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Pathogen distribution and microbial resistance pattern in endotracheal aspirate samples of intensive care unit patients before and after the COVID-19 pandemic

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ABSTRACT

Aims: The aim of this study is to evaluate the distribution of pathogen microorganisms and antimicrobial resistance rates isolated from endotracheal aspirate (ETA) samples of SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) polymerase chain reaction (PCR) positive and negative patients followed and treated in the intensive care unit (ICU) of our hospital, and to examine the effect of the COVID-19 (coronavirus disease 2019) pandemic on this.

Methods: In this study, ETA samples sent to the microbiology laboratory from hospitalized patients in Tekirdağ Namık Kemal University Hospital general ICU-1 and general ICU-2 between March 11, 2018 and March 10, 2022 were retrospectively analyzed. During the COVID-19 pandemic, it was used to follow up patients with SARS-CoV-2 PCR positive in ICU-1 and SARS-CoV-2 PCR negative patients in ICU-2. The working period is divided into two parts as pre-pandemic (2018 - 2019) and post-pandemic (2020 - 2021). Bacterial identification and antibiotic susceptibility tests were performed using conventional methods and automated systems. Colistin sensitivity was studied by broth microdilution, and ceftazidime avibactam (CZA) sensitivity was studied by disk diffusion method. Statistical analysis was performed with the chi-square test, $p<0.05$ was considered significant.

Results: A total of 1669 ETA samples from 856 patients were sent to our laboratory over a four-years period, and culture positivity was detected in 63.6% of the samples. With the COVID-19 pandemic, it was found that the culture positivity increased significantly in ETA samples of patients hospitalized in ICU-1, and there were no significant difference in ICU-2. 836 isolates from 1061 specimens were included to the study. The three most commonly isolated pathogens were *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, respectively. While *P. aeruginosa* was the most frequently isolated microorganism in both ICU-1 and ICU-2 in the pre-pandemic period, it was replaced by *A. baumannii* in both clinics with the pandemic, and the increase in the frequency of *A. baumannii* in ICU-1 was statistically significant. Antibiotic resistance rates were generally found to be higher in ICU-1 than in ICU-2, and even in ICU-2, resistance rates to some antimicrobials were found to be decreased. In *A. baumannii*, a statistically significant increase was observed in the resistance rates against all antibiotics, including colistin, in ICU-1, and a significant increase was found in resistance only against amikacin in ICU-2. In *P. aeruginosa*, a significant increase was found in the resistance rates against cephalosporins and carbapenems in ICU-1, ceftazidime, ciprofloxacin and colistin in ICU-2, and a significant decrease in resistance to amikacin in ICU-2. In *K. pneumoniae*, a significant increase was found in the resistance rates against amoxicillin-clavulanate (AMC), ceftriaxone, ertapenem, amikacin and colistin in ICU-1, ertapenem and amikacin in ICU-2, and a significant decrease in resistance to AMC and all cephalosporins in ICU-2. CZA susceptibility in *K. pneumoniae* isolates was examined in 2020 and 2021, and no resistance was found in either clinic.

Conclusion: In our study, it was determined that the culture positivity rate in ETA samples increased, the distribution of pathogen microorganisms and antimicrobial resistance rates changed with the COVID-19 pandemic. For this reason, it is important to follow up possible pathogen microorganisms and antimicrobial resistance rates during similar pandemic periods such as COVID-19.

Keywords: antimicrobial resistance, COVID-19, endotracheal aspirate, pathogen microorganism

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INTRODUCTION

COVID-19 (coronavirus disease 2019) infection, a part of SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2), was first detected in Wuhan, China in December 2019, and then caused a pandemic all over the world. In our country, the first case was reported in March 2020, and many hospitals were determined as pandemic hospitals in order to make treatment of COVID-19 patients.[1,2] COVID-19 may cause many cases such as respiratory tract infection, severe acute respiratory syndrome (SARS), sepsis and multi-organ failure.[3] Bacterial or fungal co-infection is frequently encountered in viral respiratory tract infections. These co-infections negatively affect the state of the existing disease, cause long-term hospitalization, and increase morbidity and mortality. Because of this, rapid diagnosis and initiation of treatment are important for the course of the disease.[4,5]

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Resistance to antimicrobial drugs appears as an increasing public health problem nowadays. Antimicrobial therapy is involved in the treatment of co-infections in COVID-19 patients and is used empirically or for the ruling of nosocomial infection acquired during hospitalization. Though antibacterial agents have no effect on the treatment of the disease, some case series recommend the use of broad-spectrum antibiotics in COVID-19 patients. Unfortunately, the use of broad-spectrum antibiotics brings with the risk of resistance development.[6,7]

The aim of this study; to evaluate the agents isolated from endotracheal aspirate (ETA) samples and antimicrobial resistance rates of patients with positive and negative SARS-CoV-2 polymerase chain reaction (PCR) values that followed and treated in the intensive care unit (ICU) of our hospital, and also to examine the impact of the COVID-19 pandemic on this situation.

METHODS

The study was carried out with the permission of Tekirdağ Namık Kemal University Non-Interventional Clinical Researches Ethics Committee (Date: 28.06.2022, Decision No: 2022.127.06.17). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Study Design

ETA samples sent to the microbiology laboratory from patients hospitalized in general ICU-1 and general ICU-2 of Tekirdağ Namık Kemal University Hospital (430 bed capacity) between March 11, 2018 and March 10, 2022 were analyzed retrospectively. Our hospital served as a pandemic one during the COVID-19 pandemic. ICU-1 (11 bed capacity) was used for the treatment of intubated patients followed for COVID-19 infection (SARS-CoV-2 PCR positive), ICU-2 (11 bed capacity) was used for the treatment of intubated patients followed for non-COVID-19 reasons (SARS-CoV-2 PCR negative). The time interval included in the study; the date of 11 March 2020, when the first case was detected in our country, was accepted as the starting point, and it was evaluated as two years before the COVID-19 pandemic (11 March 2018 - 10 March 2020) and two years after the COVID-19 pandemic (11 March 2020 - 10 March 2022).

Nosocomial pneumonia, is a lower respiratory infection that was not incubating at the time of hospital admission and that presents clinically two or more days after hospitalization.[8] Because of this ETA samples taken on the possibility of infection 48 hours after the patients were admitted to the intensive care unit were included in the study. Demographic (age, gender) and clinical (inpatient service, clinical sample, pathogen, etc.) data of the patients were taken from the hospital information management system (HIMS).

Microbiological Evaluation

When an intubated patient has a lung infection clinic in our hospital, an ETA sample is requested. According to guidelines,[9] first, stained microscopic examination was performed and it was evaluated whether the sample was a quality sample reflecting the lower respiratory tract. The culture of the ETA sample thought to reflect the lower respiratory tract was examined. Quantitative culture method is done in the microbiology laboratory, and ≥100.000 KOB/ml growth is considered significant. In the case of one or two bacteria grown purely in culture, these microorganisms were considered as pathogens and the antibiogram was studied. ETA samples were incubated in agar with 5% sheep blood agar (Bes-Lab, Turkey), eosin methylene blue (EMB) agar (Bes-Lab, Turkey) and chocolate agar (Bes-Lab, Turkey) for 18-24 hours at 37°C and in 5-10% CO_2 environment. In the case of pure single or double colony growth in culture, isolates were identified by conventional methods (colony morphology, Gram stain, oxidase, catalase and coagulase test) and automated identification system (Vitek2 Compact, Biomerux, France and BD Phoenix System, Beckton Dickinson, USA). Antibiotic susceptibility tests were performed with manual Kirby-Bauer disc diffusion (Bioanalyse, Turkey and Oxoid, UK) and automated antibiogram systems (Vitek2 Compact, Biomerux, France and BD Phoenix System, Beckton Dickinson, USA) in accordance with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria(10). Carbapenem resistance in *Klebsiella pneumoniae* isolates was evaluated by the combined disc diffusion method (Bioanalyse, Turkey), ceftazidime avibactam (CZA) resistance has been studied since 2020 and was studied by the disk diffusion method (Bioanalyse, Turkey). Colistin resistance was studied by broth microdilution method (Micronaut-S, Merlin, Germany). Methicillin resistance was determined in staphylococcal isolates by disk diffusion method with cefoxitin disk (Oxoid, UK). Vancomycin and teicoplanin resistance detected in enterococcal isolates was confirmed by gradient test (Bioanalyse, Turkey). In case of detection of yeast in the samples, a factor-colonization distinction was made by interviewing with the relevant clinic. Antifungal susceptibility tests of isolates considered as active agents were determined by microdilution method (Mikronaut-AM, Bruker, Germany) in accordance with EUCAST guidelines. In the repetitive sample of one patient, only the first isolate was included in the study.

The SARS-CoV-2 PCR test was done by using the Bio Speedy SARS-CoV-2 RT-qPCR kit (Bioeksen, Turkey).

Statistical Analysis

The data that was obtained in the study were recorded in the SPSS 22.0 (SPSS Inc, Chicago, IL, USA) program and statistical analyzes were made. Categorical data

were given as percentages. Chi-square test was used to compare independent groups with categorical variables. Cases where the p value was below 0.05 were considered as statistically significant.

RESULTS

1669 ETA samples from 856 patients were sent to our laboratory in four years. Demographic data of patients; 59% male (n=505), 41% female (n=351) (ICU-1 59% male, 41% female; ICU-2 59% male, 41% female), mean age was 66.7±16.2 (17-100) (67.6±16.3 for ICU-1, 65.7±16.1 for ICU-2). There was no difference in age and gender between the patients followed in ICU-1 and ICU-2 (p>0.05).

Significant culture positivity was detected in 63.6% of the samples, no growth was detected in 18%, and oropharyngeal flora elements (OPFE) grew in 18.4% and were considered as contamination. It was found that the frequency of culture positivity in ETA samples that was taken from patients hospitalized in ICU-1 with the COVID-19 pandemic increased statistically significantly (p=0.018). There was no difference in ICU-2 (p=0.596) (Table 1). In our study, the rates of ventilator-associated pneumonia (VAP), a nosocomial pneumonia, were also evaluated for both ICUs before and after COVID-19. VAP rates were detected as 10.6% in ICU-1 and 12.1% in ICU-2 before COVID-19, and increased to 16.7% (23.0% in 2020, 10.3% in 2021) in ICU-1 and decreased to 9.3% (10.9% in 2020, 7.7% in 2021) in ICU-2 after COVID-19. The increase observed in ICU-1 in 2020 was found to be statistically significant (p=0.015), and no significant difference was detected in ICU-2 (p=0.489).

The pathogen microorganism was isolated in 836 of 1061 specimens with culture positivity and were included in this study. Of 836 isolates, 672 (80.4%) were Gram-negative bacteria ((51.1% nonfermenter Gram-negative bacteria (n=427), 26.1% Enterobacterales species (n=218), 3.2% other Gram-negative bacteria (n=27)) , 117 (14.0%) gram-positive bacteria and 47 (5.6%) fungi were identified. Before the pandemic, 80.3% of the isolates in ICU-1 were Gram-negative bacteria, 16.9% were Gram-positive bacteria, and 2.8% were fungi. After the pandemic, 80.3% of Gram-negative bacteria, 11.7% of Gram-positive bacteria, and 8.0% of fungi were determined. In ICU-2, 84.4% Gram negative bacteria, 12.9% Gram positive bacteria, and 2.7% fungi species before the pandemic. After the pandemic, 77.7% of Gram-negative bacteria, 14.4% of Gram-positive bacteria and 7.9% of fungi were determined. In COVID-19 pandemic, it was observed that the frequency of fungal pathogens increased in ETA samples in both clinics, but, this increase was not statistically significant for both clinics (ICU-1 p=0.579, ICU-2 p=0.121) (Table 2).

The four most frequently isolated agents in our study were *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *K. pneumoniae* and *Staphylococcus aureus*, respectively. In pre-COVID-19 pandemic period, ranking of pathogens in ICU-1 *P. aeruginosa* (27.2%), *A. baumannii* (18.3%), *S. aureus* (11.7%), *K. pneumoniae* (8.5%), with the pandemic, this situation changed to *A. baumannii* (31%), *P. aeruginosa* (15.7%), *K. pneumoniae* (14.2%) and *S. aureus* (7.3%). Only the increase in the frequency of *A. baumannii* was statistically significant (p=0.033), no statistically significant difference was found for the other three pathogens (p>0.05). In ICU-2, the pre-pandemic ranking was *P. aeruginosa* (25.9%), *A. baumannii* (20.4%), *K. pneumoniae* (10.9%), *S. aureus* (7.5%). Along with the pandemic, *A. baumannii* (22.8%), *P. aeruginosa* (16.3%), *K. pneumoniae* (15.3%) and *S. aureus* (8.9%) were detected and there was no statistically significant difference in isolation frequency for all four pathogens (p>0.05). While *P. aeruginosa* was the most frequently isolated pathogen in both ICU-1 and ICU-2 before the pandemic, it was replaced by *A. baumannii* in both clinics with the COVID-19 pandemic (Table 2).

Table 1. Distribution of microbiological evaluation of ETA samples by years and clinics (n/%)

Culture result	Before COVID-19				After COVID-19				Total		
	2018		2019		2020		2021				
	ICU 1	ICU 2	ICU 1	ICU 2	ICU 1	ICU 2	ICU 1	ICU 2	ICU 1	ICU 2	Total
Culture positive											
n	124	88	164	82	230	148	128	97	646	415	1061
%	64.6	59.1	66.7	55.8	77.5	63.0	56.6	54.2	67.2	58.6	63.6
Culture negative											
n	26	33	29	23	23	42	66	59	144	157	301
%	13.5	22.1	11.8	15.6	7.7	17.9	29.2	33.0	15.0	22.2	18.0
*Cont											
n	42	28	51	42	44	45	32	23	169	138	307
%	21.9	18.8	21.5	28.6	14.8	19.1	14.2	12.8	17.8	19.2	18.4
Total											
n	192	149	246	147	297	235	226	179	961	708	1669

*Cont: Oropharyngeal Contamination, ETA: Endotracheal aspirate, ICU: Intensive care unit

Table 2. Distribution of pathogens isolated from ETA samples by years and clinics (n/%)

Microorganisms	Before COVID-19								After COVID-19								4 years period					
	2018				2019				2020				2021				Total					
	ICU1		ICU2		ICU1		ICU2		ICU1		ICU2		ICU1		ICU2		ICU1		ICU2		Total	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
***Gram Negative Bac.**																						
*NFGN																						
A. baumannii	21	22.1	17	21.5	18	15.3	13	19.1	53	31.0	26	22.4	32	31.1	20	23.3	124	25.5	76	21.8	200	23.9
P. aeruginosa	23	24.2	21	26.6	35	29.7	17	25.0	33	19.3	22	19.0	10	9.7	11	12.8	101	20.7	71	20.3	172	20.6
S. maltophilia	2	2.1	-	-	15	12.7	2	2.9	8	4.7	2	1.8	8	7.9	5	5.8	33	6.8	9	2.6	42	5.0
Other NFGN	2	2.1	1	1.3	1	0.9	-	-	5	2.9	4	3.4	-	-	-	-	8	1.6	5	1.4	13	1.6
Enterobacterales																						
K. pneumoniae	11	11.6	8	10.1	7	5.9	8	11.8	25	14.6	16	13.8	14	13.6	15	17.4	57	11.7	47	13.5	104	12.4
E. coli	8	8.4	8	10.1	5	4.2	4	5.9	7	4.1	8	6.9	1	0.9	8	9.3	21	4.3	28	8.0	49	5.9
Enterobacter spp.	3	3.3	5	6.4	2	1.7	4	5.9	5	2.9	4	3.4	4	3.9	4	4.7	14	2.9	17	4.9	31	3.7
Others	4	4.2	3	3.8	7	5.9	3	4.4	5	2.9	4	3.4	5	4.9	3	3.5	21	4.3	13	3.7	34	4.1
H. influenzaee	2	2.1	3	3.8	5	4.2	4	5.9	3	1.8	2	1.8	1	0.9	1	1.1	11	2.2	10	2.9	21	2.5
M. catarrhalis	-	-	3	3.8	-	-	-	-	-	-	2	1.8	1	0.9	-	-	1	0.3	5	1.4	6	0.7
***Gram Positive Bac.**																						
S. aureus	13	13.7	4	5.0	12	10.2	7	10.3	8	4.7	9	7.8	12	11.7	9	10.5	45	9.2	29	8.3	74	8.9
S. pneumoniae	4	4.2	1	1.3	4	3.4	4	5.9	5	2.9	4	3.4	1	0.9	3	3.5	14	2.9	12	3.4	26	3.1
*Other GPB	1	1.0	3	3.8	2	1.7	-	-	3	1.8	1	0.8	3	2.9	4	4.7	9	1.8	8	2.3	17	2.0
Fungi																						
Candida spp.	1	1.0	2	2.5	5	4.2	2	2.9	11	6.4	12	10.3	6	5.8	2	2.3	23	4.7	18	5.2	41	4.9
Other Fungi	-	-	-	-	-	-	-	-	-	-	-	-	5	4.9	1	1.1	5	1.1	1	0.3	6	0.7
Total	95	100	79	100	118	100	68	100	171	100	116	100	103	100	86	100	487	100	349	100	836	100

*Gram Negative Bac: Gram Negative Bacteria, NFGN: Non-fermenter Gram Negative Bacteria, Gram Positive Bac: Gram Positive Bacteria, GPB: Gram Positive Bacteria, ETA: Endotracheal aspirate, ICU: Intensive care unit

When the resistance rates of the isolated pathogens were compared before and after the COVID-19 pandemic; In *A. baumannii*, there was a statistically significant increase in the resistance rates against all antibiotics, including colistin in ICU-1, while a statistically significant increase was found in resistance only against amikacin in ICU-2 ($p<0.05$). Significant increase in resistance to cephalosporins and carbapenems in ICU-1 in *P. aeruginosa*, and again significant increase in resistance to ceftazidime, ciprofloxacin and colistin in ICU-2 and finally, a significant decrease in resistance to amikacin was observed in ICU-2 ($p<0.05$). In *K. pneumoniae* significant increase in resistance rates was detected against amoxicillin-clavulanate (AMC), ceftriaxone, ertapenem, amikacin and colistin in ICU-1 and against ertapenem and amikacin in ICU-2, and a significant decrease in resistance to AMC and all cephalosporins in ICU-2 ($p<0.05$). Ceftazidime-avibactam susceptibility was examined in *K. pneumoniae* isolates in 2020 and 2021, and no resistance was found in either clinic. In other Enterobacterales species, a significant increase was observed in resistance rates against ertapenem and amikacin in ICU-1, against cefepime in ICU-2. At the same time a significant decrease was detected in resistance to ceftriaxone, cefepime, meropenem and ciprofloxacin in ICU-1, and to AMC, piperacillin-tazobactam (PRP), imipenem and meropenem in ICU-2 ($p<0.05$) (**Table 3, 4**). While methicillin resistance in isolated *S. aureus* isolates was 24.0% and 27.3% before the pandemic in ICU-1 and ICU-2, respectively, it increased to 35.0% and 33.3% after the pandemic, and there was no statistically significant increase in either clinic (ICU-1 $p=0.088$, ICU-2 $p=0.355$). Before and after the pandemic, no resistance was found to vancomycin, teicoplanin and linezolid in *S. aureus* isolates.

Table 3. Antibiotic resistance rates of A.baumanni and *P. aeruginosa* isolates before and after the pandemic (%)

*Antibiotic	*A. baumannii*				p value		*P. aeruginosa*				p value	
	Before COVID-19 (2018-2019)		After COVID-19 (2020-2021)				Before COVID-19 (2018-2019)		After COVID-19 (2020-2021)			
	ICU1 (n=39)	ICU2 (n=30)	ICU1 (n=85)	ICU2 (n=46)	ICU1	ICU2	ICU1 (n=58)	ICU2 (n=38)	ICU1 (n=43)	ICU2 (n=33)	ICU1	ICU2
PTZ	-	-	-	-	-	-	55.2	44.7	58.1	33.3	0.669	0.102
CAZ	-	-	-	-	-	-	41.4	28.9	72.1	48.5	<0.001	0.004
FEP	-	-	-	-	-	-	37.9	28.9	58.1	33.3	0.005	0.541
IMP	87.2	96.7	98.8	91.3	0.001	0.074	34.5	26.3	51.2	30.3	0.022	0.529
MER	87.2	96.7	97.6	91.3	0.003	0.074	32.8	21.1	48.8	30.3	0.021	0.144
GEN	71.8	80.0	98.8	87.0	<0.001	0.182	-	-	-	-	-	-
AK	69.2	76.7	97.6	93.5	<0.001	0.001	24.1	15.8	23.3	6.1	0.868	0.024
CIP	87.2	96.7	98.8	91.3	0.001	0.074	37.9	15.8	48.8	30.3	0.117	0.019
TMP-SXT	71.8	83.3	91.8	84.8	<0.001	0.700	-	-	-	-	-	-
COL	0.0	13.3	13.0	6.5	0.001	0.106	6.9	0.0	9.3	12.1	0.602	0.002

*TZP: Piperacillin-tazobactam, CAZ: Ceftazidime, FEP: Cefepime, IMP: Imipenem, MER: Meropenem, GEN: Gentamicin, AK: Amikacin, CIP: Ciprofloxacin, TMP-SXT: Trimethoprim-sulphamethoxazole, COL: Colistin, ICU: Intensive care unit

Table 4. Antibiotic resistance rates of *K. pneumoniae* and other Enterobacterales isolates before and after the pandemic (%)

Antibiotic	*K. pneumoniae*				p value		Other Enterobacterales				p value	
	Before COVID-19 (2018-2019)		After COVID-19 (2020-2021)				Before COVID-19 (2018-2019)		After COVID-19 (2020-2021)			
	ICU1 (n=18)	ICU2 (n=16)	ICU1 (n=39)	ICU2 (n=31)	ICU1	ICU2	ICU1 (n=29)	ICU2 (n=27)	ICU1 (n=27)	ICU2 (n=31)	ICU1	ICU2
AMC	66.7	87.5	87.2	54.8	0.001	<0.001	79.3	70.4	81.5	48.4	0.592	0.002
PTZ	66.7	50.0	76.9	45.2	0.115	0.479	34.5	37.0	25.9	22.6	0.167	0.031
CRO	66.7	68.8	79.5	51.6	0.037	0.014	62.1	44.4	40.7	45.2	0.003	0.887
CAZ	66.7	68.8	76.9	48.4	0.115	0.003	55.2	37.0	44.4	45.2	0.120	0.250
FEP	66.7	68.8	74.4	48.4	0.278	0.003	55.2	22.2	37.0	38.7	0.011	0.009
ERT	50.0	25.0	66.7	45.2	0.015	0.003	13.8	11.1	25.9	12.9	0.034	0.663
IMP	50.0	25.0	59.0	29.0	0.201	0.524	10.3	3.7	3.7	0.0	0.096	0.043
MER	44.4	25.0	54.4	29.0	0.157	0.524	10.3	3.7	0.0	0.0	0.001	0.043
GEN	61.1	56.3	69.2	45.2	0.236	0.120	24.1	22.2	14.8	32.3	0.108	0.111
AK	11.1	18.8	61.5	32.3	<0.001	0.035	0.0	3.7	11.1	3.2	0.003	0.700
CIP	72.2	43.8	74.4	45.2	0.750	0.887	34.5	37.0	22.2	35.5	0.042	0.883
TMP-SXT	38.9	50.0	74.4	48.4	<0.001	0.777	34.5	40.7	37.0	35.5	0.883	0.467
COL	5.6	18.8	28.2	19.4	<0.001	1.000	0.0	0.0	0.0	0.0	1.000	1.000

*AMC: Amoxicillin-clavulanate, TZP: Piperacillin-tazobactam, CRO: Ceftriaxone, CAZ: Ceftazidime, FEP: Cefepime, ERT: Ertapenem, IMP: Imipenem, MER: Meropenem, GEN: Gentamicin, AK: Amikacin, CIP:Ciprofloxacin, TMP-SXT: Trimethoprim-sulfamethoxazole, COL: Colistin, ICU: Intensive care unit

DISCUSSION

COVID-19 is considered an pandemic that effects all over the world. Secondary infections added to the existing one effect the patient's prognosis and treatment process, causing patients to stay in the hospital longer than normal and use broad-spectrum antibiotics. In studies evaluating the comorbidity of COVID-19 and infection, it has been reported that the frequency of bacterial and/or fungal co-infection is increased in patients followed in the ICU.[7,11-13] In our study, compared to the pre-pandemic period; In the ETA samples of patients followed up for COVID-19 with the pandemic, culture positivity rates were found to increase statistically significantly. But also it was observed that there was no change in culture positivity rates in the samples of patients followed for reasons other than COVID-19. In addition, with the COVID-19 pandemic, there was a statistically significant increase in VAP rates in ICU-1. This evidence reveals that the frequency of respiratory tract infections increased with COVID-19 in our hospital. In our study, it was also observed that the frequency of fungal isolation in ETA samples increased with the COVID-19 pandemic, although it was not statistically significant. But, this increase is seen not only in ICU-1 where COVID-19 patients are followed, but also in ICU-2 where other patients are followed. We can interpret this result in two different ways. First, COVID-19 may increase the frequency of fungal infections, and second, in the last two years, patients may be receiving less treatments to suppress fungal isolation.

In case of suspicion of infection, it is important to first predict which microorganism is the pathogen and to initiate appropriate empirical treatment. *A. baumannii* and *P. aeruginosa* are reported to be frequently isolated agents in ETA samples found in our country.[14,15] In a study from Iran, *A. baumannii* was isolated most frequently in respiratory samples of patients followed in the ICU due to COVID-19, followed by *S. aureus*.[16] In a study conducted in Colombia, *S. aureus* (34%) and *K. pneumoniae* (26%) were detected.[17] In a study conducted in Siirt, Turkey, ETA samples of COVID-19 positive patients hospitalized in the ICU were compared with the pre-pandemic period and it was determined that the three most commonly isolated pathogens were *A. baumannii*, *K. pneumoniae* and *P. aeruginosa* in both periods, respectively. Again in the same place, while the frequency of *A. baumannii* was 28.5% in the pre-pandemic period, it was found that this rate increased to 54% during the pandemic period, and it was reported that the frequency of *K. pneumoniae* and *P. aeruginosa* decreased.[12] Again, in another study conducted in İzmir, Turkey, it was determined that the isolation frequency of *A. baumannii* in respiratory samples increased statistically significantly during the pandemic period compared to the pre-pandemic period.[18] In our study, the most frequently isolated pathogens were found to be *A. baumannii*, *P. aeruginosa*, *K. pneumoniae* and *S. aureus*, respectively. While *P. aeruginosa* was the most frequently isolated pathogen in both ICU-1 and ICU-2 in the pre-COVID-19 pandemic period, it was observed that *P. aeruginosa* was replaced by *A. baumannii* in both clinics with the COVID-19 pandemic. If we examine the isolation frequency of *A. baumannii* as a percentage, it was seen that it increased from 18.3% to 31% in ICU-1 and from 20.4% to 22.8% in ICU-2. But only the increase in ICU-1 was found to be statistically significant. In addition, with the pandemic, an increase in the frequency of *K. pneumoniae* isolation was detected in both clinics. We can interpret these results in two different ways. First of all, the increase in the frequency of multi-drug resistant (MDR) Gram-negative

bacteria such as *A. baumannii* and *K. pneumoniae* with the pandemic, especially the significant increase in *A. baumannii* in ICU-1, may be because of the increased rates of broad-spectrum antibiotic use with COVID-19. Secondly, we think that the reason for the similar changes in both clinics may be due to the transfer of flora between clinics and/or the new flora being a source of infection in patients after the change of the colonized flora in the ICU. In this respect, it should not be forgotten that good and complete implementation of hospital infection control measures is one of the most important steps in breaking this vicious circle.

While carbapenems are used as the first choice in the treatment of Gram-negative bacterial infections, combined treatment options and colistin are often preferred in the treatment of MDR Gram-negative bacterial infections.[19,20] Cayci et al.[21] found carbapenem resistance at a rate of 35-50% in *K. pneumoniae* isolates, 86.6% in *A. baumannii*, and 11.1% in *P. aeruginosa* in patients diagnosed with COVID-19 in a tertiary hospital in 2020. Rao et al.[22] in which they evaluated samples of COVID-19 patients, found that multidrug resistance rates were high in *K. pneumoniae* and *A. baumannii* isolates, while the rate of susceptible isolates was higher in *P. aeruginosa* isolates. In our study, it was found that carbapenem resistance in *A. baumannii* isolates increased in ICU-1 and decreased in ICU-2. It was determined that in *P. aeruginosa*, it reached the level of 50% by showing a statistically significant increase in ICU-1, but in ICU-2 it increased to 30% and this increase was not significant. Statistical increase in resistance to ertapenem was observed in both clinics in *K. pneumoniae*, and the resistance rates detected against imipenem and meropenem were found to be higher in ICU-1 than in ICU-2. In addition, in our study, it was determined that the resistance rates detected in *A. baumannii* and *K. pneumoniae* isolates, whose frequency increased with the pandemic, were higher than *P. aeruginosa*. Therefore, it should be kept in mind that these two isolates may cause MDR and difficult-to-treat infections in patients hospitalized in the ICUs of our hospital.

In a study conducted in Samsun, Turkey, colistin and tigecycline were found to be the most effective antibiotics in *A. baumannii* isolates isolated from ETA samples of patients hospitalized in the ICU between 2019-2020. In this study, it was reported that the COVID-19 pandemic did not change the resistance rates.[23] In different studies, it has been reported that the rates of colistin resistance are 2.1-42.4% in *A. baumannii*, 2.3-9.0% in *P. aeruginosa*, and 20.6-42.9% in *K. pneumoniae*.[24-27] In the study of Bahçe et al.[12] in which they evaluated the ETA samples of patients hospitalized in the ICU between the years 2019-2021, as before and after the pandemic; They found no resistance to meropenem and colistin in *A. baumannii* isolates in both periods, while resistance to meropenem increased from 65% to 71.4% and colistin resistance from 9.5% to 42.9% in *K. pneumoniae*. In addition, they found that the resistance rates in *P. aeruginosa* isolates increased 31.6-50% to meropenem and 5.3-16.7% to colistin before and after the pandemic, respectively, and, lastly, they reported that resistance rates increased with the pandemic and that it could pose a challenge in treatment. In our study, colistin resistance rates showed a statistically significant increase in *A. baumannii* in ICU-1 and decreased in ICU-2. In *P. aeruginosa*, it was increased in both clinics, but only the increase in ICU-2 was significant. In *K. pneumoniae*, it was observed that while it showed a statistically significant increase in ICU-1, it remained at similar levels in ICU-2. Although the colistin resistance rates we determined for all three isolates are consistent with the literature, it can be thought that the COVID-19 pandemic has affected the colistin resistance rates in our hospital in the form of an increase in resistance, as detected in carbapenems.

Ceftazidime-avibactam (CZA) is a new antibiotic combination with good efficacy against carbapenem-resistant Enterobacterales species and *P. aeruginosa* isolates. Although CZA is a newly used drug, unfortunately, resistance has been reported against this antibiotic.[28,29] In our study, CZA suscebtibility test was evaluated only in *K. pneumoniae* isolates, and between the dates 2020-2021, and no resistance was detected during the pandemic in both clinics.

In summary, antibiotics should be used with caution as they trigger the formation of resistance as well as treat bacterial infections. Some guidelines recommend the use of broad-spectrum antibiotics in patients with COVID-19, and studies have reported that antibiotic use rates increase with the pandemic.[6,30] In our study, it was determined that the resistance rates determined for the three most frequently isolated pathogens in ICU-1 increased in almost all antibiotics with the pandemic compared to the pre-pandemic period. On the other hand, in ICU-2, it was determined that some antibiotics decreased instead of increasing, and the resistance rates in ICU-1 were relatively higher than in ICU-2. Although it is not statistically significant in some antibiotics, we think that the increase in resistance observed in ICU-1 with the pandemic was not detected in ICU-2, which may be due to the intensive antibiotic use policy applied in COVID-19. In addition, the decrease in resistance rates may also have been caused by less use of these antibiotics in practice than before.

In our study, ETA samples were preferred because COVID-19 disease primarily affects the respiratory system and both the change in antibiotic resistance over

the years and the effect of COVID-19 on resistance were evaluated. The number of studies conducted in this way is limited in the literature and there is no study containing data for Region Thrace. For this reason, our study is meaningful because it can be representative for our region. The limitation of our study is that it was designed retrospectively.

COCLUSION

In summary, in our study, it was found that culture positivity rates increased significantly in ETA samples of COVID-19 patients with the pandemic. In addition, it was determined that the distribution of isolated pathogen microorganisms changed, and the antibiotic resistance rates were found to be higher in the ICU-1, where COVID-19 positive patients were followed, compared to the ICU-2, where COVID-19 negative patients were followed. It has even been found that the rates of resistance to some antibiotics show a decrease in the COVID-19 negative patient group. Under all these results, it can be said that the COVID-19 pandemic adversely affected the success of treatment by causing a change in the distribution of pathogen microorganisms isolated in ETA samples in our hospital and an increase in the rates of resistance to some antibiotics. For this reason, it can be said that possible pathogen microorganisms and antimicrobial resistance rates should be followed up during similar epidemic periods such as COVID-19.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Tekirdağ Namık Kemal University Non-Interventional Clinical Researches Ethics Committee (Date: 28.06.2022, Decision No: 2022.127.06.17).

Informed consent: Due to the nature of the study, informed consent is not required.

Referee Evaluation Process: Externally peer reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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