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Frequency and antimicrobial resistance pattern of bacterial isolates from patients with COVID-19 in two hospitals of Zanjan

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ABSTRACT

Background and Objectives: The outbreak of COVID-19 has been challenging the global health systems. As one of the major associated concerns, microbial co-infections and antimicrobial resistance play critical roles in the prognosis of the disease. This study aims to evaluate co-infections in COVID-19 patients regarding drug resistance.

Materials and Methods: A total number of 5530 Real Time PCR-confirmed COVID-19 cases, who were admitted to two major educational Hospitals in Zanjan, Iran, from February 2019 to February 2020 were included. Respiratory, blood and urine specimens were collected and cultured on selective media. Subsequently, isolates identification, disc diffusion susceptibility tests, and data analysis were carried out.

Results: Bacterial and fungal co-infections were confirmed in 423 patients (8.1%). Co-infections were more prevalent among females (53.2%) than males (46.8%). Coinfected patients had a significantly higher mortality rate compared to those without co-infections (54.8% vs. 12.2%, P<0.001). *Acinetobacter baumannii* was the most prevalent bacteria isolated from respiratory tract (15.4%) and blood (2.1%). *Escherichia coli* (12.5%) was the most frequent bacteria in urine. Fungal co-infection was confirmed in 174 (3.36%) patients. Gram-negative bacteria were highly sensitive to colistin (97.85%) and widely resistant to cefixime (91.79%) and trimethoprim-sulfamethoxazole (89.64%). Gram-positive bacteria were considerably sensitive to vancomycin (68%) and nitrofurantoin (66%). Tetracycline and ampicillin were the least effective antibiotics for Gram-positive bacteria with respective resistance rates of 90.91% and 83.33%.

Conclusion: Given the high incidence of bacterial co-infections in COVID-19 patients, it is important to develop rapid and efficient diagnostic, therapeutic and disinfection guidelines to control these infections in the hospitals.

Keywords: Bacterial co-infections; COVID-19; Susceptibility testing; SARS-CoV-2

INTRODUCTION

Pneumonia is an inflammatory condition of the lung, which is characterized by the involvement of pulmonary alveoli. Following the swelling of the air sacs, the respiratory capacity decreases. Viruses and bacteria are of great importance in causing pneumonia; however, pneumonia can be caused by fungal, parasitic, or autoimmune diseases (1). *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* are the most common causes of bacterial pneumonia (2). Although rare cases of pulmonary viral infections are associated with factors such as adenoviruses, metapneumoviruses, and hantaviruses, influenza A and B, respiratory syncytial virus (RSV), and human

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parainfluenza virus are the most common viral respiratory infections, and in addition to this list, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-COV-2) has brought about several health problems to the therapeutic community (1). One of the regarding problems is the simultaneous occurrence of bacterial and fungal infections with the mentioned virus.

On December 31, 2019, seven cases of acute respiratory syndrome were reported to the Municipal Health Commission of Wuhan, China. The early symptoms included fever, dry coughs, and shortness of breath, and radiological findings indicated lung involvement. The causative agent of this airborne infection, which is a beta-coronavirus with RNA and envelope, was named SARS-COV2, and the disease was named COVID-19 by the World Health Organization (WHO). Rapid person-to-person transmission of the disease occurs either directly or indirectly. In the direct way, close contact with infected individuals (at a distance of less than one meter) transmits the nasal secretions of the infected person during sneezing, coughing, and talking. However, the new coronavirus can also cause the disease indirectly through contact with infected objects (3).

Following the spread of the severe viral pneumonia from China and the first cases outside China (Thailand, Japan, South Korea) on January 20, 2020, and the first European (France) report on January 24, 2020, the disease became a widespread pandemic and affected more than 135 million people worldwide by April 2021, killing almost three million patients (4).

By April 2021, the total number of cases was estimated at 2.05 million. Disease-related deaths in Iran were estimated at 64,000; the mortality rate in the country (about 3.1%) is significantly higher than the global average (about 2.1%) (P<0.001) (5).

According to various studies, clinical information and lung tissue examinations of deceased patients are indicative of presence of respiratory bacterial infections. Incidence of secondary infections and co-infections (especially bacterial infections) are very likely in infected patients because of the weakening nature of SARS-CoV-2. The virus manifests more severely in patients with underlying diseases or immunocompromised individuals. Furthermore, the outbreak of secondary bacterial infections in Wuhan was reported to be 15% (6). According to the previous literature, 14.3% and 3.5% of patients were diagnosed with secondary bacterial infections and co-infections, respectively. Early detection of co-infections and proper prognosis are of great importance (7).

Bacterial infection is defined as growth of pathogenic bacteria on the body surface or inside the body. Bacterial respiratory infections affect respiratory organs, leading to mild to severe pneumonia. *S. pneumoniae, H. influenzae, S. aureus,* and *P. aeruginosa* are the most common causes of bacterial pneumonia. Meanwhile, 15% of bacterial pneumonias are caused by less common microorganisms including *Klebsiella pneumoniae, Chlamydia pneumoniae, Chlamydia psittaci, Mycoplasma pneumoniae, Legionella pneumophila, Coxiella burnetii, Bordetella pertussis, Mycobacterium tuberculosis,* and *Moraxella catarrhalis* (2).

Coinfections are common among people with viral infections; they are in fact one of the major reasons behind involvement and mortality. In other words, in several cases the patients may face acute conditions or even die due to secondary bacterial infection or dealing with more than one infectious agent at a time. Infectious pneumonias have similar clinical symptoms attaching importance to identification of the causative pathogen in early stages, effective treatment, and prevention of ineffective and improper prescription of antibiotics, which contributes to antibiotic resistance. Antibiotics are compounds that can destroy some of bacteria by killing them or inhibiting the growth and multiplication of microorganisms. The novelty of this condition, obscures the precise link between bacterial infections and the novel coronavirus. This is while the medical community needs treatment guidelines as soon as possible in order to diagnose and evaluate the effect of potential antibiotics on these co-infections considering geographical diversity (7).

The ever-rising spread of secondary infections can cause property damage and casualty to patients and medical staff. Nosocomial infections, which were one of the main problems medical facilities were facing before the outbreak of coronavirus, have gained more importance during the pandemic due to the dramatic increase in the vulnerable population. As a result, the incidence increases among hospitalized patients and the medical staff.

Studies carried out on COVID-19 coinfections and the associated antibiotic resistance pattern in every province provide a clearer prognosis of the disease and finally, reduce antibiotic resistance and the cost of treatment, which happens to be very high. The aim

of our study was to determine frequency of bacterial strains in clinical specimens in COVID-19 hospitalized patients based on demographic characteristics of patients and developing an antibiotic prescribing guideline for COVID-19 patients in Zanjan province, Iran.

MATERIALS AND METHODS

Ethics approval and consent to participate. The current study is in accordance to the ethical principles and the standards for conducting medical research in Iran and approved by the Research Ethics Committee of Zanjan University of Medical Sciences (IR.ZUMS.REC.1399.377).

Sample collection and culture. In this cross-sectional study, a total number of 2856 respiratory tract, blood, and urine samples of clinical origin were collected from Real Time PCR confirmed COVID-19 patients hospitalized in Valie-Asr and Mousavi hospitals in Zanjan, Iran, from February 2019 to February 2020. Identification of bacterial isolates was don based on the standard biochemical and microbiological tests (8).

Identification of bacterial isolates. Isolate identification was carried out based on Bergeys microbiology book guidelines (8). Gram staining, various media and conventional biochemical tests including oxidase, catalase, Triple Sugar Iron Agar (TSI), Sulfide Indole Motility (SIM), citrate, Methyl Red (MR)/Voges-Proskauer (VP), urease, sensitivity to specific antibiotic disks, Mannitol Salt Agar, Dnase, etc. (Merck, Germany) were used to confirm the growth of the bacteria.

Antibiotic susceptibility testing. Disc diffusion susceptibility tests were carried out for positive clinical cultures according to the clinical and laboratory standards institute (CLSI) guidelines (Kirby–Bauer method) (9). Subsequent to dipping a sterile swab in each of the 0.5 McFarland suspensions of the isolates, inoculation was done on Muller-Hinton agar (MHA) plate surface by streaking the swab in back-and-forth motion. The following disks were applied for Gram-negative bacteria within 15 min of inoculation: amikacin (AN) (30 µg), gentamicin (GM) (10 µg), imipenem (IMP) (10 µg), nitrofurantoin (FM) (300 µg), trimethoprim/sulfamethoxazole (SXT) (25 [1.25/23.75] µg), ciprofloxacin (CP) (5 µg), cefepime (FEP) (30 µg), ceftriaxone (CRO) (30 µg), cefixime (CFM) (5 µg), cefazolin (CZ) (10), ceftazidime (CAZ) (30 µg), meropenem (MEN) (10 µg), nalidixic acid (NA) (30 µg), ampicillin (10 µg), and colistin (CL) (10 µg). The following disks were applied for Gram-positive bacteria within 15 min of inoculation: amikacin (AN) (30 µg), gentamicin (GM) (10 µg), imipenem (IMP) (10 µg), nitrofurantoin (FM) (300 µg), trimethoprim/sulfamethoxazole (SXT) (25 [1.25/23.75] µg), ciprofloxacin (CP) (5 µg), penicillin (P) (10 µg), tetracycline (TE) (30 µg), vancomycin (V) (30 µg), cefazolin (CZ) (10), clindamycin (CC) (2 µg), meropenem (MEN) (10 µg) and ampicillin (10 µg). The MHA plates were incubated at a temperature of 37°C for 24 h (9).

Statistical analysis. Data analysis was performed using SPSS 26 (SPSS Inc., Chicago, IL., USA) and P-values of less than 0.05 were defined as statistically significant. Variables in independent groups were compared using the Chi-square test.

RESULTS

From February 20, 2019 to February 20, 2020, a total number of 5530 Real Time PCR-confirmed COVID-19 cases were admitted to Vali-Asr Educational Hospital and Mousavi Educational Hospital in Zajan, Iran. The admitted patients consisted of 2728 (49%) females and 2802 (51%) males with an overall mortality rate of 14.6% (n=812). Our study included COVID-19 patients who were hospitalized for one or more days. Therefore, out of 5530 admitted patients, 5166 cases (93%) were investigated for bacterial and fungal co-infections of respiratory tract, blood, and urine samples. According to the laboratory identification of isolates, bacterial and/or fungal co-infections were confirmed in 423 patients (8.1%) who consist the population of our study. Fig. 1 depicts the aforementioned information.

Blood, urine, and respiratory tract cultures were respectively ordered for 472 (9.2%), 2132 (41.6%) and 242 (4.6%) patients. Patients aging from 60 to 70 years (26.2%, n=111) had the highest ratio in this study and co-infections were the least prevalent among patients younger than 10 (1.2%, n=5). Bacterial and fungal co-infections were more prevalent among females (53.2%, n=225) than males (46.8%,

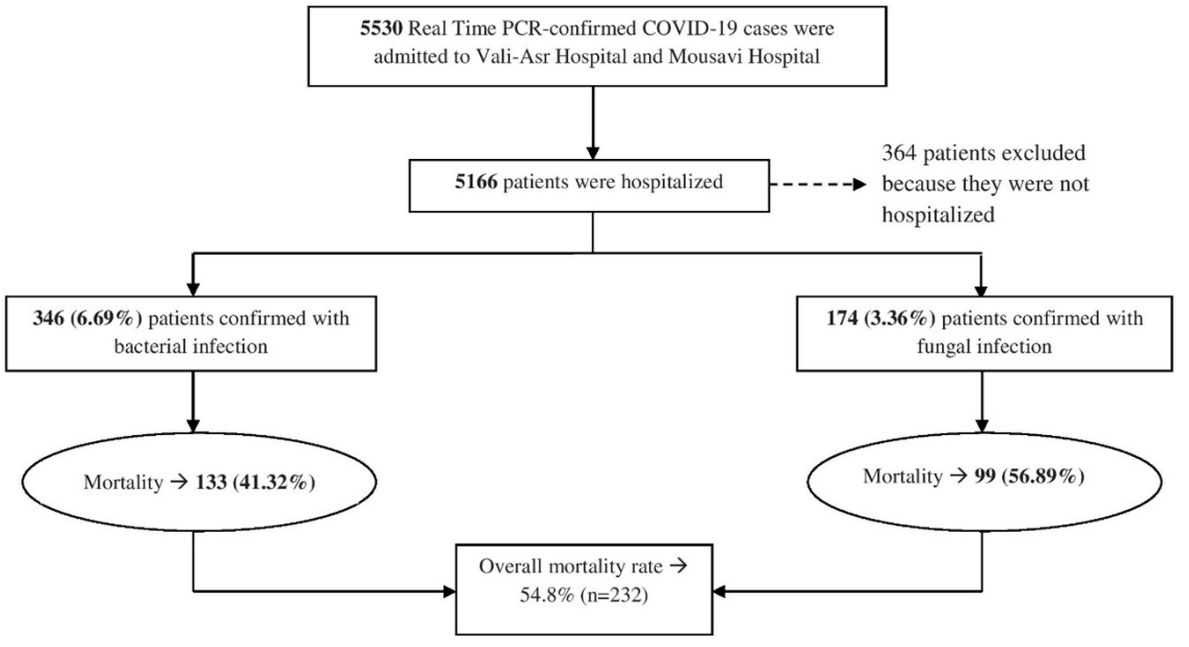

Fig. 1. Co-infections and mortality rates in the admitted patients.

n=198); nevertheless, the difference was not significant (P=0.383). Co-infection in COVID-19 patients was associated with longer length of stay. The median length of stay for these patients was 11 days with an average of 13.91 days. Mortality rate was significantly higher in coinfected patients compared to non-coinfected patients (54.8% vs. 12.2%, *P*<0.001).

ICU admission was significantly higher in patients diagnosed with bacterial and fungal co-infections compared non-coinfected COVID-19 patients (55.8% vs. 16%, P *P*<0.001). The most frequent wards of hospitalization for the coinfected patients were as follows: internal (13.9%, n=59), infectious diseases (6.9%, n=29), cardiology (5.7%, n=24), surgery (5.2%, n=22), respiratory (4.7%, n=20), coronary care unit (2.8%, n=12), gynecology (2.6%, n=11), and neurosurgery (2.4%, n=10).

A significant proportion of patients (70%, *P*<0.001) had at least one comorbidity alongside SARS-COV-2 infection, with hypertension (38.8%) and diabetes (24.3%) being the most common coexisting medical conditions followed by ischemic heart diseases (13%, n=55) and other cardiovascular dysfunctions (6.9%, n=29), hyperlipidemia (5.9%, n=25), Alzheimer's disease and other cognitive impairments (4.7%, n=20), Cerebrovascular accident (3.8%, n=6), thyroid disorders (3.1%, n=13), cancer (3.1%, n=13), rheumatoid arthritis (2.8%, n=12), asthma (1.9%, n=8), Chronic obstructive pulmonary disease (1.4%, n=6), obstructive airway disease (.02%, n=1), and other previous medical history (14.7%, n=62).

Respiratory (40%, n=169), urinary tract (32.6%, n=138) and blood (9.2%, n=39) infections were respectively the most prevalent co-infections confirmed in the included COVID-19 patients. Table 1 provides information on the types and frequencies of the bacteria isolated from the mentioned specimens. *Escherichia coli* was the main Gram-negative (12.5%, n=53) and Group D streptococci (GDS) were the main gram-positive (5.7%, n=24) pathogen isolated from the urine samples of the included patients. Yeast was isolated from the urine samples of 125 patients (29.6%). Among the Gram-negative and Gram-positive organisms isolated from respiratory samples of COVID-19 hospitalized patients, *Acinetobacter baumannii* (15.4%, n=65) and *Staphylococcus saprophyticus* (1.7%, n=7) had the highest prevalence rates, respectively. Fungal respiratory co-infections were reported in 46 (10.9%) patients. Bacteremia was identified in 39 patients (9.2%) with *A. baumannii* (2.1%, n=9) and *Staphylococcus epidermidis* (1.7%, n=7) being the most commonly identified Gram-negative and Gram-positive bacteria, respectively. Yeast was not isolated from the blood samples of the patients. Stool co-infections were rare with a total rate of 0.7% (n=3) and yeast was the only organism isolated from the stool samples. *Morganella morganii* and

Table 1. Prevalence of clinically isolated bacteria based on the sample type in a total number of 423 coinfected cases.

	Bacteria	Urine samples	Respiratory samples	Blood samples
Gram Negative	*Acinetobacter baumannii*	1.9% (n=8)	15.4% (n=65)	2.1% (n=9)
	Escherichia coli	12.5% (n=53)	2.1% (n=9)	1.4% (n=6)
	Klebsiella pneumoniae	5% (n=21)	12.5% (n=54)	1.4% (n=6)
	Klebsiella oxytoca	0.5% (n=2)	-	-
	Citrobacter freundii	-	1.9% (n=8)	0.2% (n=1)
	Pseudomonas aeruginosa	3.1% (n=13)	1.9% (n=8)	0.2% (n=1)
	Serratia marcescens	0.7% (n=3)	2.1% (n=9)	0.5% (n=2)
	Morganella morganii	0.2% (n=1)	-	-
	Proteus mirabilis	-	0.2% (n=1)	-
Gram Positive	*Staphylococcus aureus*	0.2% (n=1)	0.9% (n=4)	0.7% (n=3)
	Staphylococcus epidermidis	0.7% (n=3)	0.5% (n=2)	1.7% (n=7)
	Staphylococcus saprophyticus	1.7% (n=7)	1.7% (n=7)	0.2% (n=1)
	Other coagulase-negative *Staphylo-cocci*	0.5% (n=2)	-	0.2% (n=1)
	Group D *streptococci*	5.7% (n=24)	0.5% (n=2)	0.5% (n=2)

Proteus mirabilis were the least frequent organisms with a 0.2% isolation rate.

The clinically isolated Gram-negative bacteria were highly sensitive to colistin with an average sensitivity rate of 97.85%, making it the drug of choice. They nevertheless, were the most resistant to cefixime (91.79%) and trimethoprim/sulfamethoxazole (89.64%). The isolated Gram-positive bacteria were majorly sensitive to vancomycin and nitrofurantoin. Ciprofloxacin and tetracycline were the least effective antibiotics for Gram-positive bacteria with respective resistance rates of 90.91% and 83.33%. The results from antibacterial susceptibility tests in gram-negative and Gram-positive microorganisms are provided in Tables 2 and 3.

DISCUSSION

The emergence and outbreak of SARS-COV-2 infection has been challenging the global public health systems since December 2019. As one of the major concerns associated with the novel coronavirus disease, bacterial and fungal co-infections and antimicrobial resistance (AMR) play a critical role in the prognosis of the disease. No studies have addressed bacterial and fungal co-infections and the relative antimicrobial resistance patterns in COVID-19 patients in Zanjan, Iran. Given the fact that less than 10% of the patients experience co-infections, but over 70% receive antibiotics (10, 11), it is necessary to obtain regional data on superinfections in COVID-19 patients in order to limit the overuse of antibiotics and develop efficient antimicrobial therapy guidelines.

In this cross-sectional study, 8.1% of the patients who were hospitalized in two major Zanjan hospitals over a one-year period, experienced bacterial and/or fungal co-infections. Lansbury et al. (12) conducted a meta-analysis on the burden of co-infections in COVID-19 patients by including 30 studies and 3834 hospitalized patients. They reported an overall 7% bacterial co-infection in the patients which is almost in consistence with our study. Rawson et al. (11) also conducted a study on the coronavirus-associated superinfections. The results were indicative of an 8% (62 of 806 patients) bacterial and fungal co-infection rate in COVID-19 patients which is consistent with our study.

In general, older ages are associated with higher rates of COVID-19 morbidity, mortality and subsequently, co-infection occurrence (13). Our results were indicative of higher risk for severe illness and higher rates of bacterial and fungal co-infections in older patients and negligible risk for patients aging less than 20. According to our data, only 11 patients aging from 0-20 years were hospitalized due to COVID-19 during this period, from whom only one patient deceased. Co-infections were the most prevalent among patients aging from 60 to 70. This data is in accordance with a previously conducted study by Sharifpour et al. (14), which reported an average age of 67 for the COVID-19 patients with bacterial co-in-

Table 2. Antibiotic susceptibility testing results of a total number of 280 patients (N), who were coinfected with Gram-negative bacteria and the associated resistance rates.

		A.	*K.*	*E.*	*P.*	*S.*	*C.*	*K.*	*M.*	*P.*	Total	Resistance
	Number of	***baumannii***	***pneumonia***	***coli***	***aeruginosa***	***marcescens***	***freundii***	***oxytoca***	***morganii***	***mirabilis***	**(N)**	**Rate (%)**
	Isolates (n)	**82**	**81**	**68**	**22**	**14**	**9**	**2**	**1**	**1**	**280**	
Amikacin	Susceptible	0	19	34	0	4	3	0	0	0	60	78.21
	Intermedi-ate	0	0	0	0	1	0	0	0	0	1	
	Resistant	82	62	34	22	9	6	2	1	1	219	
Gentamicin	Susceptible	2	8	39	3	2	2	1	0	0	57	79.64
	Intermedi-ate	0	0	0	0	0	0	0	0	0	0	
	Resistant	80	73	29	19	12	7	1	1	1	223	
Imipenem	Susceptible	2	8	39	2	0	1	0	1	0	53	79.64
	Intermedi-ate	0	1	3	0	0	0	0	0	0	4	
	Resistant	80	72	26	20	14	8	2	0	1	223	
Nitrofurantoin	Susceptible	21	35	58	0	0	2	0	1	0	117	57.14
	Intermedi-ate	0	0	2	0	0	1	0	0	0	3	
	Resistant	61	46	8	22	14	6	2	0	1	160	
Trime-thoprim/	Susceptible	3	5	9	6	3	0	0	1	0	27	89.64
Sulfamethox-	Intermedi-ate	0	0	2	0	0	0	0	0	0	2	
azole	Resistant	79	76	57	16	11	9	2	0	1	251	
Ciprofloxacin	Susceptible	2	9	24	4	0	2	0	1	0	42	83.93
	Intermedi-ate	0	1	2	0	0	0	0	0	0	3	
	Resistant	80	71	42	18	14	7	2	0	1	235	
Cefepime	Susceptible	0	9	18	0	0	0	0	1	0	28	87.5
	Intermedi-ate	0	0	7	0	0	0	0	0	0	7	
	Resistant	82	72	43	22	14	9	2	0	1	245	
Ceftriaxone	Susceptible	3	4	16	0	1	2	0	1	0	27	82.14
	Intermedi-ate	3	1	0	0	0	0	0	0	0	4	
	Resistant	76	57	52	22	13	7	2	0	1	230	
Cefixime	Susceptible	0	10	9	0	2	1	0	1	0	23	91.79
	Intermedi-ate	0	0	0	0	0	0	0	0	0	0	
	Resistant	82	71	59	22	12	8	2	0	1	257	
Cefazolin	Susceptible	0	0	68	0	1	0	0	1	1	71	74.29
	Intermedi-ate	0	0	0	0	1	0	0	0	0	1	
	Resistant	82	81	0	22	12	9	2	0	0	208	
Ceftazidime	Susceptible	0	14	16	0	4	2	0	1	0	37	85.36
	Intermedi-ate	0	0	3	0	1	0	0	0	0	4	
	Resistant	82	67	49	22	9	7	2	0	1	239	
Meropenem	Susceptible	16	20	33	0	5	2	0	0	0	76	71.07
	Intermedi-ate	0	0	2	0	1	1	0	1	0	5	
	Resistant	66	61	33	22	8	6	2	0	1	199	
Nalidixic acid	Susceptible	0	10	7	11	0	3	0	0	0	31	88.93
	Intermedi-ate	0	0	0	0	0	0	0	0	0	0	
	Resistant	82	71	61	11	14	6	2	1	1	249	
Ampicillin	Susceptible	0	0	34	0	0	0	0	0	0	34	87.86
	Intermedi-ate	0	0	0	0	0	0	0	0	0	0	
	Resistant	82	81	34	22	14	9	2	1	1	246	
Colistin	Susceptible	82	75	68	22	14	9	2	1	1	274	0.00
	Intermedi-ate	0	6	0	0	0	0	0	0	0	6	
	Resistant	0	0	0	0	0	0	0	0	0	0	

Table 3. Antibiotic susceptibility testing results of a total number of 66 patients (N), who were coinfected with Gram-positive bacteria and the associated resistance rates.

	Number of Isolates (n)	Group D *Streptococci* 28	*S. saprophyticus* 15	*S. epidermidis* 12	*S. aureus* 8	Coagulase-Negative Staph-ylococci 3	Total (N) 66	Resistance Rate (%)
Amikacin	Susceptible	8	4	2	0	1	15	69.70
	Intermediate	2	1	1	0	1	5	
	Resistant	18	10	9	8	1	46	
Gentamicin	Susceptible	12	2	2	5	2	23	63.64
	Intermediate	0	0	0	1	0	1	
	Resistant	16	13	10	2	1	42	
Imipenem	Susceptible	16	0	0	4	2	22	57.58
	Intermediate	4	2	0	0	0	6	
	Resistant	8	13	12	4	1	38	
Nitrofurantoin	Susceptible	18	7	12	4	3	44	33.33
	Intermediate	0	0	0	0	0	0	
	Resistant	10	8	0	4	0	22	
Trime-tho-prim/Sulfame-thoxazole	Susceptible	7	1	2	5	1	16	71.21
	Intermedi-ate	0	0	3	0	0	3	
	Resistant	21	14	7	3	2	47	
Ciprofloxacin	Susceptible	0	1	0	2	1	4	90.91
	Intermediate	0	0	0	2	0	2	
	Resistant	28	14	12	4	2	60	
Penicillin	Susceptible	3	8	0	1	0	12	81.82
	Intermediate	0	0	0	0	0	0	
	Resistant	25	7	12	7	3	54	
Tetracycline	Susceptible	3	0	2	0	2	7	83.33
	Intermediate	2	0	2	0	0	4	
	Resistant	23	15	8	8	1	55	
Vancomycin	Susceptible	11	15	12	5	2	45	30.30
	Intermediate	1	0	0	0	0	1	
	Resistant	16	0	0	3	1	20	
Cefazolin	Susceptible	0	0	12	4	3	19	54.55
	Intermediate	9	0	0	2	0	11	
	Resistant	19	15	0	2	0	36	
Clindamycin	Susceptible	14	13	12	2	1	42	33.33
	Intermediate	0	2	0	0	0	2	
	Resistant	14	0	0	6	2	22	
Meropenem	Susceptible	0	4	2	3	0	9	74.24
	Intermediate	1	2	3	2	0	8	
	Resistant	27	9	7	3	3	49	
Ampicillin	Susceptible	3	9	0	8	0	20	68.18
	Intermediate	1	0	0	0	0	1	
	Resistant	24	6	12	0	3	45	

fections of the respiratory tract. Superinfections have been reported to be dominant in males in similar studies (14-16). However, females consisted a greater portion of the population in this study. This is probably due to the inclusion of urinary tract infections in this study, which are more common in women, and the focus of the most of the other conducted studies on respiratory tract and blood infections. Severe

COVID-19 is associated with high rates of admission to the ICU (17). ICU admission rate in the patients included in our study was 55.8%. Chronic respiratory diseases, immuno-inflammatory response, corticosteroid therapy, and mechanical ventilation are the main risk factors for bacterial and fungal co-infections in ICU-admitted patients. In this study, 70% of the patients had at least one underlying disease with hypertension and diabetes being the most frequent ones. This is aligned with the results of other similar studies (16, 17). Co-infections were not significantly associated with respiratory comorbidities compared to other types of comorbidities (P=0.361).

Bacterial and fungal co-infections were associated with longer lengths of stay, which has been observed in previous studies (16, 18, 19). This is mainly because hospital-acquired infections are more likely to occur in patients with a longer hospitalization period. Coinfected patients had poorer prognosis with a mortality rate of 58.4%, while mortality rate in non-coinfected patients was 12.2%. After conducting a meta-analysis including four studies providing data on 852 patients, Martins-Filho et al. (20) reported a 2.5-fold increase of the COVID-19-associated lethality risk in patients coinfected with bacteria and fungi, which is a smaller ratio compared to the 4.7-fold increase observed in our study.

In this study, blood, urine, and respiratory tract cultures were respectively ordered for 472 (9.2%), 2132 (41.6%) and 242 (4.6%) patients. A global crisis is whether initial challenges regarding the management of COVID-19 have potentially reduced the requests for microbiologic tests from the physicians. We are concerned about the quality and quantity of performed microbiologic tests and their authentic documentation.

Ever since the first COVID-19 case appeared, some regional studies have highlighted the burden of bacterial and fungal co-infections in the pandemic considering the responsible microorganisms and AMR patterns. In Qom, Iran, Sharifpour et al. reported a 100% respiratory bacterial co-infection rate in 19 ICU-admitted COVID-19 patients. *A. baumannii* was the most prevalent organism isolated from 90% of the patients and 10% of the cases were confirmed with *S. aureus* co-infection (14). Antimicrobial susceptibility testing in this study revealed that *A. baumannii* isolates were highly resistant of to all tested antibiotics, except for colistin with a 52% resistance rate. Methicillin resistance was reported in 25% of the *S. aureus* samples. Similarly, *A. baumannii* was the most prevalent isolated bacteria from the respiratory samples of patients in this study and colistin was reported to be the most effective antibiotic as well with a sensitivity rate of 97.85%. Methicillin resistance in our study nevertheless, was estimated at 12%. Neto et al. conducted a retrospective analysis on bacterial genitourinary, skin and respiratory infections and antibiotic use patterns in 242 COVID-19 patients. The rate of concomitant bacterial infection was 19%, having *E. coli* as the most frequently isolated organism (26%). *E. coli* was also the most prevalent organism in the urine samples of our patient population (64%). Ceftriaxone (54%), vancomycin (48%), azithromycin (47%), and cefepime (45%) were among the most prescribed antibiotics in the mentioned study (15). Among the antibiotics in this list however, only vancomycin had a promising performance in our study with a sensitivity rate of 68%. A study by Garcia-Vidal et al. assessed the incidence of respiratory, blood and urine co-infections in 989 hospitalized COVID-19 patients in Barcelona, Spain. A total number of 88 non-COVID-19 infections were confirmed including 74 bacterial, seven fungal and seven viral co-infections. The most common isolated bacteria were *S. pneumoniae* (12 cases) and *S. aureus* (12 cases) (21). In 2020, Kamaliaghdam et al. reported that *E. coli* strains isolated from the urine samples of hospitalized patients in Zanjan are highly sensitive to nitrofurantoin with a 4.5% resistance rate. *E. coli* showed the highest resistance to cefixime (34.9%). This is in accordance with our findings, which were indicative of an 85.29% nitrofurantoin sensitivity rate and an 86.76% cefixime resistance rate in *E. coli* strains isolated from the urine samples (22). Keihanian et al. evaluated the epidemiology of antibiotic resistance of blood cultures in educational hospitals in Rasht, Iran, in 2018. *P. aeruginosa* 29.3% was the most prevalent organism in patients with septicemia. Ceftriaxone and ciprofloxacin were respectively the most resistant (44%) and the most sensitive (54%) antibiotics in this study (23). This result is different from that of our study. Differences in the types of isolated bacteria and AMR patterns are probably due to different geographical distribution of bacteria and antibiotic regimens in different regions. Exclusively applying biochemical tests for bacterial identification and not determining resistance-associated genes are the limitations of this study.

CONCLUSION

It is essential to conduct further studies on the prevalence of bacterial co-infections and possible interactions between COVID-19 and AMR. Realizing how AMR trends are affected by COVID-19 and tracking the alterations in these trends are of great help in tackling AMR and developing rapid and efficient diagnostic, therapeutic and disinfection strategies in order to control these infections in the hospitals. Medical settings need to take more measures to identify the COVID-19 patients with bacterial and fungal co-infections who might benefit from antibiotics.

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REFERENCES

1. Freeman AM, Leigh, Jr TR. Viral Pneumonia. In: *StatPearls*. StatPearls Publishing. Treasure Island (FL); 2020. PMID: 30020658.
2. Wagner K, Springer B, Imkamp F, Opota O, Greub G, Keller PM. Detection of respiratory bacterial pathogens causing atypical pneumonia by multiplex Lightmix ® RT-PCR. *Int J Med Microbiol* 2018;308:317-323.
3. Coronavirus disease (COVID-19) How is it transmitted 2020 [2020-10-02]. Available from: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/question-and-answers-hub/q-a-detail/q-a-how-is-covid-19-transmitted?
4. Timeline of ECDC's reponse to COVID-19 2020 [2020-10-02]. Available from: https://www.ecdc.europa.eu/en/covid-19/timeline-ecdc-response
5. worldometer. coronavirus cases 2020 [2020-10-02]. Available from: https://www.worldometers.info/coronavirus/
6. Ruan Q, Yang K, Wang W, Jiang L, Song J. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. *Intensive Care Med* 2020;46:846-848.
7. Langford BJ, So M, Raybardhan S, Leung V, Westwood D, MacFadden DR, et al. Bacterial co-infection and secondary infection in patients with COVID-19: a living rapid review and meta-analysis. *Clin Microbiol Infect* 2020;26:1622-1629.
8. Whitman WB (2012). The Actinobacteria. In: *Bergey's Manual of Systematic Bacteriology*. Eds, WB Whitman, M Goodfellow, P Kämpfer, HJ Busse, ME Trujillo, W Ludwig, KI Suzuki. Springer, 2nd ed. New York, USA, pp.1-34.
9. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 26th ed. CLSI supplement M100-S26. Wayne, PA: Clinical and Laboratory Standards Institute; 2016.
10. Clancy CJ, Nguyen MH. Coronavirus disease 2019, superinfections, and antimicrobial development: what can we expect? *Clin Infect Dis* 2020;71:2736-2743.
11. Rawson TM, Moore LSP, Zhu N, Ranganathan N, Skolimowska K, Gilchrist M, et al. Bacterial and fungal coinfection in individuals with coronavirus: a rapid review to support COVID-19 antimicrobial prescribing. *Clin Infect Dis* 2020;71:2459-2468.
12. Lansbury L, Lim B, Baskaran V, Lim WS. Co-infections in people with COVID-19: a systematic review and meta-analysis. *J Infect* 2020;81:266-275.
13. Kang SJ, Jung SI. Age-Related morbidity and mortality among patients with COVID-19. *Infect Chemother* 2020;52:154-164.
14. Sharifipour E, Shams S, Esmkhani M, Khodadadi J, Fotouhi-Ardakani R, Koohpaei A, et al. Evaluation of bacterial co-infections of the respiratory tract in COVID-19 patients admitted to ICU. *BMC Infect Dis* 2020;20:646.
15. Goncalves Mendes Neto A, Lo KB, Wattoo A, Salacup G, Pelayo J, DeJoy R 3rd, et al. Bacterial infections and patterns of antibiotic use in patients with COVID-19. *J Med Virol* 2021;93:1489-1495.
16. Bardi T, Pintado V, Gomez-Rojo M, Escudero-Sanchez R, Azzam Lopez A, Diez-Remesal Y, et al. Nosocomial infections associated to COVID-19 in the intensive care unit: clinical characteristics and outcome. *Eur J Clin Microbiol Infect Dis* 2021;40:495-502.
17. Elabbadi A, Turpin M, Gerotziafas GT, Teulier M, Voiriot G, Fartoukh M. Bacterial coinfection in critically ill COVID-19 patients with severe pneumonia. *Infection* 2021;49:559-562.
18. Asmarawati TP, Rosyid AN, Suryantoro SD, Mahdi BA, Windradi C, Wulaningrum PA, et al. The clinical impact of bacterial co-infection among moderate, severe and critically ill COVID-19 patients in the second referral hospital in Surabaya. *F1000Res* 2021;10:113.
19. Karaba SM, Jones G, Helsel T, Smith LL, Avery R, Dzintars K, et al. Prevalence of co-infection at the time of hospital admission in COVID-19 patients, a multicenter study. *Open Forum Infect Dis* 2020;8:ofaa578.
20. Martins-Filho PR, Tavares CSS, Santos VS. Factors associated with mortality in patients with COVID-19. A quantitative evidence synthesis of clinical and laboratory data. *Eur J Intern Med* 2020;76:97-99.
21. Garcia-Vidal C, Sanjuan G, Moreno-García E, Puer-

ta-Alcalde P, Garcia-Pouton N, Chumbita M, et al. Incidence of co-infections and superinfections in hospitalized patients with COVID-19: a retrospective cohort study. *Clin Microbiol Infect* 2021;27:83-88.

22. Kamaliaghdam M, Sadeghzadeh M, Jalilvand A, Eftekhari K, Rezaei Z. Antibiotic sensitivity patterns of *Escherichia coli* isolated in urine samples of patients referred to Ayatollah Mousavi hospital in Zanjan. *J Adv Med Biomed Res* 2020;28:124-131.
23. Keihanian F, Saeidinia A, Abbasi K, Keihanian F. Epidemiology of antibiotic resistance of blood culture in educational hospitals in Rasht, north of Iran. *Infect Drug Resist* 2018;11:1723-1728.