ORİJİNAL ARAŞTIRMA ORIGINAL RESEARCH

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Bacteria Isolated from Endotracheal Aspirate Samples and Antibiotic Resistance Rates: 5-year Analysis: 5-year Retrospective Analysis

Endotrakeal Aspirat Örneklerinden İzole Edilen Bakteriler ve Antibiyotik Direnç Oranları: 5 Yıllık Analiz: 5 Yıllık Retrospektif Analiz

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ABSTRACT Objective: Ventilator-associated pneumonia (VAP) is a significant nosocomial infection occurring in patients treated in intensive care units (ICU). This study aimed to determine the antimicrobial resistance rates in microorganisms isolated from endotracheal aspirate (ETA) samples and evaluate the changes in 5 years. **Material and Methods:** ETA specimens sent to our laboratory from the ICU between 2016-2020 were evaluated retrospectively. Bacterial identification and antimicrobial sensitivity tests were made using conventional methods and automated systems. **Results:** A total of 3,943 ETA specimens examined during the study period were evaluated. Significant bacteriological growth was detected in 46.4%, and these were included in the study. Growth of Gram-negative bacteria was observed in 94.5%, and Gram-positive bacteria were found in 5.5%. The most frequently isolated organism was Acinetobacter baumannii, followed by Pseudomonas aeruginosa. A. baumannii was found as the agent having the highest rates of resistance in our study. The resistance rates were generally below 40% in P. aeruginosa strains, and Staphylococcus aureus' resistance rates were below 25%, except for penicillin and methicillin. Resistance against many antimicrobials was observed in Escherichia coli, and Klebsiella pneumoniae strains with a rate above 50%. In addition, resistance rates have been found to vary over the years. **Conclusion:** VAP is a frequently encountered infection in ICU, generally caused by resistant microorganisms. Treatment with antibiotics that are effective against causative agents as soon as possible is essential in decreasing mortality. Thus, antimicrobial resistance patterns should be followed up regularly, and treatment protocols should be updated according to this.

Keywords: Intensive care unit; pneumonia; antibiotic resistance

ÖZET Amaç: Yoğun bakım ünitelerinde (YBÜ) tedavi edilen hastalarda, ventilatörle ilişkili pnömoni [ventilator-associated pneumonia (VAP)] önemli bir hastane enfeksiyonudur. Bu çalışmanın amacı, YBÜ'de yatan hastaların endotrakeal aspirat (ETA) örneklerinden izole edilen mikroorganizmaların, antimikrobiyal direnç oranlarını belirlemek ve 5 yıl içindeki değişimini değerlendirmektir. **Gereç ve Yöntemler:** 2016 ve 2020 yılları arasında YBÜ'den laboratuvarımıza gönderilen ETA örnekleri retrospektif olarak incelenmiştir. Bakteri tanımlaması ve antibiyotik duyarlılık testleri, konvansiyonel yöntemler ve otomatize sistemler kullanılarak yapılmıştır. **Bulgular:** Çalışma süresince 3.943 ETA örneği değerlendirilmiş, %46,4'ünde bakteriyolojik açıdan anlamlı üreme tespit edilmiş ve çalışmaya dâhil edilmiştir. Bunların %94,5'inde Gram-negatif bakteri; %5,5'inde ise Gram-pozitif bakteri ürediği saptanmıştır. En sık Acinetobacter baumannii, 2. sırada Pseudomonas aeruginosa izole edilmiştir. Çalışmamızda, A. baumannii en yüksek direnç oranlarına sahip etken olarak saptanmıştır. P. aeruginosa suşlarında direnç oranlarının genel olarak %40'ın, Staphylococcus aureus'da penisilin ve metisilin hariç %25'in altında olduğu belirlenmiştir. Escherichia coli ve Klebsiella pneumoniae suşlarında ise birçok antimikrobiyale %50'nin üzerinde direnç gözlenmiştir. Ayrıca direnç oranlarının yıllar içinde değişkenlik gösterdiği görülmüştür. **Sonuç:** VAP, YBÜ'de sık karşılaşılan, genellikle de dirençli mikroorganizmaların etken olduğu bir enfeksiyondur. Etkene uygun antibiyotiğin bir an önce başlanması mortalitenin azaltılması açısından oldukça önemlidir. Bu nedenle, antimikrobiyal direnç paternleri düzenli olarak izlenmeli ve buna göre tedavi protokolleri güncellenmelidir.

Anahtar Kelimeler: Yoğun bakım ünitesi; pnömoni; antibiyotik direnci

Frequent use of broad-spectrum antimicrobials, prolonged hospitalization durations, need for ventilation assistance, and regular use of invasive procedures cause a significant increase in the predisposition of patients hospitalized at intensive care units (ICU) against infections. Ventilator-associated pneu-

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monia (VAP) in patients hospitalized in ICUs is a severe hospital infection that increases morbidity and mortality.[1-5]

A delay in detecting the causative agent in VAP and not initiating an appropriate antibiotic regimen as soon as possible is associated with a worse prognosis. Detection of the causative agent at the microbiology laboratory and the rapid determination of antibiotic sensitivity provide guidance to clinicians.[1,2,4] Bronchoalveolar lavage and brush specimens have high sensitivity and specificity, but they are invasive and relatively hard-to-perform procedures. Endotracheal aspirate (ETA) is relatively non-invasive and easy to perform.[1,2,6] Non-invasive and semi-quantitative procedures are reported to be more frequently preferred than those that are invasive and quantitative.[7] There are differences of opinion in the frequency of obtaining ETA samples and evaluating their results. Also, it is known that differentiation between colonization and infection in ETA samples is not always possible.[3,8,9]

The most frequently isolated microorganisms in VAP are Gram-negative bacteria, including *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*. At the same time, *Staphylococcus aureus* is the most frequently detected Gram-positive bacteria.[2,8,10] Widespread use of broad-spectrum antibiotics in empirical treatment causes dominance of isolates with multi-drug resistance, widespread resistance or pan-resistance, and cause considerable problems in management. The presence of multiple causative agents in the etiology further complicates the therapeutic approach and requires up-to-date data to determine appropriate antimicrobials.[2,5,8] Antimicrobial resistance rates are known to differ between different centers. Analysis of local antimicrobial resistance rates at each center is very important for selecting the most appropriate treatment.[5]

We aimed to analyze microorganisms isolated from ETA samples sent to our laboratory of adult patients hospitalized at the ICU retrospectively to determine the antimicrobial resistance distribution, evaluate the changes in resistance rates in five years, and provide guidance for the selection of antimicrobials that may be used in empirical treatment.

MATERIAL AND METHODS

After obtaining approval from the Balıkesir University Faculty of Medicine Clinical Research Ethics Committee (approval date: 21 October 2020; approval number: 2020/189), microorganisms isolated from ETA culture samples were sent to the microbiology laboratory between 2016 and 2020 of adult patients hospitalized at the ICU were analyzed retrospectively. This study was performed according to the Declaration of Helsinki. According to guidelines, microorganisms that were considered causative agents on ETA samples and simultaneous microscopic examinations of stained specimens and their respective antimicrobial sensitivity evaluations were included in this study.[11] First, microscopic examination was performed with the Bartlet scoring method and it was evaluated whether the sample was a quality sample reflecting the lower respiratory tract. The culture of the sample thought to reflect the lower respiratory tract was examined. In the case of one or two bacteria grown purely in culture, these microorganisms were considered as agents and the antibiogram was studied. In cases of recurrent growth in the same patient, only the first isolate was included in the study.

The samples were incubated in agar with 5% sheep blood and eosin methylene blue agar for 18-24 hours at 37 °C and 5-10% CO_2. Isolates that showed pure growth at culture were identified with conventional methods (colony morphology, Gram staining, oxidase, catalase, and coagulase test) and BD Phoenix 100 automated identification system (BD Phoenix System, Beckton Dickinson, US). The antimicrobial sensitivity of isolates was determined with Phoenix TM 100 automated identification system (BD Phoenix System, Beckton Dickinson, US), according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria (based on the guide of the year the bacteria were isolated).[12] Colistin and tigecycline were not included in the study, as they could not be evaluated according to EUCAST criteria. Confirmatory tests could not be done for methicillin resistance in *Staphylococcus* spp. and the presence of extended-spectrum beta-lactamase (ESBL) in Enterobacterales species, and prob-

able rates were reported according to the results obtained via the automated identification system, which are limitations of the present study.

STATICAL ANALYSIS

The statical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 22.0 (SSPS INC, Chicago, IL, USA). Categorical variables were given as a percentage. Chi-square test was used to compare the antimicrobial resistance rates for each antibiotic among the five years (2016 to 2020). The p-value <0.05 was considered statistically significant.

RESULTS

A total of 3,943 ETA samples were evaluated. All of the ETA samples were obtained from adult patients hospitalized at the ICU. Bacteriologically significant growth was detected in 46.4% of these samples and included in the study. Out of these samples, 57.7% were observed from male patients, and 42.3% were observed from female patients. Growth of Gram-negative bacteria was detected in 94.5% of ETA samples included in this study, and Gram positives were detected in 5.5%. *A. baumannii* was the most frequently isolated organism, followed by *P. aeruginosa* (Table 1).

Among microorganisms that were tested for antibiotic susceptibility, *A. baumannii* was found to possess the highest rates of resistance, there also resistance rates showed variability between years. A statistically significant increase in resistance to amikacin, gentamicin and trimethoprim-sulfamethoxazole (TMP-SXT) was detected over the years (p<0.001). The resistance rates in *P. aeruginosa* strains were generally found to be below 40%, and they also remained at this level during the changing years. Between 2016 and 2020, there was a statistically significant increase in resistance rates to amikacin, gentamicin, carbapenems, piperacillin-tazobactam (TZP) and ciprofloxacin (p 0.016, p<0.001, p<0.001, p 0.002, p<0.001) (Table 2). In *E. coli* and *K. pneumoniae* strains, rates of resistance against amoxicillin-clavulanic acid (AMC), ceftriaxone, ciprof-loxacin, TMP-SXT were found to be

TABLE 1: Bacteria isolated from endotracheal aspirate samples and their frequency (%).

Microorganism	Frequency of detection (%)
Acinetobacter baumannii	29
Pseudomonas aeruginosa	24.8
Klebsiella pneumoniae	18.4
Escherichia coli	11.9
Other Enterobacterales members	7.3
Staphylococcus aureus	4.5
Other nonfermenter Gram-negative bacteria	3.1
Other Gram-positive bacteria	1
Total	100

TABLE 2: Resistance rates of *Acinetobacter baumannii and Pseudomonas aeruginosa* srains isolated from endotracheal aspirate samples according to years (%).

	Acinetobacter baumannii							*Pseudomonas aeruginosa*						
Antibiotic	**2016**	**2017**	**2018**	**2019**	**2020**	***Total**	**p value**	**2016**	**2017**	**2018**	**2019**	**2020**	***Total**	**p value**
AK	67.3	44	86.5	93.4	83.2	**74.7**	**<0.001**	6.5	14.9	3.2	4.9	6.7	**7.5**	**0.016**
G	89.4	99.1	89.4	79.2	89.7	**89.4**	**<0.001**	5.4	22.8	30.1	69.1	25.6	**29.6**	**<0.001**
CARB	96.2	100	96.2	97.2	97.2	**97.4**	**0.410**	16.3	38.6	47.3	30.9	29.1	**32.7**	**<0.001**
CAZ	-	-	-	-	-	-		29.3	27.7	22.6	25.9	34.9	**28**	**0.395**
FEP	-	-	-	-	-	-		27.2	27.7	22.6	25.9	32.6	**27.2**	**0.605**
TZP	-	-	-	-	-	-		29.3	25.7	9.7	24.7	31.4	**24.1**	**0.002**
TMP-SXT	67.3	65.1	69.2	89.6	82.2	**74.7**	**<0.001**	-	-	-	-	-	-	
CIP	98.1	92.7	95.2	95.3	98.1	**95.8**	**0.200**	26.1	50.7	46.2	61.6	43	**36.9**	**<0.001**

*Total: Mean resistance between 2016-2020; AK: Amikacin; G: Gentamicin; CARB: Carbapenems; CAZ: Ceftazidime; FEP: Cefepime; TZP: Piperacillin-tazobactam; TMP-SXT: Trimethoprim-sulfamethoxazole; CIP: Ciprofloxacin.

above 50%, but carbapenems and aminoglycosides were lower. Also, these rates showed a fluctuation over the years. In *E. coli* strains, a statistically significant increase in resistance rates against amikacin, gentamicin, carbapenems, ceftriaxone, TZP, TMP-SXT and ciprofloxacin over the years (p 0.004, p 0.015, p 0.026, p 0.001, p 0.009, p 0.022, p<0.001). In *K. pneumoniae*, there is a statistically significant increase between years in resistance to amikacin, gentamicin, carbapenems, ceftriaxone, AMC and TZP, and ESBL positivity (p 0.001, p 0.006, p 0.019, p 0.001, p 0.007, p 0.025, p<0.001) (Table 3). Rates of resistance in *S. aureus* strains were below 25%, except penicillin and methicillin. In addition, there was a statistically significant increase in the resistance rates against penicillin, gentamicin, clindamycin, erythromycin, TMP-SXT, daptomycin and methicillin over the years (p<0.001, p<0.001, p<0.001, p 0.001, p 0.018, p<0.001) (Table 4).

TABLE 3: Resistance rates of *Escherichia coli and Klebsiella pneumoniae* strains isolated from endotracheal aspirate samples according to years (%).

	Acinetobacter baumannii							*Pseudomonas aeruginosa*						
Antibiotic	**2016**	**2017**	**2018**	**2019**	**2020**	***Total**	**p value**	**2016**	**2017**	**2018**	**2019**	**2020**	***Total**	**p value**
AK	1.7	3	11.6	4	2.1	3.7	**0.004**	**9.8**	29.3	13.5	12.3	12	**15.8**	**0.001**
G	35	24.2	34.6	44	25	33.2	**0.015**	**39.3**	44	32.7	24.7	24	**32.7**	**0.006**
CARB	0	0	3.8	0	4.2	1.4	**0.026**	**16.4**	30.7	17.3	31.5	20	**23.8**	**0.019**
CRO	63.3	45.5	61.5	74	66.7	63.6	**0.001**	**47.5**	53.3	61.5	71.2	70.7	**61.3**	**0.001**
AMC	68.3	72.7	73.1	80	81.3	70.5	**0.164**	**54.1**	69.3	59.6	68.5	66.7	**61.3**	**0.007**
TZP	38.3	27.3	26.9	16	33.3	29	**0.009**	**45.9**	61.3	46.2	56.2	41.3	**50.6**	**0.025**
TMP-SXT	53.3	57.6	46.2	68	50	55.8	**0.022**	**44.3**	46.7	50	57.3	49.3	49.7	0.412
CIP	51.7	39.4	46.2	74	66.7	57.6	**<0.001**	**45.9**	50.7	46.2	61.6	53.3	**52.1**	**0.156**
ESBL	63.3	45.5	61.5	58	62.5	59	**0.062**	**31.1**	53.3	63.5	50.7	50.7	**49.7**	**<0.001**

*Total: Mean resistance between 2016-2020; AK: Amikacin; G: Gentamicin; CARB: Carbapenems; CRO: Ceftriaxone; AMC: Amoxicillin-clavulanic acid; TZP: Piperacillin-tazobactam; TMP-SXT: Trimethoprim-sulfamethoxazole; CIP: Ciprofloxacin; ESBL: Extended-spectrum beta-lactamase.

TABLE 4: Resistance rates of Staphylococcus aureus strains isolated from endotracheal aspirate samples according to years (%).

	Staphylococcus aureus						
Antibiotic	**2016**	**2017**	**2018**	**2019**	**2020**	***Total**	**p value**
G	20	33.3	0	18.8	14.3	17.3	<0.001
PEN	100	83.3	81.3	93.4	90.5	88.9	<0.001
CC	10	11.1	6.3	25	33.3	18.5	<0.001
E	10	16.7	18.8	25	33.3	22.2	0.001
TE	10	22.2	18.8	25	23.8	21	0.062
CIP	10	16.7	12.5	18.8	9.5	13.6	0.257
TMP-SXT	0	0	6.3	6.3	4.8	3.7	0.018
VA	0	0	0	0	0	0	-
TEI	0	0	0	0	0	0	-
DAP	0	0	0	6.3	0	1.2	<0.001
LIN	0	0	0	0	0	0	-
MET	20	38.9	31.3	37.5	52.3	38.3	<0.001

*Total: Mean resistance between 2016-2020; G: Gentamicin; PEN: Penicillin; CC: Clindamycin; E: Erythromycin; TE: Tetracycline; CIP: Ciprofloxacin; TMP-SXT: Trimethoprim-sulfamethoxazole; VA: Vancomycin; TEI: Teicoplanin; DAP: Daptomycin; LIN: Linezolid; MET: Methicillin.

DISCUSSION

Longer hospitalization durations, broad-spectrum antibiotics, and invasive procedures set the stage for developing many infections. Lower airways infections such as VAP are frequently seen and increase morbidity and mortality. ETA culture is an essential diagnostic method in the evaluation of intubated patients.[13,14] Gram-negative bacteria and *S. aureus* are among frequently isolated agents.[8] Many studies have reported that the most commonly isolated microorganisms in ETA cultures are *A. baumannii* and *P. aeruginosa*.[9,14-16] Gram-negative bacteria have grown in most of the samples, *A. baumannii* and *P. aeruginosa* were also among the most frequent agents in the present study. The frequency of detection of Gram-positive bacteria was found not to exceed 6%. The most frequently detected microorganism among Gram-positive bacteria was *S. aureus*.

Correct determination of the causative agent of infection and rapid initiation of an appropriate antibiotic for this agent is very important to decrease the mortality.[17] *A. baumannii* strains seem to be more resistant to all antimicrobials among Gram-negative bacteria.[18-20] We also found *A. baumannii* strains to be the agent with the highest degree of resistance in our study. Except for amikacin and TMP-SXT, the rate of resistance to all other antibiotics that were used in the sensitivity test was above 90%. Resistance to TMP-SXT was 74.7%. Colistin and tigecycline were not included in the study, as they could not be evaluated according to EUCAST criteria, which is a limitation of our research. Rates of resistance in *P. aeruginosa* strains isolated in our study were generally lower than 40%, and various antibiotic resistance rates are reported in the medical literature for *P. aeruginosa* strains.[21-23] The resistance was found to be 24.1% to TZP, 32.7% to carbapenems and 28% to ceftazidime, which are important in antipseudomonal therapy.

Widespread use of broad-spectrum antibiotics has caused occurrence of resistant strains among *E. coli* and *K. pneumoniae* species.[20,24,25] It is apparent in the present study that antibiotic resistance in *E. coli* and *K. pneumoniae* isolates has reached considerable levels. While similar levels of resistance rates to many antibiotics were observed in *E. coli* and *K. pneumoniae*, resistance rates to carbapenems and TZP were found to be much higher in *K. pneumoniae*. When we look at the resistance rates against AMC, ceftriaxone, TMP-SXT and ciprofloxacin, which are frequently used in the treatment of infections caused by Enterobacterales; the resistance rates in *E. coli* and *K. pneumoniae* was found 70.5% and 61.3% to AMC, 63.6% and 61.3% to ceftriaxone, 55.8% and 49.7% to TMP-SXT, 57.6% and 52.1% to ciprofloxacin, respectively. *E. coli* strains showed resistance to carbapenems and TZP at a rate of 1.4% and 29%, while resistance was found to be 23.8% and 50.6% in *K. pneumoniae*. We believe that frequent use of carbapenems and TZP in *K. pneumoniae* infections recently have increased resistance.

Sağmak-Tartar et al. have detected significant growth in 42% of samples in their one-year study evaluating ETA cultures.[23] This rate is similar to our findings. 93.2% of samples that growth was detected were Gram-negative bacteria, and nearly half (49.5%) of them were *A. baumannii*. *P. aeruginosa* was isolated in 20.5% of the samples, *Klebsiella* spp. was isolated in 16.3%, *E. coli* in 2.4%, and *S. aureus* in 2.1%. Resistance rates in *A. baumannii* against TMP-SXT was 98.4%. In comparison, it was 97.7% against imipenem and ciprofloxacin and 89.2% against amikacin. Resistance rates in *P. aeruginosa* were 89.8% against amikacin, 70.9% against imipenem, 70.1% to TZP, and 63.8% against ceftazidime and ciprofloxacin. In *Klebsiella* spp. and *E. coli*, resistance against TMP-SXT was 90.1% and 12%, respectively, and 78.2% and 60% against ciprofloxacin, 82.2% and 20% against TZP, 58.4% and 20% against imipenem, and 87.1% and 60% against amikacin.[23]

In the study by Koçak et al. in Adana between 2016-2018, where respiratory specimens were evaluated, 80.8% of isolated pathogens were found to be Gram-negative bacteriae.[26] Agents that were most frequently detected were *A. baumannii*, *Klebsiella* spp., *P. aeruginosa* and *E. coli*, respectively. *S. aureus* was also among the common isolates, with a rate of 5.5%. They found resistance to imipenem, meropenem, ciprofloxacin, amikacin and gentamicin above 84% in *A. baumanii*, and below 27% in *P. aeruginosa*.

Also, they found TZP resistance of 25.6%, ceftazidime resistance of 19.2%, cefepime resistance of 25.3% in *P. aeruginosa*. Resistance to TMP-SXT, AMC and ceftriaxone were above 60.9% in *K. pneumoniae* and below 53.2% in *E. coli*. A ciprofloxacin resistance of 51.2% and 57.4%, TZP resistance of 55.8% and 14.8%, imipenem resistance of 42.4% and 1.7%, amikacin resistance of 30.6% and 1.6%, gentamicin resistance of 52.9% and 18% in *K. pneumoniae* and *E. coli*, respectively. They reported antibiotic resistance below 28.6% except penicillin in the isolated *S. aureus* strains. When we compare this study with our study, isolated agents are similar. On the other hand, there are considerable differences between the antibiotic resistance rates found in these studies that investigated similar samples in the same period. Resistance rates found by Koçak et al. are similar to ours, while those found by Sağmak-Tartar et al. are different.[23,26] Thus, each center needs to determine the microorganisms and their resistance profiles and create an empirical treatment protocol according to this data.

In our study, the change in the antibiotic resistance rates for five bacteria over the years was examined and it was evaluated whether there was a statistically significant increase in resistance rates. Carbapenem resistance in *A. baumannii* was observed over 96% in all years, but no statistically significant increase in resistance was found between years (p 0.410). Unlike *A. baumannii*, a statistically significant increase in resistance to carbapenems has been detected in *E. coli*, *K. pneumoniae* and *P. aeruginosa* strains over the years (p 0.026, p 0.019, p<0.001). Similarly, a statistically significant increase in resistance to TZP was detected in *P. aeruginosa*, *E. coli* and *K. pneumoniae* strains between years (p 0.002, p 0.009, p 0.025). ESBL, a vital resistance mechanism for Enterobacterales isolates, was found to be 59% in *E. coli* and 49.7% in *K. pneumoniae*.[20] While there was a statistically significant increase in ESBL positivity in *K. pneumoniae* strains over the years, no difference was found in *E. coli* strains (p<0.001; p 0.062). In addition, there was a statistically significant difference between years in resistance to TMP-SXT and ciprofloxacin in *E. coli* strains, but no difference was found in *K. pneumoniae* (p 0.022, p<0.001; p 0.412, p 0.156). Methicillin resistance, which is an essential problem of *S. aureus* strains, was detected at a rate of 40%.[27] Also, there was a statistically significant increase in resistance rate over the years (p<0.001). The resistance rates detected have shown that the resistance problem observed in bacteria growing in ETA samples in our hospital is not a new phenomenon. In the absence of more meticulous restriction programs, we feel that serious problems will occur in the future in empirical treatment options.

In the study by Caskurlu et al., where ETA cultures of patients hospitalized at the ICU were investigated, changes in resistance rates according to years were examined.[28] They also have found that resistance rates in some agents have increased in time, while rates of others have shown a fluctuating course, with increases and decreases in time. Long-term hospitalization of patients in the ICU, where a local flora creates a predisposition for infection due to this flora. We believe that the fluctuating antibiotic resistance course is due to the increase and decrease of infections due to ICU flora. We also believe that good hygiene and infection control measures are essential and limit antibiotic utilization in preventing antibiotic resistance. Decreasing colonization of patients with hospital microorganisms will also avoid unnecessary use of antibiotics.

CONCLUSION

ICUs are hospital departments where many infections such as VAP are seen, and frequently the causative agents are resistant microorganisms. In our study, it was found that resistance to ceftriaxone, ciprofloxacin and TMP-SXT, which are frequently used in the treatment of Gram-negative bacterial infections, changed over the years and showed a statistically significant increase. In addition, a statistically significant increase was observed in the rates of resistance to carbapenems and TZP, which are important drugs in the treatment of patients hospitalized in the ICU. Similarly, methicillin resistance was found to increase statistically significantly in *S. aureus* strains over the years. Considering all these results, antimicrobial resistance patterns of agents should be regularly examined, and treatment

protocols should be updated according to this data. Also, effective hygiene and infection control measures should be regularly employed in order to decrease nosocomial infections.

ETA samples that we evaluated in our study were taken from patients with fever or findings suggestive of lower respiratory tract infection. It was not evaluated in the study whether treatment was given to the patients who have culture antibiogram results. This is a limitation of our study.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

All authors contributed equally while this study preparing.

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