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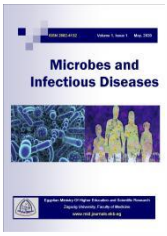

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Original article

Antimicrobial resistance patterns of bacterial and fungal lower respiratory tract infections among COVID-19 patients and their impact on mortality

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ABSTRACT

Background: Secondary infections and antibiotic resistance pattern in COVID-19 patients have drawn attention. Patients who were infected with COVID-19 were prone to subsequent infections. This study aimed to study the profile and antimicrobial susceptibility patterns of secondary bacterial and fungal lower respiratory tract infections in confirmed COVID-19 patients and analyze their impact on mortality. **Methods:** From May to October 2022, 128 sputum and endotracheal aspirates samples were collected from hospitalized confirmed COVID-19 patients suspected of having bacterial and/or fungal secondary infection. The etiologic agents and antimicrobial resistance profiles were identified using conventional and Vitek 2 methods. **Results:** Out of the 128 samples collected, 103 samples were cultivated; eighty four samples revealed growth of pathogenic micro-organisms, in which Gram negative bacteria were prevalent accounting for 50% of pathogens detected. *Klebsiella pneumoniae* was the predominant pathogen detected, followed by *Streptococcus pneumoniae* accounting for about (23.8% and 15.5%) respectively. The majority of bacteria detected were resistant to more than two classes of antibiotics. However almost, no resistance was detected among *Candida* strains. Mortality among COVID-19 patients was significantly associated with older age, positive RT-PCR results for SARS-COV-2 at the time of secondary infection, and infection with drug resistant organisms. **Conclusion:** Gram-negative bacterial infections account for the majority of secondary infections in COVID-19 cases and exhibit high rates of resistance to different categories of antimicrobials agents. Additionally, the high mortality rate in patients with secondary infections necessitates special vigilance in improving infection control procedures and antimicrobial stewardship strategies.

Introduction

The COVID-19 pandemic is attributed to the novel virus known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Infections caused by SARS-CoV-2 can vary widely, from showing no symptoms at all to causing severe, acute respiratory distress syndrome (ARDS), pneumonia, life-threatening sepsis, resulting in numerous hospitalizations and intensive care unit stays [1].

Similar to other viral pneumonia, hospitalized COVID-19 patients frequently develop

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fungal and bacterial infections [2], because associated viral infection damages ciliated cells, which worsens mucociliary clearance, increases bacterial adhesion to mucins, and enhances bacterial colonization of the airways [3]. Furthermore, ageing causes changes that influence many aspects of the body. Specifically, the immune system's age-induced adaptation increases susceptibility to infections and contributes to the failure to regulate pathogen development and tissue damage [4].

Secondary infections caused by bacteria and/or fungi have a significant impact on the prognosis and death rate of COVID-19 patients. The primary objective of the majority of infection control protocols is to avert the spread of the COVID-19 virus, whereas the significance of preventing subsequent fungal or bacterial infections is frequently disregarded. Actually, secondary infections were identified in 50% of patients who did not survive COVID-19 [5].

Antibiotics are used to treat bacterial secondary infections in proven or suspected COVID-19 patients for a variety of causes even though they are ineffective for treating this disease [6]. The challenge of excluding bacterial coinfection at presentation as well as the potential for additional microbial infections as the illness progress are among the key causes. However, incorrect antibiotic delivery will increase antibiotic resistance, which will raise COVID-19 patient mortality [6], Therefore, a key component of treatment is the identification of pathogens. Ensuring antibiotic stewardship is an additional imperative to avert any potential misuse of antibiotics beside reducing adverse effects from antibiotic overuse [7].

The study aims to identify the microbes responsible for secondary fungal and/or bacterial infections in COVID-19 patients and to evaluate antimicrobial resistance (AMR) patterns of the collected isolate.

2. Methods

2.1 Study design and location

Our study is a cross-sectional, prospective, single center study, performed at COVID-19 isolation units in Zagazig University hospitals in Egypt in collaboration with the Medical Microbiology and Immunology Department, the Scientific and Medical Research Center and the Clinical Pathology Department, Zagazig University hospitals, during the fifth wave of COVID-19 in Egypt, from May 2022 to October 2022[8]. The study has been approved by the Institutional Review Board (IRB) and the Ethical Committee of the Faculty of Medicine, Zagazig University (IRB#: 10179). It was conducted according to the revised declaration of Helsinki. All study subjects or their legal' representatives provided written informed consent.

2.2 Sample size calculation:

Assuming that prevalence rate of secondary pulmonary infections is 20% [9] and that the attendance rate of COVID-19 patients in isolation units per six months is 218 cases, the sample size was therefore determined to be 116 patients. In order to account for any dropouts or inappropriate samples, 10% of the estimated sample was added; as a result, 128 patients were included in total. Using the open Epi program, the sample size was computed with a study power of 80% and a confidence level of 95%.

2.3 Data collection:

From patients' medical records, demographic information including age, sex, date of admission, empiric antibiotic treatment, comorbidities, and outcome information were retrieved.

2.4 Patients, samples collection and processing:

We included one hundred twenty-eight adult laboratory-confirmed COVID-19 patients admitted at COVID-19 isolation units of Zagazig University hospitals suspected of having secondary pulmonary infection after at least 48 hours of admission. The patients were presented with one or more of the following: purulent sputum, hemodynamic unsteadiness. and a persistent fever (above 38.3 degrees Celsius), and radiographic abnormalities that, in the judgement of a pulmonologist, indicate a lung fungal and/or bacterial infection.

2.5 RT- PCR for SARS-CoV2 diagnosis:

At the isolation units, skilled health personnel obtained oropharyngeal (OP) and nasopharyngeal (NP) swabs, and RT-qPCR was implemented at the Scientific and Medical Research Center at the Zagazig University hospitals twice for every patients, one after patient admission for COVID-19 confirmation and repeated when there was a suspicion of secondary lung infection to assess active viral replication at this point.

2.6 Laboratory investigations supporting secondary pulmonary infections:

All COVID-19 patients suspected clinically of having secondary pulmonary infection underwent complete blood count, C-reactive protein (CRP), and procalcitonin (PCT) measurements. Upon request from the clinician, certain patients underwent assessment of serum inflammatory markers such as Interleukin-6 (IL6) and D-dimer, ferritin levels.

2.7 Bacterial and Fungal Culture:

For suspected patients with secondary pulmonary infections Sample collection and processing (For every patient, single respiratory sample was collected), either from sputum or from endotracheal aspirate, according to the patients' conscious state. A sterile wide-mouth container was utilized to collect approximately 2 mL of purulent sputum from each patient after instructing them to rinse their mouths with water beforehand. Samples were promptly moved to the microbiology lab; Specimen processing was conducted in strict adherence to the guidelines set forth by the Centers for Disease Control and Prevention (CDC) and the utilization of recommended personal protective equipment. Samples were smeared and analysed to see if it was acceptable for culture. Samples with more than 10 epithelial cells per high power field, independent of leukocyte count, were not processed. Blood, MacConkey, and chocolate agar plates (Oxoid, UK) were inoculated with sputum and endotracheal aspirates, and then incubated at 37°C for one to two days. [9]. To identify bacterial isolates; Gram stain beside common biochemical tests like coagulase, catalase, urease, mannitol salt agar, oxidase, DNase, bile esculin, citrate, triple sugar iron agar and others were employed [10]. Direct lactophenol-cotton blue smears were examined under a microscope to identify fungal isolates. The specimens were subsequently cultivated on Sabouraud dextrose agar (Oxoid UK), and incubated at room temperature [10]. Furthermore, the identification of bacterial and *Candida* species was validated using an automated Vitek 2 Compact system (bioMerieux, France), in accordance with the guidelines provided by the manufacturer.

2.8 Antimicrobial susceptibility testing:

All the bacterial and fungal strains were analysed by automated Vitek 2 Compact (bioMerieux, France), by applying AST-YST, Gram-negative, and Gram-positive cards, antimicrobial breakpoints were interpreted as per CLSI 2021[11]. Phenotypic detection of resistance pattern was based on advanced expert system on Vitek 2. Multidrug resistant (MDR) is defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories, extensively drug-resistant (XDR) is defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories Extended spectrum beta-lactamase (ESBL) producers is defined as the bacteria that are able to hydrolyze extended spectrum cephalosporins, carbapenamase producers is defined as the bacteria that are able to hydrolyse carbapenem antibiotics [12].

Statistical analysis

2015 IBM Corp. was used for data collection, tabulation, and statistical analysis. Version 23.0 of IBM SPSS Statistics for Windows. New York, Armonk: IBM Corp. The mean ± SD was used to convey quantitative data, whereas the terms number and (%) were used to express qualitative data. The Chi-square test was utilized to compare the percentage of categorical variables. Logistic regression was used to characterize data and elucidate the connection between one or more independent factors and one or more dependent categorical variables. Every test had two sides. A p-value of less than 0.05 was deemed statistically significant, whereas a p-value of more than 0.05 was deemed statistically insignificant.

3. Results

3.1. Demographic and clinical characteristics

The present study enrolled 128 confirmed COVID-19 patients who were suspected of having secondary pulmonary infection at least 48 hours after admission, as determined by systematic random sampling. A total of 128 (sputum and endotracheal aspirate) specimens were obtained. Twenty-five sputum samples were excluded. The study comprised thirty endotracheal aspirate samples and seventy-three sputum samples. The participants were all adults. Males comprised the majority (54.4%). Microbial growth was found in 80 (77.7%) of the cultured samples. **Table 1** provides an illustration of the demographic and clinical characteristics of the patients under study.

3.2 Etiological distribution of Secondary lower respiratory tract infections among hospitalized confirmed COVID-19 patients:

Overall, 84 bacterial and fungal isolates were collected from the 80 patients, where the Gram-negative bacteria was more prevalent than the Gram-positive with a number and percentage of 42 (59.2%), 29 (40.8%) respectively. Candida species were found in all 13 isolated fungi. *K.pneumoniae* was the main isolated pathogen (24 %), followed by *S.pneumoniae* (15.5%), the results are shown in **(Fig. 1)**. Mixed infections involving two pathogens were identified in the same sample from four patients; the pathogens were either *K. pneumoniae* and *Burkholderia cepacia* or *K. pneumoniae* and *pseudomonas aeruginosa.*

3.3 Antibacterial susceptibility

The antimicrobial resistance rate of isolated bacteria in patients with secondary bacterial infections (SBIs) was generally high. The percentages of carbapenem-resistant *P.aeruginosa* and *B.cepacia* were 100%, While that of *K. pneumoniae* reached 90% **(Table 2)**. Moreover, almost all Gram-negative bacteria showed either multidrug resistance (MDR) or extreme drug resistance patterns (XDR). While among Gram-positive bacteria, 61.5% of *S. pneumoniae* were MDR **(Table 3).**

3.4 Antifungal susceptibility

C. albicans, C.glabrata isolates were tested against fluconazol, voriconazole, caspofungin, micafungin, amphotericin B and flucytosine. All *C.glabrata* isolates (n=4) were susceptible to all antifungal drugs tested, while *C. albicans* (n=9) showed lower susceptibility pattern to fluconazole and caspofungin with percentages (67% and 89%) respectively and 100% susceptibility to the rest of drugs.

3.5 Risk factors associated with mortality in COVID-19 patients with secondary bacterial infection:

In univariate analysis, mortality was significantly associated with older patients, positive RT-PCR results for SARS-Cov2 at the time of secondary infection, and infection with drug resistance organisms (P<0.001), (P=0.004) and (P=0.006) respectively. While no association was found between patients' sex and mortality **(Table 4)**.

In the multivariate analysis (including all the variables significantly associated with the mortality in the univariate analysis), mortality was significantly associated with older patients and infection with drug resistance organisms (P<0.001) and (P=0.024) **(Table 5)**.

There was no significant difference in mortality rate among patients with bacterial or fungal infection **(Table 6)**.

Table 1. Demographic and clinical characteristics of the studied patients**.**

Variables	(n=103)	%
Gender		
Males	56	54.4
Females	47	45.6
Age		
≤60 years	54	52.4
>60years	49	47.6
Mean ±SD.	59.7±10.8	
Procalcitonin Mean ±SD.	3.1±5.6	
CRP Mean ±SD.	89.5±55.9	
Culture		
Endo-tracheal aspirate	30	29.1
Sputum	73	70.8
COVID-19 RT qPCR (at the time of culture sampling)		
Positive	56	54.4
Negative	47	45.6
Secondary infection		
Yes	80	77.7
No Associated comorbidity	23	22.3
Yes	88	85.4
No	15	14.6

*Associated comorbidity included hypertension, diabetes, cardiac and renal disease

Table 2. Antibiotic resistance of Gram-negative bacteria (n=42). *

	K. pneumoniae (n=20)		*Escherichia coli* (n=10)		*P. aeruginosa* (n=6)		*B. cepacia* (n=3)		*Proteus mirabilis* (n=3)	
	N	%	N	%	N	%	N	%	N	%
Amikacin	15	75	4	40	6	100	-	-	0	0
Ampicillin-sulbactam	18	90	5	50	6	100	-	-	3	100
Cefepime	20	100	5	50	2	33	-	-	3	100
Cefotaxime	18	90	5	50	4	66.7	-	-	3	100
Ceftazidime	18	90	9	50	6	100	0	0	3	100
Ceftriaxone	18	90	8	80	6	100	-	-	3	100
Ciprofloxacin	18	90	4	40	6	100	-	-	3	100
Impenem	18	90	9	90	6	100	-	-	3	100
Gentamicin	15	75	5	50	6	100	-	-	0	0
Levofloxacin	18	90	7	70	6	100	3	100	3	100
Meropenem	16	80	5	50	6	100	3	100	0	0
Moxifloxacin	19	95	10	100	6	100	-	-	3	100
Piperacillin_tazobactam	20	100	10	100	6	100	-	-	0	0
Tigecycline	13	65	5	50	-	-	-	-	3	100
Trimethoprim/Sulfamethoxazole	16	80	10	100	6	100	3	100	3	100
Colistin	0	0	0	0	0	0	-	-	0	0
Aztreonam	20	100	10	100	6	100	-	-	3	100
MDR	8	40	10	100	-	-			3	100
XDR	10	50	-	-	6	100	3	100	-	-
Carbapenemase producer	18	90	5	50	6	100	3	100	3	100
ESBL producer	18	90	5	50	4	67	-	-	3	100

*The numbers and percentages in this table represent the resistance rates
(-): Not tested.

Table 3. Antibiotic susceptibility of Gram-positive bacteria (n=29) *

	S.pneumoniae (n=13)		*Coagulase negative staphylococci* (n=9)		*Streptococcus viridans* (n=3)		*Enterococcus faecalis* (n=2)		*Staphylococcus aureus* (n=2)	
	N	%	N	%	N	%	N	%	N	%
Amoxacillin-clavulinic acid	-	-	9	100	-	-	2	100	0	0
Ampicillin	9	69	6	67	0	0	2	100	0	0
Clindamycin	8	62	5	56	3	100	2	100	2	100
Erythromycin	6	46	4	44	3	100	0	0	2	100
Levofloxacin	11	85	6	78	3	100	2	100	2	100
Linezolid	13	100	6	67	3	100	2	100	2	100
Moxifloxacin	_	-	9	67	-	-	2	100	2	100
Oxacillin	-	-	7	78	-	-	2	100	0	0
Penicillin	9	69	6	67	0	.0	2	100	0	0
Tetracycline	12	92	0	0	3	100	0	0	2	100
Tigecycline	-	-	6	67	-	-	2	100	2	100
Trimethoprim/Sulfamethoxazole	9	69	9	100	3	100	2	100	2	100
Vancomycin	13	100	4	44	3	100	2	100	2	100
Gentamycin	-	-	9	100	-	-	2	100	2	100
Benzylpenicillin	9	69	6	67	0	0	-	-	-	-
Cefotaxime	9	69	-	-	0	0	-	-	-	-
Ceftriaxone	9	69	-	-	0	0	-	-	-	-
Rifampicin	11	85	-	-	3	100	-	-	-	-
MDR	8	62	4	44	0	0	0	0	0	0

*The numbers and percentages in this table represent the susceptibility rates.
(-): Not tested.

Table 4. Univariate analysis of the association of risk factors with mortality in COVID-19 patients

Variables	**Outcome**				**N**	**P**	**Odds(95%CI)**
	Survival		**Died**				
	No.	**%**	**No.**	**%**			
Age per years							
≤60	29	65.9	15	34.1	44	0.0001*	9.7(3.3-28.3)
>60	6	16.7	30	83.3	36		
Gender							
Males	18	38.3	29	61.7	47	0.24	0.77(0.24-1.4)
Females	17	51.5	16	48.5	33		
The second RT-PCR for SARS-Cov2							
Positive	12	28.6	30	71.4	42	0.004*	3.8(1.5-9.7)
Negative	23	60.5	15	39.5	38		
Multiple drug resistance							
No	20	62.5	12	37.5	32	0.006*	3.7(1.4-9.4)
Yes	15	31.3	33	68.7	48		
Comorbidities							
No	4	28.6	10	71.4	14	0.21	0.45(0.13-1.6)
Yes	31	47	35	53	66		

Abbreviations: CI, confidence interval; COVID-19, coronavirus disease, *P: significant

Table 5. Multivariate analysis of the association of risk factors with mortality in COVID-19 patients

Variables	Sig.	Exp(B)	95% C.I. for EXP(B)	
			Lower	Upper
Age>60 years	0.0001*	8.359	2.571	27.180
Second RT-PCR positive	0.26	1.9	0.6	5.7
MDR drug resistance	0.024*	3.6	1.18	11.28

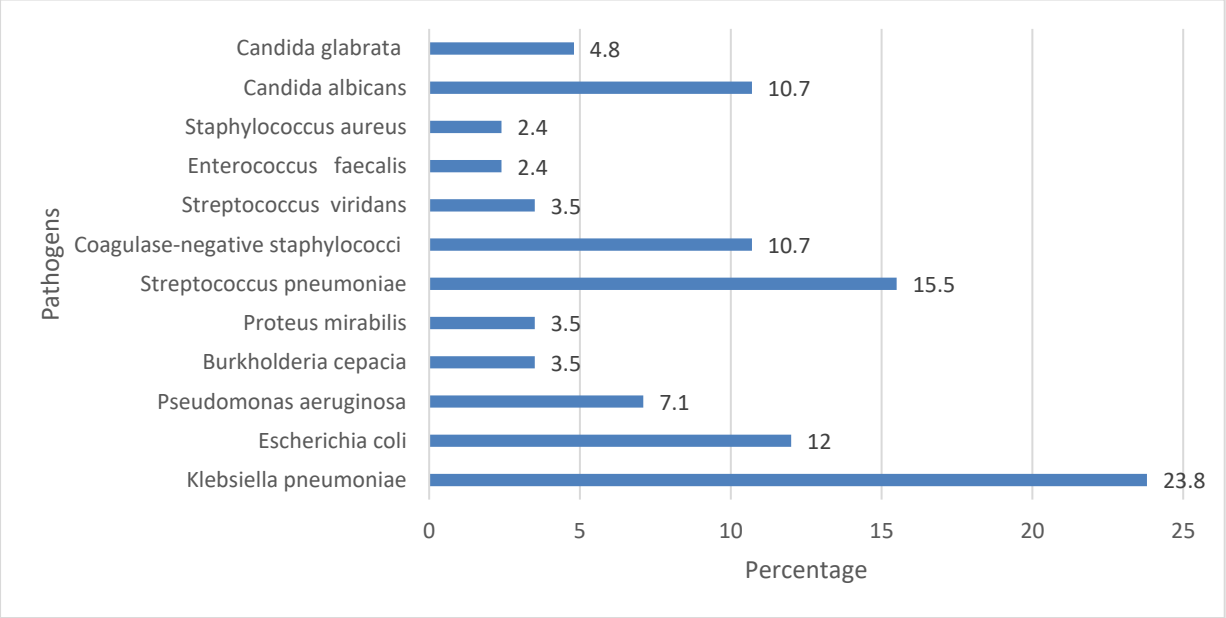
Abbreviations: Exp(B); odds ratio, CI; confidence interval

Table 6. Prognosis of COVID-19 patients with bacterial and fungal infection.

	Bacterial n.67		Fungal n.13		χ^2	P
	n.	%	n.	%		
Survived	31	46.2	4	30.8	1.1	0.3
Died	36	53.7	9	69.2		

χ^2 Chi square test P >0.05: no-significant

Figure 1. The isolated pathogens from secondary pulmonary infections in COVID-19 patients with their percentages



Discussion

Secondary infection of SARS-CoV-2 with other microorganisms, particularly bacteria and fungi, plays a crucial role in the progression of COVID-19, complicating diagnosis, treatment, and prognosis. Inpatients with COVID-19 who develop bacterial superinfections experience worsened disease progression and prognosis, leading to increased admissions to intensive care units, higher antibiotic use, and elevated mortality rates [13].

In this study, we described the characteristics of secondary pulmonary infections in COVID-19 patients. Culture results from sputum or endotracheal aspirate as well as antibiotic sensitivity patterns and their association with an increased risk of mortality in hospitalized patients are discussed.

In the present study, bacterial infection represented 84.5 % of the isolated pathogen while fungal infection represented 15.5% .These finding are consistent with those of Hegazy et al. who reported that from all collected samples; 85.8%

were positive for bacterial growth and 14.2% were positive for fungal growth [14]. Moreover, Sonam et al. observed that there was a minimal incidence of fungal infection among COVID-19 patients and that Gram-negative bacteria dominated COVID-19 secondary infections accounting for 50 % of the isolated organisms, which aligns with our findings. Our findings were consistent with other studies demonstrating that bacterial infections in COVID-19 cases are likely to be Gram-negative bacteria (14-17). Due to the limited number of new antibiotics that have been effectively developed to combat this particular category of infection, Gram-negative resistant bacteria are more prevalent [18].

K.pneumoniae, *S. pneumoniae* and *E. coli* were the pathogens most frequently identified in COVID-19 patients. In partial agreement with our finding, Hegazy et al. observed that *K. pneumoniae* and *Acinetobacter baumannii* were found to be the most commonly isolated bacteria from COVID-19 patients [14]. Carolina et al. reported that the most common bacteria isolated were *S. pneumoniae*, followed by *S. aureus* [19], while Karatas et al. reported that *E. coli* was more prevalent followed by *K. pneumonieae* [20].

The varied findings can be attributed to a number of different factors; the antimicrobial policy applied in the hospital, the locality where the study was done, healthcare-associated infection monitoring programs beside drug resistance status appear to have a substantial influence on the occurrence of fungal or bacterial infections in coronavirus patients [21]. The potential cause for the high occurrence of Gram-negative bacteria among these individuals is the acquisition of infections associated with invasive devices while hospitalized [22], and the incorporation of azithromycin, which is predominantly efficacious against Gram-positive bacteria into the COVID-19 treatment regimen [23].

Notably, In our investigation, the preponderance of isolated bacteria exhibited a consistent pattern of multidrug resistance (MDR) or extensively drug-resistant (XDR) accounting for about 73.2 % of all bacterial isolates. This finding was in line with another study from Assiut University Hospital in Egypt which also reported that most strains isolated from COVID- 19 patients had pattern of multidrug resistance [15]. The majority of Gram-negative clinical isolates were extended-spectrum beta-lactamase (ESBL), and/or carbapenemase producer, all isolates of *P.aeruginosa*, *B.cepacia* and *Proteus mirabilis* were carbapenemase producers while it reaches 90% among *K.pneumoniae* strains. The drug with the highest sensitivity was colistin. This agreed with the findings by Hegazy et al. who reported high levels of antibiotic resistance in the bacterial isolates from COVID-19 patients[14]. Moreover. Pourajam et al. reported that bacterial superinfection in COVID-19 patients was mostly due to carbapenem *resistant K. pneumoniae* (CRKP) and carbapenem resistant *A. baumannii* (CRAB) [17]. Furthermore Lie et al. demonstrated that a majority of the *K. pneumoniae* and *A. baumannii* strains isolated from their patients were multidrug-resistant bacteria, isolation rates for CRAB were 91.7% and for CRKP were 76.6% [24]. Among Gram-positive bacteria, isolated *S.aureus* were resistant to methicillin but not to vancomycin. This observation matched the results that were reported by Ramadan et al. who reported that all staph strains found were vancomycin-susceptible [15]. Therefore, in the event of a secondary infection, vancomycin appears to be the best empirical choice for Gram-positive bacteria. These results all highlight the fact that antibiotic abuse or misuse leading to antimicrobial resistance, is a major global health issue that has arisen during the pandemic[17]. Another explanation for the increase of drug-resistant strains is that during the outbreak of COVID-19, It was almost mandatory for healthcare workers to wear gloves as part of their personal protective equipment and do not consider the need to practice hand washing prior to and following patient care, and there was a shortage of worry for patient-to-patient transfer of XDR pathogens in hospitalized patients [16].

In our study, all *Candida* species were susceptible to at least one drug of each class of antifungals drugs tested. This was concordance with other researches by Hegazy et al. [14], He reported that most of the *Candida* isolates exhibited susceptibility to every antifungal drug tested.

In this study, old age, sustained positivity of RT-qPCR results for COVID-19 infection at the time of respiratory sample collection for culture and infection with multiple drug resistance bacteria exhibited a statistically significant correlation with mortality in patients who were infected with COVID-19.

Interestingly, advanced age has been identified as a substantial independent predictor of mortality in cases of severe acute respiratory syndrome [25].This was confirmed in our study

which indicated that increasing age was related to mortality in COVID-19 patients, and was consistent with the finding of other studies by Zhou et al. and Du et al. [26,27]. Possible negative outcomes in old age may be attributed to an inability to regulate viral replication and longer proinflammatory responses due to age-related declines in T-cell and B-cell activity and an overabundance of type 2 cytokines. [28]. In the present study more than half (56.3%) of the hospitalized patients with secondary bacterial infections died. This finding is in line with prior studies demonstrating closely related percentages of mortality (50%) [26] and (49.0%)[24]. Possible causes of the higher mortality rate include patients' ages, immunosuppression, critical illness, and need for mechanical ventilation [28]. It has been suggested that a hyperinflammatory response and cytokine storm, which result in immunological dysregulation and ARDS, are responsible for the increased fatality rate in severely sick COVID-19 patients [29]. Hence early corticosteroid therapy in critically ill patients is thought to improve outcomes and decrease mortality . However, due to immunosuppression, individuals receiving corticosteroid treatment had a higher risk of contracting a bacterial infection [30].

As far as we are aware, this is the first inv estigation assessing the relationship between positive RT-qPCR results for COVID-19 patients when the respiratory sample was taken for culture and raising the potential for death among COVID-19 patients with secondary bacterial infection. Patients with positive RT-qPCR results had significantly greater mortality rate than patients with negative results. This finding may emphasize the possible role of the viral load in indicating the severity of the disease which was demonstrated in an investigation by Dadras et al. who found that the viral load was a key factor in determining the severity of the illness and was particularly significantly connected with lung damage [31].

Moreover, our study is the first to examine the association between infection with multiple drug resistant microbes and increased risk of death among COVID-19 patients. We found a significant association between infection with the resistant pathogen and the mortality rate in both univariate and multivariate analysis. This finding is in line with that of a study from Saudia Arabia which revealed that bacterial colonization lengthened ICU hospital stays and increased the mortality rate [32].

On the other hand, our study did not reveal any relation between sex and the risk of mortality in patients with COVID-19, which was in agreement with the finding of other study from Italy [33]. Opposing this finding a meta-analysis conducted by Du et al. found that male patients with COVID-19 have a 2.5 times greater mortality rate than that of females [34]. One explanation is that men are more likely than women to be infected with the coronavirus, which is likely due to greater levels of ACE2 expression in men which is attacked by the coronavirus [35]. Furthermore, There is an important function for sex hormones and the female X chromosome in adaptive and innate immunity [36]. Discrepancy between results may be due to differences in sample size or study population. So, results need to be verified by additional studies with larger sample sizes.

In our study comorbidities were not significantly associated with mortality. In a line with our study Soto et al. didn’t find any relation between comorbidities and mortality risk [37], another study by Grasselli et al. could not detect any association between mortality and hypertension [38]. On the other side some studies found association between only certain risk factors and mortality. In hospitalized patients, Diabetes and obesity were linked to death, but not hypertension, according to Mexican research [39], while a Brazilian investigation found that diabetes and hypertension, but not obesity were an independent risk factors for mortality[40]. One explanation for these findings is that, although these conditions may increase the likelihood of hospital stay in the general population, they may not necessarily elevate the mortality risk among patients with severe COVID-19[37], which was the predominant condition in most of our patients. Finally, there was no discernible difference in fatality rates between patients who had bacterial or fungal infections.

Our study has certain limitations, although it produced significant data about the effects of secondary bacterial infections on COVID-19 patients. First of all, because the data came from a single hospital, it is possible that the microbes that were found there had a unique microbiological profile. Secondly, despite having highly resistant phenotypes (such ESBL or CRE) on the surface, we were unable to corroborate the genotypic resistance profiles of the bacterial isolates in our investigation. Therefore, additional multicentral investigations with a larger sample size are required to determine

the connection between COVID-19 and subsequent bacterial infections. However, our results could contribute to a better understanding of secondary infection in patients with COVID-19.

In conclusion, this study identifies multi-drug resistant Gram-negative bacteria as the most common pathogens responsible for secondary bacterial infections in hospitalized COVID-19 patients. Hospitalized COVID-19 patients who developed secondary infections caused by a resistant strain had a high mortality rate. The risk factors identified in this research may aid clinicians in identifying patients with poor prognosis while caution against bacterial or fungal infections is critical in the care and treatment of COVID-19 patients, prompt characterization of associated infections is likely to reduce fatal outcomes while improving antimicrobial stewardship during the COVID-19 pandemic.

Disclosure of potential conflicts of interest

The authors report that there were no conflicts of interest.

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