



Microbes and Infectious Diseases

Journal homepage: <https://mid.journals.ekb.eg/>

Original article

Prevalence of respiratory bacterial co-infection among COVID-19 patients

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ARTICLE INFO

Article history:

Received 13 August 2023

Received in revised form 19 August 2023

Accepted 22 August 2023

Keywords:

COVID-19

Bacterial co-infection

Multidrug resistance

Prevalence

Respiratory infection.

ABSTRACT

Background and rationale: Since the beginning of COVID-19 pandemic, the number of cases and deaths increased. Bacterial co-infection was reported as one of the complications associated with increased mortality. This work aimed at exploring and describing the prevalence of respiratory bacterial co-infection among COVID-19 patients in Ain Shams University Isolation Hospitals. **Methodology:** This study included 160 lower respiratory samples collected from 80 COVID-19 positive patients, and 80 COVID-19-negative patients admitted to Ain Shams University Isolation Hospitals between February and June 2021. Samples were cultured, and all recovered isolates were identified and tested for antibiotic susceptibility using Vitek2C. **Results:** Our results showed high prevalence of respiratory bacterial infections in males (106/160, 66.2%) than in females (54/160, 33.8%). The age ranged from 25-88 years (mean age 58.24 ± 14.19). In COVID-19-positive patients, 16/80 (20%) samples showed negative bacterial growth, and 64/80 (80%) were positive. In COVID-19-negative patients, 33/80 (41.25%) samples showed negative bacterial growth, and 47 (58.75%) showed bacterial growth. *Klebsiella* spp. was the most common isolated organism (51/148, 25.9%), followed by *Acinetobacter* spp. (50/148, 25.4%), and *Stenotrophomonas* (1/148, 0.5%) was the least common one. As per the Antibiotic susceptibility testing, a high resistance pattern was noticed among the isolated bacteria against all the tested antibiotics. **Conclusion:** COVID-19-positive patients showed higher positive bacterial growth than COVID-19 patients. Generally, a high resistance pattern was noticed among the isolated bacteria. The obtained results are alarm to the clinicians that they should halt the usage of empirical antimicrobials promptly and resort to culture and antimicrobial susceptibility testing.

Introduction

The coronavirus disease of 2019 (COVID-19) appeared in Wuhan, China, in December 2019. Since then, it has spread worldwide, resulting in a pandemic that confers a defy to healthcare systems everywhere [1].

Most patients present with mild manifestations, such as fever and cough. However, 6.5% of patients rapidly progress to grave complications, such as acute respiratory distress syndrome (ARDS) and acute respiratory failure [2].

Viral pneumonia is the most common indication for hospital admission among COVID-19 patients. Co-infection with bacterial pathogens was found among 6.8% of patients with viral respiratory infections and is a significant reason for morbidity and mortality [3].

COVID-19 patients are in increased jeopardy of developing secondary infections because they are in continuous need of mechanical ventilation, sedatives, and muscle relaxants for long periods. *Staphylococcus aureus*, *Streptococcus pneumoniae*, and Gram-negative bacteria have been recognized as the most frequently isolated

pathogens from respiratory samples of hospitalized COVID-19 patients [4].

Antimicrobials, such as azithromycin, have several potential roles in treating suspected or confirmed bacterial or fungal respiratory co-infections in COVID-19 patients [5].

Antimicrobials are used empirically or targeted for managing community-acquired infections and nosocomial infections, such as hospital-acquired or ventilator - associated pneumonia [5]. Such a high rate of prescribing antibiotics for almost all hospitalized COVID-19 patients can augment the problem of antimicrobial resistance [6].

The current work aimed at exploring and describing the prevalence of respiratory bacterial co-infection among COVID-19 patients in Ain Shams University Isolation Hospitals (Field and geriatric hospitals).

Methods

Study design and study population

This cross-sectional study included a total of 160 lower respiratory samples (LRT) (bronchoalveolar lavage) submitted for routine culture and sensitivity in the Central Microbiology Laboratory of Ain Shams University Hospitals during the period from February 2021 to June 2021. The samples were collected from 80 COVID-19 positive patients and 80 COVID-19 negative patients confirmed by RT-PCR, admitted to Ain Shams University Isolation Hospitals (Field and geriatric hospitals). The study was performed according to the guidelines published by **Vandenbroucke et al.** [7].

Ethical approval

All study procedures were as per ethical guidelines of the 1975 Declaration of Helsinki and approved by the ethical committee of Faculty of Medicine, Ain Shams University. (No. FWA 000017585) (FMASU MS 283/2021).

All the lower respiratory samples were cultured, according to standard operating procedures of the microbiology laboratory, using the streak plating technique on each of the routine microbiology plates; blood agar, MacConkey agar, and chocolate agar (Oxoid, UK) [8]. After 24-48 hours incubation, the culture plates were examined for growth. Media with no viable growth were interpreted as sterile/ no growth. Dominant colony, suspected as a pathogen, was selected.

1.All the selected isolates were subjected to the following:

a. Identification by conventional microbiological techniques as colonial morphology, Gram stain characteristics and biochemical reactions according to standard microbiological identification methods [9].

b. Antimicrobial susceptibility testing by MICs, according to the CLSI (2017) [10], using the correspondent cards (AST GN222 and AST GN73) of Vitek2C automated system (BioMerieux, France). Multidrug-resistant organisms (MDROs) were designated as non-susceptibility to at least one or more drugs in three grouping of antibiotics.

2.Relevant clinical and laboratory data were obtained from electronic medical records:

a. Demographic data (age and gender).

b. Laboratory values (D-dimer, C-reactive protein (CRP), total leucocytic count (TLC), neutrophil count, lymphocyte count, ferritin, and lactate dehydrogenase (LDH)).

Statistical analysis

Data were analyzed using the Statistical Package for Social Science (IBM SPSS) version 23. The quantitative data were presented as mean, standard deviations and ranges when parametric and median, inter-quartile range (IQR) when data found non-parametric. Also, qualitative variables were presented as number and percentages.

The comparison between groups regarding qualitative data was done by using Chi-square test and/or Fisher exact test when the expected count in any cell found less than 5.

The comparison between two independent groups with quantitative data and parametric distribution was done by using independent t-test while with non-parametric distribution were done by using Mann-Whitney test.

The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered significant as the following:

p -value > 0.05: Non-significant (NS)

p -value < 0.05: Significant (S)

p -value < 0.01: Highly significant (HS).

Results

1-Age and gender distribution

As regard the demographic data of the patients in the current study, age ranged between 25-88 years with mean age 58.24 (\pm 14.19). The

majority of the patients were males (106/160, 66.2%) followed by females (54/160, 33.8%). **Table (1)** shows that there was no statistically significant relation between the presence of COVID-19 with age and gender of the studied patients with *p* -value = 0.855 and 0.181 respectively.

2- Laboratory data

As regard laboratory data of the patients, LDH, Ferritin, and neutrophils showed a statistically significant increase in COVID-19 positive patients in comparison with COVID-19 negative patients (**Table 2**).

3-Bacterial coinfection

As for the results of LRT cultures among the studied groups, 49 (24.9%) showed negative bacterial growth and 111 (75.1%) showed positive bacterial growth, 35 (31.5%) of them had mixed bacterial growth. A total of 148 organisms were isolated from the total number of positive patients' cultures.

Klebsiella spp. was the most common isolated organism (51/148, 25.9%) followed by *Acinetobacter* spp. (50/148, 25.4%), and *Stenotrophomonas* (1/148, 0.5%) was the least common isolated one. *Acinetobacter* spp. and *Klebsiella* spp. had the highest percentage of (40%) among the mixed organisms, followed by *Acinetobacter* spp. and *Pseudomonas* spp. (14.3%) (table 3).

In COVID-19 positive patients, 16/80 (20%) LRT samples cultures showed negative bacterial growth and 64/80 (80%) were positive. A

total of 90/148 organisms were isolated. On the other hand, in COVID-19 negative patients 33/80 (41.25%) LRT samples cultures showed negative bacterial growth and 47(58.75%) showed bacterial growth, A total of 58/148 organisms were isolated. A statistically high significant difference between COVID-19 positive and negative patients regarding the results of cultures where 80% of COVID-19 positive patients were associated with positive bacterial growth with *p* -value of 0.0035.

Acinetobacter spp. was only organism showing statistically significant difference between COVID-19 positive and negative patients (**Table 4**).

4-Antibiotic susceptibility testing

As per the Antibiotic susceptibility testing (AST) among the studied patients, there was a high resistance pattern noticed among the isolated bacteria against all the tested antibiotics. In COVID-19 negative patients, 42 (72.4%) organisms showed MDR pattern and in COVID-19 positive patients, 63 organisms (70.0%) displayed MDR pattern. The isolated bacteria exhibited the highest susceptibility for Amikacin (AK) (11.7%) followed by Doxycycline (DO) (10.3%). Isolates with intermediate results were scarce, so they were set in the group of resistant isolates. No statistically significant difference was found regarding the antibiotic susceptibility pattern in all the studied organisms between COVID-19 positive and negative patients (**Table 5**).

Table 1. Relation of presence of COVID with demographic data and characteristics of the studied patients

		COVID-19 negative	COVID-19 positive	Test value	<i>p</i> -value	Sig.
		No. = 80	No. = 80			
Age	Mean ± SD	58.04 ± 15.05	58.45 ± 13.37	-0.183•	0.855	NS
	Range	25 – 87	30 – 88			
Sex	Female	23 (28.8%)	31 (38.8%)	1.789*	0.181	NS
	Male	57 (71.2%)	49 (61.2%)			

p-value > 0.05: Non-significant (NS); *P*-value < 0.05: Significant; *P*-value < 0.01: Highly significant*; Chi-square test; •: Independent t-test.

Table 2. Relation of presence of COVID-19 with laboratory data the studied patients.

		Total no. = 160	COVID-19 negative	COVID-19 positive	Test value	p-value	Sig.
			No. = 80	No. = 80			
LDH	Median (IQR)	480 (303 – 718)	400 (300 – 560)	538.5 (379 – 795)	-2.476#	0.013	S
	Range	104 – 5500	128 – 1400	104 – 5500			
Ferritin	Median (IQR)	812.5 (375 – 1744)	700 (270 – 1126)	1044 (445 – 2000)	-2.247#	0.025	S
	Range	43 – 7400	43 – 7400	110 – 6726			
WBCs	Median (IQR)	11.85 (7.7 – 18.2)	10 (5.75 – 18.5)	14.4 (8.85 – 18.2)	-1.884#	0.060	NS
	Range	1.1 – 125	1.2 – 125	1.1 – 43.6			
Neutrophil	Median (IQR)	10.05 (6.15 – 16.6)	8.35 (4.5 – 16.75)	12.85 (7.35 – 16.55)	-2.092#	0.036	S
	Range	0.8 – 38	0.8 – 38	0.9 – 37.5			
Lymph	Median (IQR)	0.95 (0.4 – 1.2)	1 (0.5 – 1.25)	0.8 (0.4 – 1.15)	-1.221#	0.222	NS
	Range	0 – 13	0 – 11	0.02 – 13			
CRP	Median (IQR)	100 (40 – 204)	102.5 (32.5 – 175)	100 (45 – 210)	-0.701#	0.483	NS
	Range	2 – 505	2 – 505	5 – 500			
D-dimer	Median (IQR)	2.5 (1.1 – 5)	2.5 (1 – 5)	2.5 (1.1 – 5)	-0.549#	0.583	NS
	Range	0.1 – 75	0.1 – 25	0.2 – 75			

p-value > 0.05: Non-significant (NS); P-value < 0.05: Significant; P-value < 0.01: Highly significant. #: Mann-Whitney test.

Table 3. Distribution of different organisms among the lower respiratory specimens

		Total no. of organisms = 148
Culture results	Negative bacterial growth	49 (24.9%)
	Positive bacterial growth	148 (75.1%)
	<i>Klebsiella spp.</i>	51 (25.9%)
	<i>Acinetobacter spp.</i>	50 (25.4%)
	<i>Pseudomonas spp.</i>	16 (8.1%)
	<i>Proteus spp.</i>	15 (7.6%)
	<i>Candida</i>	10 (5.1%)
	<i>E. coli</i>	5 (2.5%)
	<i>Stenotrophomonas</i>	1 (0.5%)
	Mixed growth	35 (31.5%)
	<i>Acinetobacter spp.</i> + <i>Klebsiella spp.</i>	14 (40.0%)
	<i>Acinetobacter spp.</i> + <i>Pseudomonas spp.</i>	5 (14.3%)
	<i>Klebsiella spp.</i> + <i>Pseudomonas spp.</i>	5 (14.3%)
	<i>Proteus spp.</i> + <i>Klebsiella spp.</i>	3 (8.6%)
	<i>E. coli</i> + <i>Proteus spp.</i>	2 (5.7%)
	<i>Acinetobacter spp.</i> + <i>Proteus spp.</i>	1 (2.9%)
	<i>Klebsiella spp.</i> + <i>E. coli</i>	1 (2.9%)
	<i>Klebsiella spp.</i> + <i>Proteus spp.</i>	1 (2.9%)
<i>Acinetobacter spp.</i> + <i>Stenotrophomonas</i>	1 (2.9%)	
<i>Proteus spp.</i> + <i>Klebsiella spp.</i> + <i>Pseudomonas spp.</i>	2 (5.7%)	

Table 4. Relation of presence of COVID-19 with presence and type of organism

Organism	COVID negative	COVID positive	Test value	p -value	Sig.
	No. = 58	No. = 90			
<i>Klebsiella spp.</i>	20 (22.0%)	31 (29.2%)	1.348*	0.246	NS
<i>Acinetobacter spp.</i>	17 (18.7%)	33 (31.1%)	4.008*	0.045	S
<i>Pseudomonas spp.</i>	7 (7.7%)	9 (8.5%)	0.042*	0.838	NS
<i>Proteus spp.</i>	8 (8.8%)	7 (6.6%)	0.333*	0.564	NS
<i>Candida</i>	4 (4.4%)	6 (5.7%)	F	0.755	NS
<i>E. coli</i>	2 (2.2%)	3 (2.8%)	F	1.000	NS
<i>Stenotrophomonas</i>	0 (0.0%)	1 (0.9%)	F	1.000	NS
Mixed growth	9 (19.1%)	26 (40.6%)	5.789	0.016	S

p -value > 0.05: Non-significant; P-value < 0.05: Significant; P-value < 0.01: Highly significant *: Chi-square test; F: Fisher's Exact test

Table 5. Relation of presence of COVID-19 with Antibiotic susceptibility Test

		COVID-19 negative	COVID-19 positive	Total (%)	Test value	p -value	Sig .
		No. = 58	No. = 90				
Multidrug resistance (MDR)	Positive	42 (72.4%)	63 (70.0%)	105 (70.9%)	0.100*	0.752	NS
(Amoxicillin/Clavulanic) AMC	Susceptible	1 (2.1%)	2 (2.8%)	3 (2.5%)	F	1.000	NS
	Resistant	46 (97.9%)	70 (97.2%)	116 (97.5%)			
(Cefotaxime) CTX	Susceptible	1 (2.0%)	4 (5.2%)	5 (4.0%)	F	0.648	NS
	Resistant	48 (98.0%)	73 (94.8%)	121 (96.0%)			
Ceftazidime (CAZ)	Susceptible	1 (2.0%)	3 (3.9%)	4 (3.2%)	F	1.000	NS
	Resistant	48 (98.0%)	74 (96.1%)	122 (96.8%)			
(Ceftriaxone) CRO	Susceptible	1 (2.0%)	3 (4.0%)	4 (3.2%)	F	1.000	NS
	Resistant	48 (98.0%)	72 (96.0%)	120 (96.8%)			
(Ciprofloxacin) CIP	Susceptible	3 (6.0%)	6 (7.8%)	9 (7.1%)	0.148*	0.701	NS
	Resistant	47 (94.0%)	71 (92.2%)	118 (92.9%)			
(Meropenem) MEM	Sensitive	2 (4.3%)	9 (11.5%)	11 (8.8%)	1.938*	0.164	NS
	Resistant	45 (95.7%)	69 (88.5%)	114 (91.2%)			
(Trimethoprim/Sulphamethoxazole) SXT	Susceptible	2 (4.0%)	5 (6.5%)	7 (5.5%)	F	0.703	NS
	Resistant	48 (96.0%)	72 (93.5%)	120 (94.5%)			
(Ampicillin/Sulbactam) SAM	Susceptible	0 (0.0%)	1 (1.5%)	1 (0.9%)	F	1.000	NS
	Resistant	45 (100.0%)	66 (98.5%)	111 (99.1%)			
(Doxycycline)	Susceptible	2 (4.1%)	11 (14.3%)	13 (10.3%)	0.370*	0.066	NS

DO	Resistant	47 (95.9%)	66 (85.7%)	113 (89.7%)			
(Imipenem)	Susceptible	2 (4.3%)	1 (1.6%)	3 (2.7%)	F	0.570	NS
IPM	Resistant	44 (95.7%)	63 (98.4%)	107 (97.3%)			
(Amikacin)	Susceptible	5 (9.6%)	10 (13.2%)	15 (11.7%)	0.375*	0.541	NS
AK	Resistant	47 (90.4%)	66 (86.8%)	113 (88.2%)			
(Levofloxacin)	Susceptible	2 (4.4%)	3 (4.4%)	5 (4.4%)	F	1.000	NS
LEV	Resistant	43 (95.6%)	65 (95.6%)	108 (95.6%)			
(Aztreonam)	Susceptible	0 (0.0%)	0 (0.0%)	0 (0.0%)	-	-	-
AZM	Resistant	42 (100.0%)	63 (100.0%)	105 (100.0%)			
(Tobramycin)	Susceptible	1 (2.3%)	2 (3.0%)	3 (2.7%)	F	1.000	NS
TOB	Resistant	43 (97.7%)	64 (97.0%)	107 (97.3%)			
(Gentamycin)	Susceptible	2 (4.2%)	9 (12.2%)	11 (9.0%)	2.269*	0.132	NS
CN	Resistant	46 (95.9%)	65 (87.8%)	111 (91.0%)			
(Piperacillin/Tazobactam)	Susceptible	1 (2.2%)	3 (4.4%)	4 (3.5%)	F	0.647	NS
TZP	Resistant	45 (97.8%)	65 (95.6%)	110 (96.5%)			
(Cefazolin)	Susceptible	0 (0.0%)	0 (0.0%)	0 (0.0%)	-	-	-
CFZ	Resistant	43 (100.0%)	63 (100.0%)	106 (100.0%)			
(Cefepime) FEP	Susceptible	1 (2.2%)	3 (4.2%)	4 (3.4%)	F	1.000	NS
	Resistant	44 (97.8%)	68 (95.8%)	112 (96.6%)			
(Ampicillin)	Susceptible	0 (0.0%)	0 (0.0%)	0 (0.0%)	-	-	-
AMP	Resistant	43 (100.0%)	62 (100.0%)	105 (100.0%)			
(Cefoperazone)	Susceptible	2 (4.4%)	1 (1.5%)	3 (2.7%)	F	0.565	NS
CFP	Resistant	43 (95.6%)	65 (98.5%)	108 (97.3%)			
(Cefoxitin)	Susceptible	0 (0.0%)	0 (0.0%)	0 (0.0%)	-	-	-
FOX	Resistant	43 (100.0%)	62 (100.0%)	105 (100.0%)			
(Cefpodoxime)	Susceptible	0 (0.0%)	0 (0.0%)	0 (0.0%)	-	-	-
CPD	Resistant	43 (100.0%)	64 (100.0%)	107 (100.0%)			

p -value > 0.05: Non-significant; P-value < 0.05: Significant; P-value < 0.01: Highly significant *: Chi-square test; F: Fisher's Exact test

Discussion

Following the start of the COVID-19 pandemic in March 2020, the total of cases and deaths increased worldwide dramatically [11]. Previous meta-analyses reported bacterial co-infections in <4% - 8% of patients with COVID-19 [12]. Bacterial co-infection is uncommon among hospitalized patients, so an empirical antibacterial therapy may not be necessary. Despite the

ineffectiveness of antimicrobials in the remediation of COVID-19, they are still prescribed because it is not easy to exclude the presence of a bacterial co-infection on presentation or predict its occurrence afterward [13,14]. Such a practice of prescribing unwanted antibiotics can increase bacterial resistance [15]. Thus, for optimum management of COVID-19 patients, the bacterial pathogen

responsible for the respiratory bacterial co-infection should be known [16].

In this study we aimed to explore and describe the most common bacterial pathogens encountered in lower respiratory tract infections among COVID-19 patients and to determine their antibiotic susceptibility.

Our study was conducted on lower respiratory samples collected from 80 confirmed COVID-19 positive patients and 80 confirmed COVID-19 negative patients admitted to Ain Shams University Isolation Hospitals (Geriatric and Field hospitals) during the period from February 2021 to June 2021.

Lower respiratory samples were subjected to routine culture and sensitivity in Central Microbiology Laboratory, Clinical Pathology Department, Ain Shams University Hospitals. Collected samples were cultured on routine microbiological media plates and all the tested isolates were identified by conventional microbiological techniques and subjected to antimicrobial susceptibility testing by MICs using Vitek2C automated system.

Relevant clinical and laboratory data were obtained from electronic medical records such as demographic data (age and gender) and laboratory values (D-dimer, C-reactive protein, total leucocytic count, neutrophils and lymphocyte count, lactate dehydrogenase, ferritin).

Our study showed a high prevalence of respiratory bacterial infections in males (106/160, 66.2%) than females (54/160, 33.8%). Our results correlate with findings of studies in India [17], and New York [18]. On the other hand, a study in Palestine showed different results, with high prevalence in females than males. This disagreement may be due to the Palestinian women bearing many duties, such as working and shopping, making them more exposed to COVID-19 [19].

The reason for the high prevalence in males in the present work is the biological differences between both genders (genetically and immunologically). SARS-CoV enters the host cell through angiotensin-converting enzyme 2 (ACE2) receptors [20]. A Chinese study found that ACE 2 in the human lung was far more expressed in Asian males than in females [21]. Additionally, the custom of smoking and drinking among males compared to females enhances the risk of severe COVID-19 [22].

In the current study, the age of the patients ranged from 25-88 years with a mean age of 58.24 (\pm 14.19) and a median (IQR) of 60 (50-70). Similarly, in the UK [23] and at Johns Hopkins University (JHU), United States [24], older adults (aged 65 and older) make up the majority of hospitalizations and mortalities due to comorbidities. This is identical with Data accumulated from patients in Iran [25] which suggests that older adults have higher mortality rates and constitute a larger proportion of the patients.

In our study, there was no statistically significant relation between the presence of COVID-19 and with age and gender of the studied patients, with p -value = 0.855 and 0.181, respectively. This differs from a study in India which reveals that the odds for infection were significantly higher among females for lower age categories, which declines with age [26].

As regard laboratory data of our patients, lactate dehydrogenase, ferritin, and neutrophils showed a statistically significant increase in COVID-19 positive patients in comparison with COVID-19 negative patients.

This was in concordance with a study in Spain, which showed that in 93% of the non-ICU hospitalized patients, fibrinogen was raised and two thirds of them showed an increase of ferritin. The levels of CRP, LDH, and D-dimer were above normal range in 89, 85, and 75% of the non-ICU hospitalized patients, respectively [27].

Similarly, a study conducted at Al Hussein Hospital, Egypt revealed that CRP, D-dimer and serum ferritin levels were more significantly increased in COVID-19 pneumonia group when compared with bacterial pneumonia group. WBCs and neutrophils were increased in the bacterial pneumonia group, while lymphocytes were significantly decreased in COVID-19 pneumonia [28].

As for the results of LRT samples cultures among the studied groups in the current work, 49 (24.9%) showed negative bacterial growth and 111 (75.1%) showed positive bacterial growth, 35 (31.5%) of them had mixed bacterial growth. A total of 148 organisms were isolated from the total number of positive culture patients.

Klebsiella spp. was the most common isolated organism (51/148, 25.9%) followed by *Acinetobacter* spp. (50/148, 25.4%), and *Stenotrophomonas* (1/148, 0.5%) was the least

common isolated one. Similarly, a study in Milan, Italy showed that lower respiratory tract infections were caused mainly by Gram-negative pathogens (14/26, 53.8%) [29].

Also, in Iran, a study conducted on COVID-19 patients admitted to the intensive care unit (ICU) revealed that the Gram-negative bacteria were more predominant than the Gram-positive pathogens. *Klebsiella pneumoniae* (28.4%) was the most common isolate followed by *Staphylococcus aureus* (22.4%) [30]. But Gram-positive organisms represented the majority of infections found in a study in both the blood and sputum cultures in USA [31].

In our COVID-19 positive patients, 16/80 (15.1%) LRT cultures showed negative bacterial growth and 64/80 (84.9%) showed positive growth. A total of 90/148 organisms were isolated. On the other hand, in COVID-19 negative patients, 33 (36.3%) LRT cultures showed negative bacterial growth and 47(63.7%) showed bacterial growth, a total of 58/148 organisms were isolated.

This is different from a study done in the United States in which positive bacterial infections were found in a higher proportion of patients with non-COVID-19 pneumonia (129, 13%) than in patients with COVID-19 (117, 8%) [31].

In the present study, regarding the results of cultures, a statistically significant difference between COVID-19 positive and negative patients, where 80% of COVID-19 positive patients were associated with positive bacterial growth. *Acinetobacter* spp. was the only organism showing a statistically significant difference between COVID-19-positive (31.1%) and COVID-19-negative patients (18.7%).

Our findings agreed well with those observed in a study performed in Saudi Arabia, ICU patients showed a significantly higher percentage of bacterial coinfections in sputum (74%) and blood (38%) samples. MDR-*Acinetobacter baumannii* (56%) and *Klebsiella pneumoniae* (56%) were the most prevalent bacterial species [32, 33].

Another study conducted in 16 ICUs in Italy found that 19% of ICU COVID-19 patients became positive for Carbapenem-resistant *Acinetobacter-baumannii* (CR-Ab), either colonization or infection, during an ICU stay. Furthermore, the death rate in patients with CR-Ab was as high as 64.7%, significantly higher than the

overall mortality in critically ill COVID-19 patients [34].

This was discordant with a study in North India, in which *Escherichia coli* was the most common isolate followed by *Pseudomonas aeruginosa* and *Klebsiella* spp. among the Gram-negative bacterial infections [35].

The reason for the dissimilar bacterial species and their frequency between the current work and other studies might be the variability in the health practices, patients' conditions, individuals' hygiene, number of participants, and laboratory methods [36].

As per the AST among our studied patients, there was a high resistance pattern noticed among the isolated bacteria against all the tested antibiotics. In COVID-19 negative patients, 42 (72.4%) organisms showed MDR pattern and in COVID-19 positive patients, 63 organisms (70.0%) displayed MDR pattern. The isolated bacteria exhibited the highest susceptibility for amikacin (AK) (11.7%) followed by doxycycline (DO) (10.3%). No statistically significant difference was found between the antibiotic susceptibility testing pattern in all the studied organisms between COVID-19 positive and negative patients.

Similarly, a study in Iran revealed increased concomitant infections by MDR bacteria (68.7%, 46/67) among COVID-19 patients. More than 70.0% of the Gram-positive and Gram-negative isolates were resistant to most antibiotics, including fluoroquinolone, carbapenems, and cephalosporins [30]. Also, in Saudi Arabia, ICU patients which had coinfections with Gram-negative bacteria, showed high resistance to all tested antibiotics except colistin [32].

Our findings were different from those reported in a study in North India. Their antibiotic susceptibility profiling revealed that colistin (99%), imipenem (78%), and fosfomycin (95%) were the most effective drugs against the Gram-negative isolates [35].

The reason behind the antimicrobial resistance is that exceeding 70% of patients were on antimicrobials, mostly empiric broad-spectrum ones. The marked increase in antibiotic prescribing during the pandemic challenges antimicrobial stewardship programs and risks emergence of multi-drug resistant bacteria, with their associated impact on morbidity, mortality and costs [35, 37].

The present study had several limitations. The recovered bacterial pathogens were not identified to the species level. We did not follow up with the patients to study the outcome of the bacterial co-infections, e.g., mortality. Moreover, due to financial constraints, no molecular study was performed to determine the genes responsible for the high antibiotic resistance reported in the current work.

Conclusions

COVID-19-positive patients showed higher positive bacterial growth (80%) than COVID-19 patients (58.75%). *Klebsiella* spp. was the most common organism (25.9%), followed by *Acinetobacter* spp. (25.4%). Generally, a high resistance pattern was noticed among the isolated bacteria against all the tested antibiotics.

The obtained results should be an alarm to the clinicians that they should halt the usage of empirical antimicrobials promptly and resort to culture and antimicrobial susceptibility testing as a mandatory step for the proper management of patients, and this should help slow down the progression of multidrug resistance. Moreover, we recommend sticking to the infection control principles. All these parameters should aid the implementation of robust antibiotic stewardship programs.

Declaration of conflict of interest

no conflicts of interest.

Funding

None

Author contributions

NAF and MAE designed the study. EAS collected the samples. NAF and MAE did the microbiological experiments. NAF, MAE and EAS performed the statistical analysis. NAF and EAS wrote the first draft of the manuscript. NAF and MAE provided critical suggestions on study design and manuscript writing. All authors contributed to the revision of the manuscript and approved the final version.

Acknowledgments

None.

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