkey's agar. Identification was followed by specific and standard microbiological protocols. COVID-19 was confirmed by qualitative PCR. Antimicrobial susceptibility testing was performed using the Kirby Bauer disc diffusion method.
Results: A total of 202 clinical samples, including blood, sputum, tracheal aspirates, and bronchial lavage, were collected from COVID-19-positive patients. Male patients were more common in sputum and tracheal aspirates, while female patients were more common in blood cultures. The majority of patients were over 60 years of age. Acinetobacter baumannii was predominantly isolated from blood and tracheal aspirates, exhibiting multidrug resistance, but showing complete sensitivity towards Colistin. Klebsiella pneumonia exhibited high prevalence in sputum, with complete resistance observed in Cephalosporins and Co-trimoxazole.
Conclusion: The study concludes a high frequency of superadded bacterial co-infections, caused most prominently by Acinetobacter baumannii, Klebsiella pneumonia, and Pseudomonas aeruginosa. The majority of these are multidrug-resistant pathogens, therefore, urgent action is required to control the spread of nosocomial infections by resistant strains, which are responsible for the high mortality rate among COVID-19 critical patients.
Keywords: COVID-19, bacterial coinfection, Pseudomonas aeruginosa, Acinetobacter baumannii, MRSA.

[1,2,3] Assistant Professor, Dow International Medical College; [4] Lecturer, Dow International Medical College; [5] MSc, University of Karachi; [6] Associate Professor, Dow International Medical College.
Correspondence: Dr Fouzia Zeeshan Khan, Assistant Professor, Dow International Medical College. **Email:** fouzia.zeeshan@duhs.edu.pk
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1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was initially identified in December 2019. The World Health Organization (WHO) declared it a pandemic in March 2020, and the virus has since spread globally, presenting a significant challenge to the economy and healthcare systems of both developed and developing countries[1]. The first case of COVID-19 in Karachi was reported on February 26, 2020, and the virus has subsequently become epidemic, affecting various regions throughout the country[2]. COVID-19 can manifest with varying clinical presentations ranging from asymptomatic to mild and severe disease[3]. One of the major concerns associated with COVID-19 is the potential evolution of acute viral pneumonia to acute respiratory distress syndrome (ARDS), which can result in high morbidity and mortality. COVID-19 can also cause life-threatening complications such as arrhythmias, acute cardiac injury, encephalitis, and shock[4]. The severity of the disease is not linked to any particular age group. However, it is mostly observed in elderly individuals, especially those with immune-compromised status, diabetes mellitus, hypertension, ischemic heart disease, malignancy, and chronic lung/renal disease[5]. Some patients may require intensive care with mechanical intubation[5, 6]. Studies have reported that the mortality rate is highest among elderly, immune-compromised patients[6]. Patients who are incubated are at a major risk of nosocomial pneumonia, which may worsen in the presence of lower respiratory tract infections[6]. Superimposed bacterial infections can have negative health outcomes, as seen during the H1N1 pandemic in 2009[6]. A recent study reported that bacterial infections were present on admission in 3.1-3.5% of COVID-19 patients, while 15% of patients acquired nosocomial infections during their hospital stay[7-9]. In response to the devastating spread of COVID-19, the government of Pakistan has implemented immediate and profound safety control and preventive measures, including quarantine of infected individuals, lockdowns, and upgrades to healthcare facilities[10]. The purpose of this study is to evaluate the prevalence of bacterial infections in COVID-19-positive patients admitted to a tertiary care hospital in Karachi.

2. Materials & Methods

Study Design and Sampling: This prospective cross-sectional study was conducted using a convenience sampling technique in the Department of Microbiology between December 1st, 2021, and April 30th, 2022. The study included all COVID-19 patients who tested positive via nasopharyngeal PCR. Samples of blood and respiratory tract specimens, including sputum, bronchial lavage, and tracheal aspirate, were collected from each patient.

Microbiological Testing: Clinical specimens were inoculated on Sheep blood agar, Chocolate agar, and MacConkey's agar according to standard microbiological protocols. After 24 hours of incubation, plates were examined for bacterial growth, and identification was confirmed through specific biochemical tests, followed by confirmation using API 20 E and API 20NE (bioMerieux France).

Antimicrobial Susceptibility: Antimicrobial susceptibility testing was performed using the modified Kirby Bauer's disc diffusion method on Mueller Hinton agar (MHA) (Oxoid Ltd, England) by CLSI guidelines.

Statistical Analysis: Data analysis was conducted using SPSS version 17.0 and presented as frequencies and percentages.

3. Results

A total of 202 clinical samples were collected from COVID-positive patients, including blood, sputum, bronchial lavage, and tracheal aspirates.

No bacterial growth was observed in the bronchial lavage samples.

The male population showed higher bacterial growth in sputum and tracheal aspirates, while the female population showed higher bacterial growth in blood cultures (Table 1).

The majority of the patients were aged 60 years or older (Table 1).

Table 1 Gender and Age-wise Distribution of Covid Positive Patients among Clinical Samples.

Age group	**Blood**		**Sputum**		**Trachea**		**Total**
	Gender	%	Gender	%	Gender	%	
0-20 YRS	M	16%	M	3%	M	0%	17
	F	7%	F	3%	F	0%	8
21-40 YRS	M	5%	M	13%	M	3%	11
	F	2%	F	3%	F	1%	4
41-60 YRS	M	12%	M	10%	M	19%	29
	F	18%	F	17%	F	30%	45
> 60 YRS	M	22%	M	28%	M	22%	46
	F	17%	F	23%	F	25%	42
Total	99		30		73		202

M=Male, F=Female

Table-2 Frequency and Distribution of Bacterial Isolates from Various Clinical Samples of COVID-19-Positive Patients

	Trachea		**Sputum**		**Blood**	
Organisms	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
Acinetobacter baumannii (n=85)	44	60.3%	6	20.0%	35	35.0%
Pseudomonas aeruginosa (n=15)	9	12.3%	5	16.7%	1	1.0%
Stenotrophomonas maltophilia (n=1)	1	1.4%	NF	NF	NF	NF
Elizabethkingia meningoseptica(n=5)	2	2.7%	NF	NF	3	3.0%
Enterobacter species (n=6)	3	4.1%	NF	NF	3	3.0%
Staphylococcus aureus (n=11)	3	4.1%	6	20.0%	2	2.0%
Escherichia coli (n=8)	3	4.1%	1	3.3%	4	4.0%
Klebsiella pneumoniae (n=17)	6	8.2%	8	26.7%	3	3.0%
Enterococcus species (n=4)	1	1.4%	NF	NF	3	3.0%
Streptococcus pneumoniae (n=1)			1	3.3%		
Moraxella catarrhalis(n=3)	NF	NF	3	10.0%	NF	NF
Staphylococcus species (coagulase-negative) (n=27)	NF	NF	NF	NF	27	27.0%
Serratia marcescens (n=5)	NF	NF	NF	NF	5	8.0%
Salmonella typhi(n=10)	NF	NF	NF	NF	10	10.0%
Total	73	----	30	----	100	---

*NF; Not found

The most common bacterial isolate in blood samples was A. baumannii (35%), followed by Staphylococcus species (27%). In sputum samples, A. baumannii (20%) and Staphylococcus aureus (20%) were the most prevalent bacteria. K. pneumoniae was isolated in 3.0% of cases. Among tracheal aspirates, A. baumannii (60%) and P. aeruginosa (12.3%) were the predominant bacteria identified. The antibiotic susceptibility pattern of methicillin-resistant Staphylococcus aureus isolated from sputum revealed the highest resistance to erythromycin (67%), gentamicin (36%), and tetracycline (27%), while being completely sensitive to fusidic acid and vancomycin, as shown in Table 3

Acinetobacter baumannii, the most prevalent Gram-negative bacteria isolated from Blood cultures and Tracheal aspirates, exhibited a multi-drug resistant pattern with complete resistance to Cephalosporins, Meropenems, Fluoroquinolones, and Co-trimoxazole. Only Colistin showed no resistance, while Minocycline, Tigecycline, and Amikacin displayed slight resistance (Table 4).

Klebsiella pneumonia, which was predominantly isolated from Sputum, showed complete sensitivity to Colistin, Minocycline, Ciprofloxacin, and Levofloxacin. However, complete resistance was observed in Cephalosporins and Co-trimoxazole (Table 4)

Table-4 Antibiotic Susceptibility Profile of Gram-Negative Bacteria Isolated from Clinical Samples of COVID-19 Positive Patients

Organism	AMC	AMP	CFM	SCF	CAZ	CRO	CXM	CFM	CIP	CT	SXT	AK	CN	LEV	MEM	TZP	MH	TGC	C	AZM	TOB
A baumannii (n=85)	NT	NT	NT	50%	-	90%	NT	NT	80%	8%	93%	85%	85%	98%	97%	93%	20%	15%	NT	NT	75%
K.pneumoniae (n=17)	83%	NT	83%	50%	NT	90%	93%	93%	80%	6%	60%	51%	67%	75%	50%	67%	50%	7%	NT	NT	75%
P.aeruginosa (n=16)	NT	NT	NT	NT	33%	NT	NT	NT	40%	10%	NT	20%	25%	70%	30%	20%	NT	NT	NT	NT	20%
S.typhi (n=10)	NT	90%	80%	NT	NT	85	NT	NT	100%	NT	90%	NT	NT	NT	0%	NT	NT	NT	20%	0%	NT
E.coli (n=8)	66%	96%	96%	23%	NT	89%	93%	90%	80%	0%	67%	10%	23%	80%	23%	45%	10%	0%	NT	NT	13%
Enterobacter species (n=6)	NT	NT	96%	96%	NT	97%	96%	96%	89%	15%	85%	90%	90%	80%	90%	88%	5%	8%	NT	NT	70%

Table-3 Antimicrobial Susceptibility Profile of Staphylococcus aureus Isolates from Clinical Samples of Patients with COVID-19

Antibiotics	Percentage of Resistance
Amoxicillin-clavulanic acid	64% (7)
Chloramphenicol	27% (3)
Clindamycin	45% (5)
Cloxacillin	64% (7)
Co-trimoxazole	45% (5)
Erythromycin	67% (7)
Gentamicin	36% (4)
Tetracycline	27% (3)
Vancomycin*	0%

**Amoxicillin (AMC), *chloramphenicol (C), *Clindamycin (DA), *Oxacillin (OX), * Co-trimoxazole (SXT), * Erythromycin (E), *Fusidic acid (FD), *Gentamicin (CN), *Tetracycline (TE), *Vancomycin (VA).*

S.aureus (n=11)

* Vancomycin MIC was performed on Vitek

5. Discussion

The SARS-CoV-2 virus has caused a devastating global pandemic, with over 100 million cases and 2 million deaths reported within a year[11]. Bacterial confections are known to complicate viral respiratory infections, leading to increased morbidity and mortality. Several studies have shown that secondary bacterial infections are associated with the severity and mortality of COVID-19[12-13]. In developing countries such as Pakistan, low socioeconomic infrastructure, poor health hygiene, and inadequate healthcare facilities have further aggravated the burden of the pandemic[14]. Our study showed that respiratory tract bacterial co-infections were more common in males, while females were more likely to suffer from bacteremia, which is consistent with previous research[15,16]. We also found that bacterial co-infections were more frequent in COVID-19-positive patients over the age of 60, followed by those between 41 and 60 years old. This is not surprising, as older age is associated with a decline in physiological homeostasis, which can result in decreased organ function, increased morbidity, and higher mortality rates[17].

The World Health Organization (WHO) has established guidelines for the management of Covid-19, which do not recommend the use of antibiotics for patients with suspected or confirmed mild Covid-19. However, for severe Covid-19, the use of empiric antimicrobials is recommended for treatment[18]. The current study showed a high prevalence of *A. baumannii* isolated from blood and tracheal cultures of Covid-positive patients, which is consistent with other studies[19]. Covid-19 patients admitted to intensive care units (ICUs) are at a higher risk of healthcare-associated infections, including ventilator-associated pneumonia, which may require urgent mechanical intubation[20]. *A. baumannii* has a strong ability to develop resistance to antibiotics, especially in immunocompromised and critically ill patients. The current study also reported multidrug resistance of *A. baumannii*, with slight sensitivity to Colistin, Minocycline, and Tigecycline, as observed in a previous study[21]. Carbapenem resistance in *A. baumannii* (CRAb) is a concerning issue as carbapenems are the last resort of antibiotics used to treat multidrug-resistant Gram-negative bacteria. CRAb can easily spread in hospital environments and in the hands of paramedics, and can persist for extended periods on dry surfaces. It can also be spread by asymptomatic carriers and is highly resistant to common disinfectants, resulting in outbreaks that can affect the most susceptible and critically ill patients. This highlights the urgent need for effective infection control measures and the development of new treatments to combat CRAb infections[22]. Our study found a high prevalence of multi-drug resistant Pseudomonas aeruginosa in tracheal cultures, while blood cultures exhibited a majority of Staphylococcus species, *S. Typhi*, and *S. marcescens*, which is consistent with previous findings by Tayyab *et al*.[23]. *P. aeruginosa* is known for its ability to form biofilms and cause infections in immunocompromised patients, making it a common co-infecting pathogen in COVID-19 patients and exacerbating the illness. Biofilms are notoriously resistant to antimicrobial agents, which can protect the pathogen from the immune system[24]. In underdeveloped countries like Pakistan, extensively drug-resistant (XDR) typhoid fever is a serious health concern, especially due to the indiscriminate use of azithromycin during the COVID-19 pandemic, leading to chaos [25]. The present study found a significant prevalence of carbapenem-resistant *K. pneumoniae* in the sputum of Covid-19 positive patients, followed by MRSA (methicillin-resistant *S. aureus*), which is consistent with the findings of a review study by Wioletta *et al.*[26]. The high occurrence of *K. pneumoniae* NDM is likely due to the complex respiratory pathology associated with Covid-19 infection, mechanical intubation, exposure to carbapenems and β-lactam/β-lactamase inhibitors, blood transfusions, and prolonged hospital stay [27, 28]. The current study suggests that SARS-CoV-2 infection may have a detrimental effect on the immune system of patients, and the exact mechanism by which natural killer cells and antibodies are triggered remains unclear. Steroids are often administered to reduce the cytokine storm induced by the coronavirus, but a study by Karruli *et al*[29]. proposed that steroid therapy could increase the frequency of multi-drug resistant (MDR) bacterial infections, which may be a possible reason for the high incidence of MDR bacterial infections observed in our study [29, 30]

5. Conclusion

In conclusion, our study revealed a high frequency of superimposed bacterial infections in hospitalized Covid-19 patients, caused predominantly by A. baumannii, K. pneumoniae, and P. aeruginosa. These pathogens are mostly multidrug-resistant, resulting in increased mortality and morbidity. It is imperative to implement strategic planning, including health education and infection prevention measures, to control the spread of nosocomial infections.

CONFLICTS OF INTEREST- None

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Contributions:

F.Z.K - Conception of study

F.Z.K, M.S - Experimentation/Study Conduction

M.S, S.N - Analysis/Interpretation/Discussion

F.Z.K, H.G, M.S - Manuscript Writing

A.F, S.N - Critical Review

A.F - Facilitation and Material analysis

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