



Study of bacteria isolated from COVID-19 and non-COVID-19 intensive care units and determination of their antibiotic susceptibility profiles

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Abstract

Nosocomial infections occur 48-72 hours after hospitalization, especially caused by bacteria, and pose a high risk for patients in intensive care units (ICUs), including COVID-ICUs. This study aimed to reveal bacteria distribution and antibiotic susceptibility profiles isolated from various clinical samples of non-COVID-ICU and COVID-ICU patients. We included in this study bacterial strains isolated from ICUs patients in Kastamonu Training and Research Hospital between March 2020 and October 2020. We identified the strains using the Vitek 2 compact automated system (BioMerieux, France) and standard microbiological methods. Using the Vitek 2 automated system, we analyzed antibiotic susceptibility tests and interpreted the results based on the European Committee for Antimicrobial Susceptibility Tests (EUCAST) guideline. There were 302 patients in the non-COVID-ICUs and 440 patients in the COVID-ICUs. We isolated a total of 470 strains, 370 from non-COVID-ICUs and 100 from COVID-ICUs. *Acinetobacter* spp. was the most frequently isolated strains for both ICUs. *Acinetobacter* spp. isolated from non-COVID-ICUs had higher resistance rates to meropenem ($p=0.043$), ceftazidime ($p=0.014$), and levofloxacin ($p<0.001$) antibiotics than strains from COVID-ICUs. Antibiotic susceptibility profiles of other strains were similar for both ICUs. As a result, the incidence of nosocomial infections in COVID-ICU patients was lower than in non-COVID-ICU patients. Health personnel working in COVID-ICUs may have played an important role in this, as they were more careful about using personal protective equipment and complying with hygiene rules. However, antibiotic resistance continues to be a serious problem in ICUs, including COVID-ICUs.

Keywords: *Acinetobacter*, antibiotic resistance, COVID-intensive care unit, nosocomial infections

1. Introduction

COVID-19 was described in December 2019 and, has become a pandemic in March 2020. The disease is transmitted by aerosols and shows a course ranging from asymptomatic to severe respiratory failure (1). About 20% of the patients are treated in hospitals due to severe lung involvement, and 5-10% of them are hospitalized in intensive care units (ICUs) and need respiratory support. The COVID-19 pandemic has become a severe burden on health systems, especially ICUs (2).

The widespread use of ventilators, catheters, and antibiotics and the prolonged hospital stay period predispose the development of nosocomial infections in ICUs patients. Nosocomial infections are infectious diseases that usually occur 48 hours after hospitalization and are frequently bacterial

in origin. Nosocomial infections are troublesome for all ICUs patients, including the COVID-ICUs, as they adversely affect the prognosis and increase the mortality rate (3). On the other hand, viral infections may predispose the host to secondary bacterial infections due to their effects on the immune system (4). In addition, high-dose steroid therapy can be used to alleviate the symptoms of COVID-19 patients with severe symptoms. In this case, COVID-ICU patients may become prone to nosocomial infections or secondary bacterial infections of flora (endogenous) origin (5).

Periodic monitoring of bacteria distribution and antibiotic susceptibility profiles isolated from COVID-ICU and other ICUs patients is essential for infection control. This study

aimed to reveal bacteria distribution and antibiotic susceptibility profiles isolated from various clinical samples in non-COVID-19 intensive care units (non-COVID-ICUs) and COVID-19 intensive care units (COVID-ICUs) patients.

2. Materials and Methods

This study was approved by the Kastamonu University (Turkey), Faculty of Medicine Medical Research Ethical Committee (Date: 14.12.2020 and Decision number: 2020-KAEK-143-04).

We conducted this study in the Microbiology Laboratory of Kastamonu Training and Research Hospital (TRH) and the COVID-19 diagnosis using RT-PCR (Bio-Speedy COVID-19 RT-qPCR Detection kit; Bioeksan, Istanbul, Turkey) and CORONEX (MOTAKK, Ankara, Turkey) from respiratory tract samples. According to the manufacturer's instructions, we performed qPCR using the C1000 Touch CFX96 system (Bio-Rad, USA).

We included in this study bacterial strains isolated from various clinical samples (respiratory secretions, blood, urine, wound, pleural and peritoneal fluids) of COVID-ICU and non-

COVID-ICU patients in Kastamonu TRH between March and October 2020 and the first isolates of the patients. We identified the strains using the Vitek 2 compact automated system (BioMerieux, France) besides standard microbiological methods (culture examination, Gram reaction, catalase, and oxidase tests). We analyzed antibiotic susceptibility tests using the Vitek 2 automated system and interpreted the results based on the European Committee for Antimicrobial Susceptibility Tests (EUCAST) guideline (6).

2.1. Statistical analysis

We used the chi-square test for the statistical analysis of the data on the SPSS 23.0 for Windows (IBM Inc., Armonk, NY, USA) and took the significance of the p-value as <0.05.

3. Results

There were 302 patients in the non-COVID-ICUs and 440 patients in the COVID-ICUs. We isolated a total of 470 strains, 370 from non-COVID-ICUs and 100 from COVID-ICUs, as infectious agents. Table 1 shows the distribution of bacterial strains between ICUs. *Acinetobacter* spp. was the most frequently isolated strains (33.6%) for both ICUs.

Table 1. Comparison of the distribution of inpatients and isolated bacteria in non-COVID-ICU and COVID-ICU

Bacteria	non-COVID-ICU	COVID-ICU	Total (n, %)
Gram-negative bacteria	<i>Acinetobacter</i> spp. (n=123, 26.2%)	<i>Acinetobacter</i> spp. (n=35, 7.4%)	158 (33.6%)
	<i>Klebsiella</i> spp. (n=89, 18.9%)	<i>Klebsiella</i> spp. (n=22, 4.7%)	111 (23.6%)
	<i>E. coli</i> (n=57, 12.1%)	<i>E. coli</i> (n=18, 3.8%)	75 (15.9%)
	<i>Pseudomonas</i> spp. (n=52, 11.1%)	<i>Pseudomonas</i> spp. (n=5, 1.1%)	57 (12.2%)
	Others (n=5, 1.1%)	Others (n=3, 0.6%)	8 (1.7%)
Total (n)	326 (69.4%)	83 (17.6%)	409 (87.0%)
Gram-positive bacteria	<i>S. aureus</i> (n=29, 6.2%)	<i>S. aureus</i> (n=13, 2.8%)	42 (9.0%)
	<i>Enterococcus</i> spp. (n=11, 2.3%)	<i>Enterococcus</i> spp. (n=2, 0.4%)	13 (2.7%)
	Others (n=4, 0.9%)	Others (n=2, 0.4%)	6 (1.3%)
Total (n)	44 (9.4%)	17 (3.6%)	61 (13.0%)
Overall (n)	370 (78.8%)	100 (21.2%)	470 (100%)

Among the clinical samples of ICU patients, we isolated most bacteria from respiratory secretions (n= 251), followed by blood (n= 112), urine (n= 89), wound (n= 13), pleural fluid (n= 4), and peritoneal fluid (n= 1). Of respiratory secretion samples, 214 were sent from non-COVID-ICUs and 37 from COVID-ICUs. The most common strains isolated from respiratory secretions for both ICUs were *Acinetobacter* spp. The most frequently isolated bacteria from blood and urine cultures for both ICUs were *Staphylococcus aureus* and *Escherichia coli*, respectively (Table 2).

Table 3 and Table 4 show the resistance rates of Gram-

negative and Gram-positive bacteria to commonly used antibiotics, respectively. We did not present colistin susceptibility results in this study due to EUCAST criteria. *Acinetobacter* spp. isolated from non-COVID-ICUs had higher resistance rates to meropenem (p= 0.043), ceftazidime (p= 0.014), and levofloxacin (p<0.001) than isolates from COVID-ICUs. Moreover, *Pseudomonas* spp. isolated from non-COVID-ICUs had a higher resistance rate to levofloxacin (p= 0.047) than isolates from COVID-ICUs. Antibiotic susceptibility profiles of other bacteria were similar for both ICUs.

Table 2. Bacterial distribution among clinical specimens from non-COVID-ICU and COVID-ICU

Clinical specimen	Bacteria	non-COVID-ICU (n, %)	COVID-ICU (n, %)	Total (n, %)
Respiratory secretions	<i>Acinetobacter</i> spp.	98 (20.8%)	23 (4.9%)	121 (25.7%)
	<i>Klebsiella</i> spp.	55 (11.7%)	11 (2.3%)	66 (14.0%)
	<i>Pseudomonas</i> spp.	45 (9.6%)	0 (0.0%)	45 (9.6%)
	<i>E. coli</i>	7 (1.5%)	1 (0.2%)	8 (1.7%)
	Other	9 (1.9%)	2 (0.4%)	11 (2.3%)
	Total (n, %)	214 (45.5%)	37 (7.8%)	251 (53.3%)
Blood	<i>S. aureus</i>	21 (4.5%)	11 (2.3%)	32 (6.8%)
	<i>Acinetobacter</i> spp.	21 (4.5%)	10 (2.1%)	31 (6.6%)
	<i>Klebsiella</i> spp.	13 (2.8%)	3 (0.6%)	16 (3.4%)
	<i>E. coli</i>	12 (2.6%)	4 (0.9%)	16 (3.4%)
	<i>Enterococcus</i> spp.	9 (1.9%)	1 (0.2%)	10 (2.1%)
	<i>Pseudomonas</i> spp.	2 (0.4%)	1 (0.2%)	3 (0.6%)
	Other	1 (0.2%)	3 (0.6%)	4 (0.9%)
Total (n, %)	79 (16.9%)	33 (6.9%)	112 (23.8%)	
Urine	<i>E. coli</i>	35 (7.4%)	11 (2.3%)	46 (9.8%)
	<i>Klebsiella</i> spp.	16 (3.4%)	7 (1.5%)	23 (4.9%)
	<i>Pseudomonas</i> spp.	3 (0.6%)	4 (0.9%)	7 (1.5%)
	<i>Enterococcus</i> spp.	2 (0.4%)	2 (0.4%)	4 (0.9%)
	Other	7 (1.5%)	2 (0.4%)	9 (1.9%)
	Total (n, %)	63 (13.3%)	26 (5.5%)	89 (18.8%)
Wound	<i>Klebsiella</i> spp.	4 (0.9%)	1 (0.2%)	5 (1.0%)
	<i>E. coli</i>	3 (0.6%)	2 (0.4%)	5 (1.0%)
	<i>Acinetobacter</i> spp.	1 (0.2%)	1 (0.2%)	2 (0.4%)
	<i>Pseudomonas</i> spp.	1 (0.2%)	0 (0.0%)	1 (0.2%)
	Total (n, %)	9 (1.9%)	4 (0.8%)	13 (2.7%)
Pleural fluid	<i>S. aureus</i>	3 (0.6%)	0 (0.0%)	3 (0.6%)
	<i>Stenotrophomonas maltophilia</i>	1 (0.2%)	0 (0.0%)	1 (0.2%)
	Total (n, %)	4 (0.8%)	0 (0.0%)	4 (0.8%)
Peritoneal fluid	<i>Pseudomonas</i> spp.	1 (0.2%)	0 (0.0%)	1 (0.2%)
	Overall (n, %)	370 (78.4%)	100 (21.2%)	470 (100%)

Table 3. The resistance rates of Gram-negative bacteria to commonly used antibiotics

Bacteria	ICUs	Amoxicillin clavulanic acid	Piperacillin tazobactam	Meropenem	Amikacin	Ceftazidime	Cefepime	Levofloxacin
<i>E. coli</i>	non-COVID-ICU (n=57)	19 (33.3%)	6 (10.5%)	3 (5.3%)	2 (3.5%)	14 (24.6%)	13 (22.8%)	-
	COVID-ICU (n=18)	7 (38.9%)	4 (22.2%)	0 (0.0%)	0 (0%)	4 (22.2%)	4 (22.2%)	-
<i>Klebsiella</i> spp.	non-COVID-ICU (n=89)	60 (68.2%)	66 (75.0%)	60 (67.4%)	47 (53.4%)	69 (78.4%)	66 (75.0%)	-
	COVID-ICU (n=22)	14 (63.6%)	14 (63.6%)	10 (45.4%)	9 (40.9%)	13 (59.1%)	13 (59.1%)	-
<i>Acinetobacter</i> spp.	non-COVID-ICU (n=123)	-	122 (99.2%)	120 (97.6%)¹	97 (78.9%)	120 (97.6%)²	-	122 (99.2%)³
	COVID-ICU (n=35)	-	33 (94.3%)	31 (88.6%)	26 (74.3%)	30 (85.7%)	-	27 (77.1%)
<i>Pseudomonas</i> spp.	non-COVID-ICU (n=52)	-	33 (63.5%)	36 (69.2%)	4 (7.7%)	24 (46.2%)	24 (46.2%)	36 (69.2%)⁴
	COVID-ICU (n=5)	-	2 (40.0%)	2 (40.0%)	1 (20.0%)	2 (40.0%)	2 (40.0%)	1 (20.0%)

¹p= 0.043, ²p= 0.014, ³p<0.001, ⁴p= 0.047

Table 4. The resistance rates of Gram-positive bacteria to commonly used antibiotics

Bacteria	ICUs	Methicillin	Vancomycin	Ampicillin	Ciprofloxacin	Clindamycin	Erythromycin	Tetracycline	Tigecycline
<i>S. aureus</i>	non-COVID-ICU (n=29)	14 (48.3%)	0 (0.0%)	-	10 (34.5%)	10 (34.5%)	12 (41.4%)	9 (31.0%)	2 (6.9%)
	COVID-ICU (n=13)	8 (61.5%)	0 (0.0%)	-	4 (30.8%)	6 (46.2%)	7 (53.8%)	4 (30.8%)	1 (7.7%)
<i>Enterococcus</i> spp.	non-COVID-ICU (n=11)	-	2 (18.2%)	9 (81.8%)	9 (81.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (18.2%)
	COVID-ICU (n=2)	-	0 (0.0%)	2 (100.0%)	2 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

4. Discussion

This study determined bacteria distribution and antibiotic susceptibility profiles isolated from COVID-ICUs and other ICUs patients. The results showed that non-COVID-ICU patients (78.8%) had a higher incidence of nosocomial infections than COVID-ICUs (21.2%). This striking difference in the frequency of nosocomial infections might stem from healthcare personnel working in COVID-ICUs being more careful about using personal protective equipment and complying with hygiene rules in pandemic conditions.

Considering the distribution of bacteria, we found that gram-negative bacteria were dominant in both ICUs in this study. Many researchers stated that gram-negative bacteria were dominant among bacteria isolated from ICUs patients (7-9). This can be attributed to gram-negative bacteria being more resistant than gram-positive bacteria by their structure. So, resistant gram-negative strains become dominant in the hospital environment due to the selective pressure of antibiotics (10).

The distribution of the clinical samples from which the strains were isolated evinced that involvement in the respiratory system was the most common, followed by blood, urine, and wounds in both ICUs. However, although there was risk factor such as the use of ventilators for the development of nosocomial infections in the COVID-ICUs (11), the frequency of bacteria isolated from respiratory secretions was 32.7% in COVID-ICUs and 56.7% in non-COVID-ICUs. This may indicate that the antibiotics recommended in the COVID-19 treatment protocol play an active role in protecting against respiratory system infections.

In the presented study, *Acinetobacter* spp. were the most frequently isolated bacteria from both ICUs. *Acinetobacter* spp. can survive for a long time in the hospital environment and on dry surfaces with their simple nutritional requirements, ability to grow in a broad pH and temperature range, resistance to disinfectants and antiseptics, and the ability to form biofilms on living and non-living surfaces (12-14). Therefore, they are frequently isolated from inpatients in hospitals (15). In addition, many studies reported that *Acinetobacter* spp. was the most

frequently isolated bacteria from COVID-ICU and other ICUs patients (16-19).

The antibiogram results of the strains revealed that *Acinetobacter* spp. had the highest resistance rate for both ICUs. We actually expected this result, as these bacteria, especially *Acinetobacter baumannii* strains, have intrinsic resistance to many antibiotics (20). In addition, they can easily acquire resistance to antibiotics with acquired resistance mechanisms. In particular, carbapenem-resistant *Acinetobacter* strains emerge as urgent threats (21). We found the resistance rates of *Acinetobacter* strains isolated from COVID-ICU and other ICUs patients against meropenem, a carbapenem class antibiotic, as 88.6% and 97.6%, respectively. Meropenem resistance was statistically significant in *Acinetobacter* strains isolated from other ICUs compared to those isolated from COVID-ICUs ($p= 0.043$). However, this may be due to the number of *Acinetobacter* isolated from COVID-ICUs being lower than those isolated from other ICUs.

As a result, the COVID-ICU patients had a lower incidence of bacterial infection than other ICU patients in Kastamonu TRH. While there are many risk factors for COVID-ICU patients to get bacterial infections, the lower incidence of infection than in other ICUs shows that successful infection control is implemented in COVID-ICUs of Kastamonu TRH. However, antibiotic resistance continues to be a serious problem in ICUs, including COVID-ICUs.

Conflict of interest

The authors declare that they have no conflict of interest.

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None to declare.

Authors' contributions

Concept: Ç.K., N.C., Design: N.C., M.Y.D., B.Ç., Data Collection or Processing: M.G., A.Y., V.G.S., Analysis or Interpretation: Ç.K., E.F.T., B.Ç., Literature Search: E.F.T.,

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