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Evaluation of Microorganisms Isolated from Endotracheal Aspirate Cultures in Patients with and without COVID-19 in the Intensive Care Unit: Single-Centre Retrospective Analysis

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ABSTRACT

Objective: Detection of common microorganisms from endotracheal aspirate cultures is helpful for the appropriate treatment of ventilator-associated pneumonia (VAP). We aimed to compare the growths detected in cultures of endotracheal aspiration material in patients with and without a diagnosis of COVID-19.

Methods: Patients admitted to the intensive care unit between 1 November 2020 and 1 April 2022 who required intubation for more than 48 hours were retrospectively screened. Patients with a diagnosis of VAP were included in the study. Demographic characteristics, comorbidities, clinical findings and distribution of growing microorganisms were collected and compared for patients with and without the diagnosis of COVID-19.

Results: There were 135/370 (36%) patients with microbiologically confirmed VAP in the COVID-19 group and 54/168 (32%) in the non-COVID-19 group (p=0.55). Microbiologically confirmed VAP incidence was 30.6 events per 1000 days on a mechanical ventilator (32 events/1000 days in COVID-19 patients, and 26.5 events/1000 days in non-COVID-19 patients). The most common pathogens were Acinetobacter baumannii, Klebsiella pneumoniae and Pseudomonas aeruginosa. Acinetobacter baumannii culture positivity was higher in COVID-19 patients than non-COVID-19 patients (p=0.01).

Conclusion: *A. baumannii* culture positivity was higher in COVID-19 patients than non-COVID-19 patients. Although the events of VAP per 1000 days on mechanical ventilation was higher in COVID-19 patients, no significant difference was observed in the number of patients who developed VAP among COVID-19 patients compared to non-COVID-19 patients.

Keywords: COVID-19, endotracheal culture, invasive mechanical ventilation, pneumonia

Introduction

Patients in the intensive care unit (ICU) have a higher risk of developing infection due to critical illness and invasive procedures (1,2) Bacterial co-infection or superinfection is common in viral respiratory tract infections (3). The widespread use of antibiotics in the early stages of the coronavirus disease 2019 (COVID-19) pandemic, when the frequency of bacterial co-infection in COVID-19 pneumonia was unknown, led to the inappropriate use of antibiotics and concerns about the development of resistance (4,5). Knowledge of bacterial co-infection rates and agent distribution during COVID-19 viral pneumonia is essential for empirical treatment. COVID-19 pneumonia is associated with a higher rate of intubation (6, 7). Therefore, these patients are at risk of developing ventilator-associated pneumonia (VAP) (6), which is a cause of secondary bacterial infections. Bacteraemia and need for vasopressor support were found to be more prevalent in COVID-19 patients who were mechanically ventilated than those who were not ventilated (11% vs. 1.8% and 95.4% vs. 1.5%, respectively) (7).

It is necessary to determine the microbiological agents and antibiotic resistance profile to guide the choice of treatment and reduce the rate of antibiotic resistance (1,8,9). This study aimed to determine whether there is a difference in the distribution of microorganisms grown in the endotracheal aspirate (ETA) cultures of patients with and without COVID-19.

Materials and Methods

This study was approved by the Tepecik Training and Research Hospital Non-Interventional Clinical Research Ethics Committee

(Date.15.04.2022; no: 2022/04-08) and the Ministry of Health of the Turkish Republic (Date.2022-04-06; no: T14-31-40). The ETA cultures of the patients in the ICU between 1 November 2020 and 1 April 2022 were retrospectively analyzed. Patients with ETA cultures containing a detected microorganism that was evaluated as a cause of lower respiratory tract infection by an infection specialist and an intensive care specialist were included in the study. Microbiologically confirmed VAP cases were included.

Only the first episode of VAP occurring at least 48 hours after initiation of invasive mechanical ventilation was analysed. In accordance with the European Center for Disease Control (10) definitions, VAP was defined as the combination of radiological, clinical and microbiological criteria developed in a patient who was followed on a mechanical ventilator for at least 48 hours. The radiological criterion was new or worsening infiltrates; clinical criteria were fever, leukopenia or leukocytosis, increased secretion and purulence and oxygen demand; and the microbiological criterion was microbial growth above the threshold value of 10^5 in the quantitative culture of the ETA sample taken from the respiratory tract. Polymicrobial growth was defined as the growth of more than two different microorganisms in the same model. Polymicrobial growths were excluded from the evaluation. Patients who were followed for less than 48 hours in the ICU and those who lived less than 48 hours after intubation were excluded. The quality of the ETA sample, predominant microorganisms and leukocytes were investigated. Samples with less than ten epithelium and more than 25 leukocytes in the microscopic field were evaluated. Growing bacteria were quantitatively assessed, and those without bacterial growth over 10^5 cfu/mL were excluded from the study. Organisms with poor lung pathogenicity, *Enterococcus* spp., *Candida albicans*, non-pneumococcal *Streptococci*, and coagulase-negative *Staphylococci* were not considered to be the cause of VAP (11). Microbiologically confirmed VAP incidence was defined as pneumonia developing in 1000 ventilator days and was calculated as (VAP incidence/number of ventilator days) × 1000.

The factors that were examined included age, gender, acute physiology and chronic health assessment (APACHE II) (12), comorbidities, leading causes of the need for ICU (acute respiratory failure, sepsis, post-cardiac arrest, neurological causes, acute renal failure, gastrointestinal pathologies, postoperative, and trauma), length of ICU stay, 30-day mortality and microorganisms isolated in ETA cultures. Steroid and antibiotic drugs administered before ETA sampling and tocilizumab and anakinra treatments, if administered, were recorded in COVID-19 patients. Immunodeficiency was defined when one of the following was detected: human immunodeficiency virus (HIV) infection, haematological cancer, and use of steroids (>1 mg/kg/day) or other immunosuppressant agents for at least one month. A white blood cell count, lymphocyte count, neutrophil count, C-reactive protein (CRP), and procalcitonin (PCT) results were evaluated on the day ETA samples were taken. We compared the data of the patients with and without COVID-19. The primary endpoint was the distribution of the microorganisms in ETA cultures in VAP patients with and without COVID-19. The secondary endpoint was the incidence of VAP per 1000 days on a mechanical ventilator.

Table 1. Demographic, laboratory and clinical data of patients with and without COVID-19.

	COVID-19 (n=135)	Non-COVID-19 (n=54)	p value
Age, year	66.6 ±15	65.9 ±16	0.79
Gender, Female (%)	80(59)	27(50)	0.26
APACHE II	16.8 ±3.9	17.6 ±4.2	0.61
Comorbidities,n(%)			
Diabetes mellitus	41(30)	16(29)	0.92
Hypertension	56(41)	17(31)	0.24
Heart Disease	34(25)	12(22)	0.71
Lung Disease	19(14)	9(16)	0.65
Renal Disease	16(12)	3(5)	0.19
Neurological	23(17)	7(13)	0.66
Malignancy	17(11)	8(15)	0.64
Immunosuppression	14(10)	2(4)	<0.01
Antibiotic use before ETA, n(%)	93 (68)	38 (70)	0.70
Carbapenem	15(11.1)	21(55)	0.04
Quinolone	36(26.6)	0(0)	0.02
Piperacilin tazobactam	36(26.6)	15(39)	0.89
Cephalosporin	6(4.4)	2(5,2)	0.62
Laboratory parameters			
C-reactive protein, mg/L	174±93	189± 87	0.11
Procalsitonin, ng/ml	0.9(0.2-3.9)	1.8(0.3-6.3)	0.80
White Blood Cell, (/µL) $\times10^9$	14.2±6.6	14.3±6.7	0.97
Lymphocyte, (/µL) $\times10^9$	0.6(0.3-1.0)	0.9(0.4-1.2)	0.03
Neutrophil, (/µL) $\times10^9$	11.5(8.4-16)	12(7.3-15)	0.81
Duration from intubation to ETA, day	5(3-10)	6(3-10)	0.77
Duration from ICUadmission to intubation, day	4(2-9)	3(1-9)	0.48
Duration from ICU admission to ETA, day	8 (5-12)	8(3-17)	0.64
Length of ICU stay, day	12(8-17)	9(5-17)	0.03
Length of hospital stay, day	18(12-34)	15(8-37)	0.10
30th-day mortality, n(%)	97(71.9)	37(68.5)	0.72

Data are shown as n (%), mean ± standard deviation, and median (interquartile range) APACHE II: Acute physiologic and Chronic Health Score II; ETA: endotracheal aspirate.

Statistical analysis

In continuous variables, mean and standard deviation were used for normally distributed data, and median and interquartile range values were used for non-normally distributed data. The normal distribution of the data was evaluated with the Shapiro–Wilk test. Categorical variables were reported as numbers and percentages. The Student's t-test was used if continuous variables met the parametric test assumptions, and the Mann–Whitney U test was used if they did not. For categorical variables, chi-square or Fisher's exact test was used according to the characteristics of the data for comparison between groups. Statistical analysis of the data was performed with the Statistical Package for Social Science for Windows (SPSS) v.22. A p-value of <0.05 was considered statistically significant.

Results

Between 10 January 2020 and 1 April 2022, the data of 822 patients being treated in the ICU with and without COVID-19 were screened (Figure 1). The number of patients who were

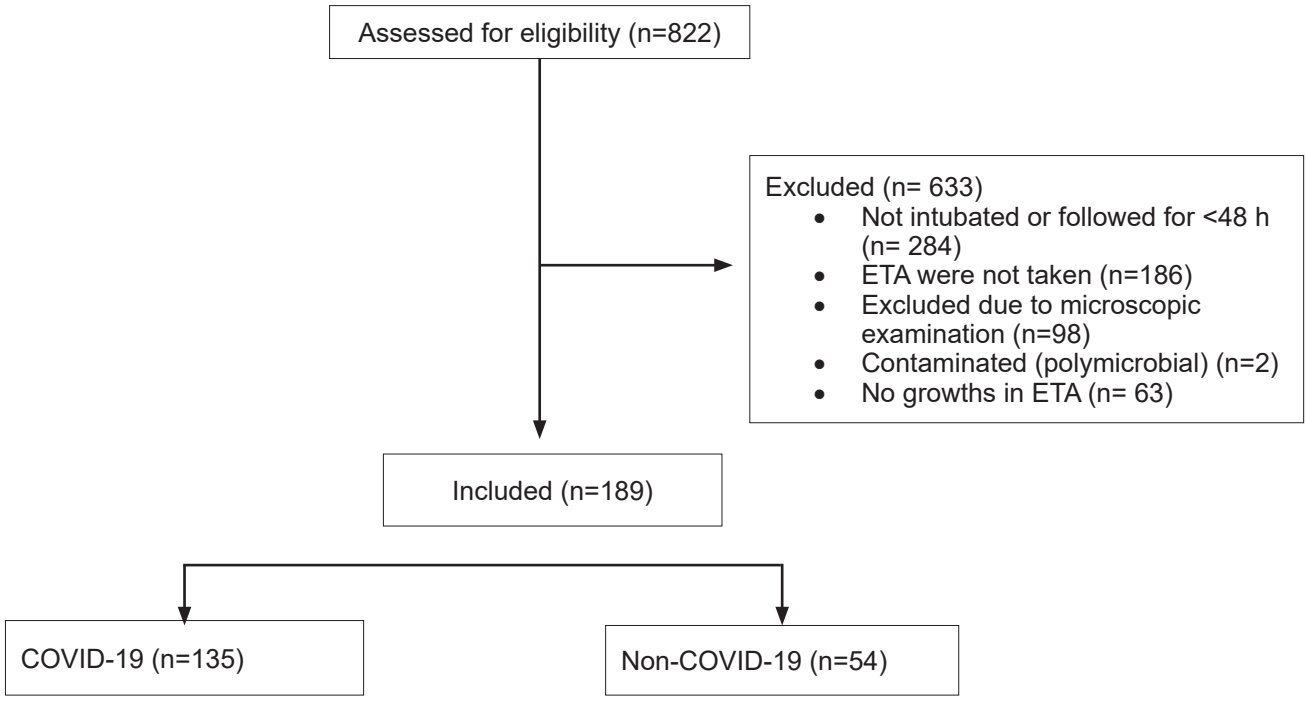

Figure 1. Flow Chart

Table 2. Microorganisms isolated from ETA in patients with and without COVID-19.

Microorganism	COVID-19 (n=135)	Non-COVID-19 (n=54)	p value
Gram-negative, n(%)			
Acinetobacter baumannii	68(50)	16(29.6)	0.01
Klebsiella pneumoniae	35(27.4)	19(35.0)	0.29
Pseudomonas aeruginosa	10(7.4)	10(18.5)	0.03
Escherichia coli	5(3.7)	4(7.5)	0.27
Serratia Marcescens	3(2.2)	0(0)	0.56
Stenotrophomonas maltophilia	4(2.9)	1(1.9)	1.00
Gram-positive, n(%)			
Staphylococcus aureus	12(8.8)	5(9,4)	0.89
Streptococcus pneumonia	4(2.9)	2(3.8)	0.67
Corynebacterium striata	8(5.9)	7(13.2)	0.13
Fungus, n(%)			
Aspergillus fumigatus	1(0.7)	1(1.8)	0.48
Non-Pathogenic, n(%)			
Candida spp.	6(4.4)	2(3.6)	1.00
Enterococcus faecium	1(0.7)	2(3.6)	0.19

Data are shown as n (%).

intubated in the ICU and followed up with for more than 48 hours was 538, with 370 (68.8%) COVID-19 patients versus 168 (31.2%) non-COVID-19 patients. ETA samples were not taken for 186 (34%) of these patients after the first 48 hours of intubation. Of the remaining 352 patients, 98 were excluded from the evaluation (64 COVID-19 and 43 non-COVID-19) due to the presence of PMNL <25 or epithelial >10 or colony count $<10^5$ cfu/ml in a microscopic examination of the samples. Two COVID-19 patients were excluded because the samples taken from their ETA were considered contaminated due to polymicrobial growth. There was no growth in the ETA cultures of 63 (25%) of the remaining 252 patients. Microbiological growth was observed in all of the remaining 189 patients. The number of microbiologically confirmed VAPs was 135/370 (36%) in patients with COVID-19 and 54/168 (32%) in non-COVID-19 ($p=0.55$). The incidence of microbiologically confirmed VAP was 30.6 events per 1000 mechanical ventilator days (32 events/1000 days in COVID-19 and 26.5 events/1000 days in non-COVID-19).

Two microorganisms were detected in the same isolate in 26 (13.7%) patients (15 COVID-19 vs. 11 non-COVID-19; $p=0.10$).

Demographic characteristics, comorbidities and laboratory characteristics of the patients included in the study are shown in Table 1. The mean age of the patients was 66.9 ± 15.4, and there were 107 male patients (56%) and 82 female patients (43%). There was no difference between the two groups in terms of age and gender ($p=0.79$ and $p=0.26$, respectively). The APACHE II mean was 17.1±4.0, and no difference was observed between the two groups ($p=0.61$). Out of 189 patients, 142 (75%) had at least one comorbid disease, the most common comorbidities were hypertension, diabetes mellitus and heart disease. These were followed by chronic lung disease, malignancy, neurological diseases and renal failure. Fourteen COVID-19 patients were diagnosed with immunosuppression (hematological malignancy (n=8), immunosuppressive drug use (n=6) at the admission to the ICU. Only two patients without COVID-19 were diagnosed with immunosuppression (malignancy (n=1), immunosuppressive drug use (n=1)) (p< 0.01). In the COVID-19 group, 115 (85%) patients admitted to the ICU, primarily due to COVID-19 related respiratory failure, while the remaining 20 patients were primarily due to sepsis ($n=6$, 4.4%), acute kidney injury ($n=4$, 2.9%), cerebrovascular accident ($n=1$, 0.7%), postoperative follow-up ($n=1$, 0.7%), acute abdomen ($n=3$, 2.1%), post-cardiac arrest follow-up ($n=5$, 3.7%), trauma ($n=1$, 0.7%), and acute decompensated heart failure ($n=1$, 0.7%) (data not shown in the table). Reasons for ICU admissions in the non-COVID-19 group were acute respiratory failure ($n=11$, 20%), sepsis ($n=12$, 22%), cerebrovascular accident ($n=10$, 18.5%), postoperative follow-up ($n=7$, 13%), post-cardiac arrest follow-up ($n=5$, 9%), acute decompensated heart failure ($n=4$, 7.4%), trauma ($n=1$, 1.8%), acute kidney injury ($n=2$, 3.6%), and acute abdomen ($n=2$, 3.6%) (data not shown in the table).

There were 93 patients (68%) with a COVID-19 diagnosis and 38 (70%) without a COVID-19 diagnosis who were receiving antibiotics before ETA sampling ($p=0.70$). The antibiotics most frequently started before ETA in patients with COVID-19 ($n=6$, 4.4%) were carbapenems ($n=15$, 11.1%), quinolones ($n=36$, 26.6%), piperacillin tazobactam ($n=36$, 26.6%) and cephalosporins ($n=36$. 26.6%). In patients without COVID-19, the most common antibiotics were carbapenems ($n=21$, 38%), piperacillin tazobactam ($n=15$, 27.7%) and cephalosporins ($n=2$, 3.7%). Carbapenems were was more common in COVID-19 patients ($p=0.02$ and $p=0.04$, respectively).

Steroids (dexamethasone, $n=121$; methylprednisolone, $n=13$; hydrocortisone, $n=1$) were used in the treatment of all COVID-19 patients. Only 9 (16%) patients without COVID-19 treated with steroids. Tocilizumab was used in 7 (5%) and anakinra in 1 (0.7%) COVID-19 patient (data not shown in the table).

There was no difference between the two groups in CRP, PCT, white blood cell count and neutrophil count on the laboratory findings obtained during ETA sampling ($p=0.11$, $p=0.80$, $p=0.97$, and $p=0.81$, respectively). The lymphocyte count was low in patients with COVID-19 ($p=0.03$).

The median interval between intubation and ETA sampling was 5 (3–10) days in patients with COVID-19 and 6 (3–10) days in non-COVID-19 patients ($p=0.77$). The median length of ICU stay was 12 (8–17) days in COVID-19 and 9 (5–17) days in non-COVID-19 patients ($p=0.03$). The median length of hospital stay was 18 (12–34) days in patients in COVID-19 and 15 (8–37) days in non-COVID-19 patients ($p=0.10$). The 30th-day mortality was 71.9% ($n=97$) in COVID-19 and 68.5% ($n=37$) in non-COVID-19 patients ($p=0.72$).

The most common microorganisms detected in ETA samples of non-COVID-19 patients were *Acinetobacter baumannii*, 16 (29.6%); *Klebsiella pneumoniae*, 19 (35%); *Pseudomonas aeruginosa*, 10 (18.5%); *Staphylococcus aureus*, 5 (9.4%); *Corynebacterium striatum*, 7 (13.2%); and *Escherichia coli*, 4 (7.5%). The most common microorganisms detected in ETA samples of COVID-19 patients were *A. baumannii*, 68 (50%); *K. pneumoniae*, 35 (27.4%); *P. aeruginosa*, 10 (7.4%); *S. aureus*, 12 (8.8%); *C. striatum*, 8 (5.9%); and *E. coli*, 5 (3.7%). *Aspergillus fumigatus* reproduced in one COVID-19 patient and one non-COVID-19 patient. The distribution of microorganisms in the ETA cultures is shown in Table 2. The number of *A. baumannii* culture positivity was higher in COVID-19 patients than in non-COVID-19 patients ($p=0.01$). The numbers of *K. pneumoniae* and *S. aureus* culture positivity were not different between groups ($p=0.29$ and $p=0.89$, respectively), and *P. aeruginosa* was observed more frequently in non-COVID-19 patients ($p=0.03$).

Out of 8 patients who received either tocilizumab or anakinra, *P. aeruginosa* was detected in 2, *A. baumannii* in 3, *K. pneumoniae* in 1, and *A. fumigatus* in 1 patient. (data not shown in the table).

A subgroup analysis of frequently detected microorganisms was performed for immunosuppression at admission, length of ICU stay, time from intubation to ETA sampling, and 30th-day mortality. The lymphocyte counts of the COVID-19 patients with *A. baumannii* culture positivity were lower than non-COVID-19 patients ($p=0.01$). Seven (10%) of COVID-19 patients with *A. baumannii* were immunosuppressant, while 1 of the non-COVID-19 patients with *A. baumannii* was (p=0.56). The length of ICU stay was 16 days (12–20) for COVID-19 patients and 11.5 days (5–36) for non-COVID-19 patients ($p=0.40$). The time from intubation to ETA culture sampling was longer in COVID-19 patients (6.5 (4–10) days vs 3.5 (2-10) days) ($p=0.04$). Thirty-day mortality of the COVID-19 patients was 72% ($n=49$) and 62.5% ($n=10$) in non-COVID-19 patients ($p=0.34$) (Table3). In the subgroup analysis of patients with *K. pneumoniae, P. aeruginosa, C. striatum* and *S. aureus* culture positivities, there were no differences in the presence of immunosuppression at baseline, time from intubation to ETA sampling, length of ICU stay and mortality between COVID-19 and non-COVID-19 patients (Table 3).

Discussion

COVID-19 patients have a higher rate of mechanical ventilation requirement in the ICU. Therefore, they are at risk of developing VAP. In this retrospective study, we examined microorganisms isolated from ETA due to VAP in COVID-19 patients and non-

Table 3. Analysis of the patients in whom Acinetobacter baumannii, Klebsiella pneumoniae, Pseudomonas aeruginosa, Corynebacterium striatum, Staphylococcus aureus were isolated.

	Acinetobacter baumannii			*Klebsiella pneumoniae*			*Pseudomonas aeruginosa*			*Corynebacterium striatum*			*Staphylococcus aureus*		
	COVID-19 (n=68)	Non-COVID (n=16)	p	COVID-19 (n=35)	Non-COVID-19 (n=19)	p	COVID-19 (n=10)	Non-COVID-19 (n=10)	p	COVID-19 (n=8)	Non-COVID-19 (n=7)	p	COVID-19 (n=12)	Non-COVID-19 (n=5)	p
Age, year	65 ±14	67 ±17	0.94	69 ±14	63 ±14	0.69	67±11	68 ±16	0.80	63±14	77 ±7.8	0.07	65±16	62 ±15	0.66
Gender, Female (%)	29(42)	9(56)	0.40	11(29)	8(42)	0.80	3(30)	6(60)	0.37	5(62)	4(57)	1.00	6(50)	3(60)	1.00
Laboratory															
C-reactive protein, mg/L	183±81	193 ±91	0.70	178±84	208 ±97	0.27	154±47	209 ±75	0.10	140±93	223 ±84	0.12	142(111-192)	170(131-336)	0.09
procalcitonin, ng/ml	0.83(0.2-3.1)	0.53(0.20-2.3)	0.37	1.04(0.4-5.2)	4.5(0.81-11.6)	0.08	3.2(1.4-6.8)	0.43(0.29-7.8)	0.17	0.25(0.1-0.59)	0.39(0.33-8.1)	0.18	0.44(0.17-13.8)	3.3(0.3-4.3)	0.41
White Blood Cell, (/µL) ×10^3	12.7(9.9-17.4)	13.2(8.7-18.3)	0.79	13.6(9.0-18.8)	10.2(8.5-17.9)	0.35	15.3(8.6-23.7)	14.2(9.4-16.8)	0.63	10.2(7-16.9)	15.8(10.2-17.3)	0.29	15(6-20)	15(11-25)	0.28
Lymphocyte. (/µL) ×10^3	0.5(0.2-0.9)	0.9(0.65-1.50)	0.01	0.7(0.4-1.4)	0.85(0.3-1.65)	0.88	0.9(0.5-1.0)	1.1(0.7-1.65)	0.12	0.6(0.2-0.8)	0.75(0.37-1.0)	0.36	0.3(0.2-1.0)	1.0(0.6-2.4	0.20
Neutrophil, (/µL) ×10^3	10.6(8.6-15.2)	11.0(5.2-15.3)	0.52	13.0(7.8-16.4)	10.8(7.3-15.5)	0.63	13.0(4.2-21.1)	12.5(7.9-16.9)	0.79	9(6.4-15.3)	12.9(8.4-16.2)	0.37	14(5-18)	14(6-21)	0.63
Immunosupression, n(%)	*7(10)*	1(6)	0.56	4(10)	0(0)	0.28	*0*	0	-	*0*	0	-	1(8)	0(0)	1.00
Length of ICU stay day	16 (12-20)	14 (7-35)	0.40	15(6-25)	11 (7-15)	0.63	17(7-26)	11 (7-19)	0.86	17(15-37)	12.5(4-33)	0.58	11(9-15)	6(3-27)	0.05
Duration fron intubation to ETA sampling, day	6.5(4-10)	3.5(2-10)	0.04	7(3-11)	11(5-36)	0.54	3.5(2-5)	5.5(3 7)	0.23	4(2-6)	3(2-9)	0.83	3(2-4)	2(2-4)	0.41
30th-day mortality, n(%)	49(72)	10(62.5)	0.54	26(70)	14(73)	1.00	8(80)	6(60)	0.62	4(50)	5(71)	0.55	10(83)	3(60)	0.53

COVID-19 patients. The number of patients who developed VAP was higher in the COVID-19 group than in the non-COVID-19 group, but it was not statistically significant. The number of microbiologically confirmed VAP events per 1000 days on the ventilator was higher in COVID-19 patients (32 events/1000 ventilator days vs. 26.5 events/1000 ventilator days). It was observed that most of the growths in ETA cultures in ICU were gram-negative bacilli. *A. baumannii* was the most common microorganism in all of the patients, but it was more common in COVID-19 patients than in non-COVID-19 patients.

There was a higher rate of culture positivity when the length of intubation was string out. Delayed ETA sampling may occur because of difficulty differentiating bacterial and viral pneumonia due to clinical and laboratory similarities, especially in the early stages of viral pneumonia, and it may be one of the reasons for the differences in microorganisms in the two groups (13). Another explanation may be the difference in the treatments recently used as antibiotic therapy before VAP development. Previous use of broad-spectrum antibiotics is a risk factor for VAP (14). When we examined the spectrum of antibiotic therapy before the ETA sampling in patients with and without COVID-19, we found that carbapenems were used more often in non-COVID-19 patients, and quinolones were used more frequently in COVID-19 patients. These data suggest that, empirically, quinolones were frequently used in COVID-19 patients as community-acquired infections were initially considered. *A. baumannii* was the most common isolated microorganism in COVID-19 patients. Longer stays in the ICU for COVID-19 patients and the longer duration between ETA sampling and intubation in these patients might contribute to this result. *P. aeruginosa* was observed more frequently in non-COVID-19 patients, but the low isolation numbers of this pathogen in both groups makes interpretation difficult.

In another study examining the patients who had follow-ups in our ICU between 2004 and 2009, *A. baumannii* (40%) and *P. aeruginosa* (40%) were found to be the most common causes of VAP (15). The distribution of microorganisms may change over the years, but *A. baumannii* has maintained its place as the most common microorganism.

Since the early period of the COVID-19 pandemic, data have been presented regarding the higher incidence of VAPs in COVID-19 patients in the ICU (16). Although the incidence of VAP is different in various studies, it is generally above 30% (range 36–86%) (11, 16, 17). Rouzé et al. (18) found in their multicentre cohort study that the incidence of VAP in COVID-19 patients was higher than in those with influenza pneumonia and viral pneumonia (36% vs 22% vs 16%, respectively). In the same study, gram-negative bacilli were the most frequently isolated bacteria in VAP patients, particularly *P. aeruginosa*, *Enterobacter spp.* and *Klebsiella spp*. Similarly, Razazi et al. (19) found higher VAP in patients with COVID-ARDS than in non-COVID patients. In a multicentre study, Giacobbe et al. (20) found that VAP was observed in 29% of the COVID-19 patients. They found the incidence rate of VAP to be 18 events per 1000 ventilator days, and the most common organisms were *P. aeruginosa* (35%) and *S. aureus* (23%). In our study, the incidence of microbiologically confirmed VAP per 1000 days on mechanical ventilator was higher in the COVID-19 group (32 vs 26.5). One reason for this difference may be that more immunosuppressed patients were found in the COVID-19 group. However, there was no statistically significant difference in the number of intubated patients who developed VAP between the COVID-19 and non-COVID-19 groups.

Critically ill patients are susceptible to nosocomial infection regardless of viral infections (21), and impaired immune cell function during COVID-19 may facilitate the development of infection (22). Lymphocytopenia poses a significant risk of infection in patients with COVID-19, and previous studies showed that low lymphocyte counts is associated with increased risk of ICUacquired infection, 28th-day septic shock development and 28th-daymortality (23,24).

After the RECOVERY study (25), steroids were used as part of standard treatment for COVID-19 patients. There was no increase in the number of COVID-19 patients in whom pathogenic microorganisms were isolated receiving dexamethasone and who had VAP. In the CODEX study, it was found that the use of steroids did not increase the risk of secondary infection in COVID-19 patients (26).

This research was a single-centre study; therefore, the results cannot be generalised. The fact that the drug resistance patterns of microorganisms are outside the scope of this study can be considered a limitation. In our clinic, the use of tocilizumab and anakinra was not frequent due to difficulty accessing the drugs. Therefore, it is not possible to comment on these agents based on our results.

In conclusion, there was no significant difference in the number of patients who developed VAP among COVID-19 patients compared to non-COVID-19 patients, despite more VAP events per 1000 ventilator days in the COVID-19 patients. *A. baumannii* was the most commonly isolated microorganism in COVID-19 patients, which may have been due to the longer stay in the ICU and the longer duration between intubation and ETA sampling in COVID-19 patients with *A. baumannii* growth. Efforts should be made to better understand the frequency and etiological causes of VAP in patients with COVID-19 to improve preventive measures in this population.

AUTHOR CONTRIBUTIONS:

Concept: KR,ÖE, TÇ, TY, GE,ÖS; **Design:** KR, GE; **Supervision:** TÇ, TY, GE; **Data Collection and/ or Processing:** KR, ÖE, TÇ, TY, ÖS; **Analysis and/ or Interpretation:** KR, GE; **Literature Search:** GE, KR; **Writing Manuscript:** KR,ÖE, TÇ, TY, GE,ÖS; **Critical Review:** GE.

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