ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Evaluation of Bacteria Isolated from Endotracheal Aspirates of Patients in Intensive Care Units: A Single-Centre Retrospective Study

Yoğun Bakım Ünitelerindeki Hastaların Endotrakeal Aspiratlarından İzole Edilen Bakterilerin Değerlendirilmesi: Tek Merkezli Retrospektif Bir Çalışma

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ABSTRACT

Aim: *This study aims to analyze the bacteriological profile and antibiotic susceptibility patterns of isolates from endotracheal aspirates (ETAs) of intensive care units (ICUs) patients to provide data for combating ventilator-associated pneumonia (VAP) and other nosocomial infections.*

Material and Methods: *A retrospective study of ETA (Endotracheal Aspirate) samples from ICU patients (January-December 2022) was conducted. Quantitative cultures were performed on the ETA samples, and the results were expressed as CFU/mL (colony-forming units per milliliter). Bacteria were identified and tested for antibiotic susceptibility according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria.*

Results: *Among 263 isolates, predominant gram-negative bacteria were Acinetobacter baumannii, Pseudomonas aeruginosa, and Klebsiella spp., gram-positive bacteria included Staphylococcus epidermidis and Staphylococcus aureus. High resistance was noted in Acinetobacter baumannii to ciprofloxacin (96.4%) and piperacillin/tazobactam (97.3%). Staphylococcus epidermidis showed resistance to oxacillin (66.6%) but none to vancomycin or linezolid.*

Conclusions: *The prevalence of multidrug-resistant pathogens in ETA cultures emphasizes the importance of improved surveillance, antimicrobial stewardship and infection control in ICUs.*

Key words: ventilator-associated *pneumonia; endotracheal aspirates; antibiotic resistance*

ÖZET

Amaç: *Bu çalışmada, ventilatör ile ilişkili pnömoni (VİP) ve diğer hastane enfeksiyonlarıyla mücadele için veri sağlamak amacıyla yoğun bakım ünitelerindeki (YBÜ) hastaların endotrakeal aspiratlarından (ETA) izole edilen suşların bakteriyolojik profili ve antibiyotik duyarlılık paternleri analiz edilmiştir.*

Gereç ve Yöntem: *YBÜ hastalarından alınan ETA örnekleri retrospektif (Ocak-Aralık 2022) olarak değerlendirilmiştir. Endotrakeal aspirat örnekleri üzerinde kantitatif kültürler yapılmış ve sonuçlar CFU/mL (mililitre başına koloni oluşturan birim) olarak ifade edilmiştir. Tanımlanmış olan bakteriler EUCAST (Avrupa Antimikrobiyal Duyarlılık Testi Komitesi) kriterlerine göre antibiyotik duyarlılığı açısından test edilmiştir.*

Bulgular: *263 izolat arasında, en sık saptanan gram-negatif bakteriler Acinetobacter baumannii, Pseudomonas aeruginosa ve Klebsiella spp. iken, gram-pozitif bakteriler Staphylococcus epidermidis ve Staphylococcus aureus idi. Acinetobacter baumannii'de siprofloksasin (%96,4) ve piperasilin-tazobaktama (%97,3) karşı yüksek direnç kaydedilmiştir. Staphylococcus epidermidis suşlarında oksasiline (%66,6) direnç tespit edilmişken vankomisin veya linezolide direnç görülmemiştir.*

Sonuç: *ETA kültürlerinde çoklu ilaca dirençli patojenlerin yüksek oranda tespit edilmesi, YBÜ'lerde gelişmiş sürveyans, antimikrobiyal yönetim ve enfeksiyon kontrolünün önemini vurgulamaktadır.*

Anahtar kelimeler: *ventilatör ilişkili pnömoni; endotrakeal aspirat; antibiyotik direnci*

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Introduction

Ventilator-associated pneumonia (VAP) is a significant hospital-acquired infection, particularly prevalent in intensive care units (ICUs) where patients are dependent on mechanical ventilation. Diagnosing VAP and determining appropriate antibiotic therapy depends on accurately identifying bacterial pathogens and their antibiotic susceptibility patterns from endotracheal aspirate (ETA) cultures. The significance of ETA cultures lies in their ability to provide crucial microbiological data that guide targeted treatment, thereby improving patient outcomes and reducing the incidence of multidrug-resistant infections.

Several studies have highlighted the prevalence and variety of bacterial pathogens isolated from ETAs. Gram-negative bacteria, including *Acinetobacter baumannii* (*A.baumannii*), *Klebsiella pneumoniae* (*K.pneumoniae*), and *Pseudomonas aeruginosa (P.aeruginosa*), are frequently identified as predominant pathogens, often exhibiting multidrug resistance (MDR). Gram-positive bacteria, such as *Staphylococcus aureus* (*S.aureus*), including methicillin-resistant strains (MRSA), are also commonly isolated[1–3].

The management of VAP involves empiric antibiotic therapy, typically starting with broad-spectrum antibiotics and later de-escalating based on culture results. Rapid identification of pathogens and their resistance profiles can significantly reduce the duration of inappropriate antibiotic use, thereby mitigating the development of resistance and improving clinical outcomes[4].

This study aims to comprehensively analyze the bacteriological profile and antibiotic susceptibility patterns of isolates from ETAs in ICU patients at our center, contributing valuable data to the ongoing efforts to combat VAP and other nosocomial infections in critically ill patients.

Material and Methods

The study was approved by the Ethics Committee of Health Sciences University Bursa Yüksek İhtisas Training and Research Hospital (Approval No: 2011-KAEK-25, Date: 2023/06/01). This retrospective study investigated the bacteria isolated from endotracheal aspirate samples of patients admitted to our hospital's Medical and Surgical ICUs between January 2022 and December 2022 and their antibiotic susceptibilities.

Inclusion criteria were hospitalization in the ICU for ≥48 hours and growth of ≥100.000 cfu/ml in endotracheal aspirate cultures. Exclusion criteria were incomplete medical records, polymicrobial growth or contamination in cultures, or age <18 years. Data on patient demographics, microbiological results, and antibiotic susceptibilities were retrieved retrospectively from the hospital's electronic database.

The hospital's microbiology laboratory routinely processed endotracheal aspirate samples. Clinical specimens were inoculated onto 5% sheep blood agar and eosin methylene blue (EMB) agar. Plates were incubated at 37°C for 18–24 hours. Pure cultures with ≥100.000 cfu/ml were identified using conventional biochemical methods. Antibiotic susceptibility testing was performed via disc diffusion and gradient tests (Etest, bioMérieux, France) following European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines.

Empirical therapy for gram-negative bacteria included broad-spectrum antibiotics such as piperacillin/tazobactam, meropenem, and ciprofloxacin. For gram-positive bacteria, vancomycin, teicoplanin, and linezolid were preferred.

Data were analyzed using IBM Statistical Package for Social Sciences (SPSS) for Windows program version 28.0. Descriptive statistics (numbers, percentages, means, and standard deviations) were used to summarize the distribution of isolated microorganisms, antibiotic resistance patterns, and patient demographics.

Results

Two hundred and fifteen patients met the inclusion criteria and were enrolled in the study. The cohort included 136 males (63.3%) and 79 females (36.7%), with a mean age of 65.2±12.8 years (range: 18–92). Overall, 263 microorganisms were isolated. Among these, *A.baumannii* was the most frequently isolated microorganism, accounting for 43.0%. *P.aeruginosa*, *Klebsiella spp*., and *Escherichia coli* (*E.coli*) followed this. *Stenotrophomonas maltophilia* (*S.maltophilia)* and *Enterobacter spp*. were less common, representing 0.8% and 1.9%, respectively (Table 1).

Among gram-positive bacteria, *Staphylococcus epidermidis* (*S.epidermidis*) and *S.aureus* were the primary isolates (Table 2). Eleven (4.2%) *Candida spp*. were detected among the isolated microorganisms.

A.baumannii (n=113) exhibited high resistance rates to multiple antibiotics, with the highest resistance observed against ciprofloxacin (96.4%) and

Table 1. *Resistance rates of gram-negative microorganisms to various antibiotics*

Microorganism (n)	Co-trimoxazole n (%)	Ceftazidime-Avibactam n (%)	Ciprofloxacin n (%)	Cefoperazone/ sulbactam n (%)	Piperacillin/ tazobactam n (%)	Meropenem n (%)	Amikacin n (%)
A.baumannii (n=113)	34 (30.0)	-	100 (96.4)	-	-	108 (95.5)	20 (17.6)
P.aeruginosa (n=34)	32 (94.1)	1 (2.9)	9 (26.4)	11 (32.3)	11 (32.3)	5 (14.7)	4 (11.7)
Klebsiella spp. (n=33)	29 (87.8)	2 (6.0)	28 (84.8)	29 (87.8)	29 (87.8)	11 (33.3)	7 (21.2)
E.coli (n=46)	11 (23.9)	0 (0)	20 (43.4)	11 (23.9)	10 (21.7)	11 (23.9)	7 (15.2)
S.maltophilia (n=2)	0 (0)	-	-	-	-	-	-
Enterobacter spp. (n=5)	0 (0)	0 (0)	2 (40.0)	1 (20.0)	2 (40.0)	1 (20.0)	1 (20.0)

Table 2. *Resistance rates of gram-positive microorganisms to various antibiotics*

Microorganism (n)	Oxacillin n (%)	Vancomycin n (%)	Teicoplanin n (%)	Linezolid n (%)	Ciprofloxacin n (%)
S.epidermidis (n=12)	8 (66.7)	0 (0)	0 (0)	0 (0)	5 (41.7)
S.aureus (n=7)	5 (71.4)	0 (0)	0 (0)	0 (0)	6 (85.7)

Table 3. *Antibiotic regimens administered to the microorganisms grown in endotracheal aspirate cultures that were considered to be VAP agents*

Treatment	*A.baumannii* (n=86)	*P.aeruginosa* (n=34)	*Klebsiella spp.* (n=28)	*E.coli* (n=41)	*S.maltophilia* (n=2)	*Enterobacter spp.* (n=5)	*S.epidermidis* (n=3)	*S.aureus* (n=6)
Monotherapy, n (%)	5 (5.8)	31 (91.2)	24 (85.7)	33 (80.5)	2 (100)	3 (60.0)	3 (100)	6 (100)
Meropenem, n (%)	-	22 (64.7)	13 (46.4)	23 (56.1)	-	3 (60.0)	-	-
Colistin, n (%)	5 (5.8)	-	-	-	-	-	-	-
Tigecycline, n (%)	-	-	4 (14.3)	-	-	-	-	-
Vancomycin, n (%)	-	-	-	-	-	-	1 (33.3)	4 (66.7)
Linezolid, n (%)	-						2 (66.7)	2 (33.3)
Co-trimoxazole, n (%)	-	-	-	-	2 (100)	-	-	-
Piperacillin/tazobactam, n (%)	-	9 (26.5)	2 (7.1)	9 (22.0)	-	-	-	-
Ceftazidime/avibactam, n (%)	-	-	5 (17.9)	1 (2.4)	-	-	-	-
Combination therapy, n (%)	81 (94.2)	3 (8.8)	4 (14.3)	8 (19.5)	0 (0)	2 (40.0)	0 (0)	0 (0)
Meropenem + Colistin, n (%)	4 (4.6)	1 (2.9)	4 (14.3)	-	-	2 (40.0)	-	-
Meropenem + Tigesiklin, n (%)	11 (12.7)	-	-	3 (7.3)	-	-	-	-
Sefaperazon/sulbaktam + Colistin, n (%)	49 (56.9)	-	-	-	-	-	-	-
Meropenem + Amikasin, n (%)	17 (19.7)	-	-	-	-	-	-	-
Piperacillin/tazobactam + Amikacin, n (%)	-	2 (5.9)	-	5 (12.2)	-	-	-	-

meropenem (95.5%). *P.aeruginosa* (n=34) showed notable resistance to cotrimoxazole (94.1%) and ciprofloxacin (26.4%), while resistance to colistin was 11.7%. *Klebsiella spp.* (n=33) had high resistance rates to multiple antibiotics, including ciprofloxacin (84.8%), cotrimoxazole (87.8%), and piperacillin/tazobactam (87.8%). *E.coli* (n=46) exhibited the highest resistance to ciprofloxacin (43.4%) and relatively lower resistance to other antibiotics. *S.maltophilia* (n=2) isolates were susceptible to co-timoxazole. *Enterobacter spp*. (n=5) isolates showed the highest resistance rates to ciprofloxacin and piperacillin/tazobactam (Table 3).

Among gram-positive bacteria, *S.epidermidis* (n=12) demonstrated a high resistance rate to oxacillin (66.6%), and ciprofloxacin (41.6%), but no resistance was observed for vancomycin,

teicoplanin, and linezolid. *S.aureus* (n=7) isolates showed a high resistance rate to oxacillin (71.4%) and ciprofloxacin (85.7%), while no resistance was detected for vancomycin, teicoplanin, and linezolid (Table 2).

Discussion

Ventilator-associated pneumonia is a severe infectious disease affected by various factors, such as the patient population in intensive care units, the length of hospital stay, and previous antimicrobial treatment[5]. It is commonly caused by microorganisms such as *P.aeruginosa*, *S.aureus*, *A.baumannii*, and *Klebsiella pneumoniae (K.pneumoniae)* in intensive care units[6,7].

In our study, gram-negative bacteria were dominant among the strains isolated from ETA samples of ICU patients. These pathogens are frequently associated with healthcare-associated infections and are usually multidrug resistant, which makes treatment difficult[4,8]. Among the gram-negative bacteria identified in our study, the high resistance rates observed in *A.baumannii*, *Klebsiella spp*., and *P.aeruginosa* are consistent with reports from other regions and underline the global challenge posed by these MDR organisms. In a study investigating the microorganisms causing VAP in Türkiye, gram-negative organisms were found in 76.5% of cases[9]. In another study conducted in Türkiye, *A.baumannii* and *P.aeruginosa* were found in 49.5% and 20.5% of ETA samples, respectively[10]. In another study conducted in India, Pseudomonas Spp. (18%), Escherichia coli (25%) and Klebsiella pneumonia (36%) were identified as the common pathogens causing VAP[11]. In our study, gram-negative microorganisms were found in 88.6% of the cases, and the most frequently grown agent was *A.baumannii*, which had a remarkable rate.

Our study identified *A.baumannii* strains as the agent with the highest degree of resistance. In a study conducted in Türkiye in which VAP agents were evaluated, ciprofloxacin resistance was found in 63.6% of *A.baumannii*. In another study conducted in our country, the meropenem resistance rate was 90% in *A.baumannii*[8,12]. In our study, ciprofloxacin (96.4%) and meropenem (95.5%) resistance rates for *A.baumannii* strains are particularly alarming and similar to the resistance patterns recorded in studies conducted in Türkiye. Colistin, tigecycline and piperacillin/tazobactam susceptibility profiles of *A.baumannii* could not be evaluated using the E-test method according to EUCAST criteria.

Studies have highlighted the role of *P.aeruginosa* in the pathogenesis of VAP, emphasizing its presence as a common pathogen in both early and late-onset VAP cases[13,14]. In a study conducted in Türkiye in which VAP agents were evaluated, cotrimoxazole resistance was found in 65.2% of *P.aeruginosa*. In another study conducted in our country, the ciprofloxacin resistance rate was 39% in *P.aeruginosa*[8]. In our study, the high resistance rate of *P.aeruginosa* to cotrimoxazole (94.1%) and ciprofloxacin (26.4%) is remarkable and reflects the trends observed in studies in our country[12]. The intrinsic resistance of *P.aeruginosa* to many antimicrobial agents underlines the difficulty in treating infections caused by this pathogen[15]. In our study, carbapenem resistance was relatively low in *P.aeruginosa* isolates. Although known to be effective against *P.aeruginosa* strains, empirical use of carbapenem group antibiotics is limited due to increasing resistance rates[16]. Resistance rates of *P.aeruginosa* strains isolated from ICU patients have been shown to be higher[17].

Multidrug resistant *K.pneumoniae* strains are common in cases of VAP, and these strains often produce extended-spectrum beta-lactamase (ESBL), especially the bla CTX-M-15 gene, which is common[18]. Ventilator-associated pneumonia due to multidrug-resistant *Klebsiella spp*. can be monobacterial or polybacterial, with polybacterial cases associated with higher mortality rates and distinct clinical characteristics[19]. In a study conducted in Türkiye, *K.pneumoniae* was found to be the 3rd most common VAP agent with 18.4%[20]. In our study, *Klebsiella spp*. strains, which ranked third in frequency (12.5%) among gram-negative strains, showed high resistance to multiple antibiotics, including ciprofloxacin (84.8%) and piperacillin/tazobactam (87.8%), consistent with ICU findings reported in the literature[4].

These resistance patterns in gram-negative agents require regular updating of local antibiograms to guide empirical treatment effectively. Due to our study's low colcystin resistance in gram-negative bacteria, colistin-containing regimens can be considered a priority in the empirical treatment plan of severe septic patients in our unit.

The resistance patterns of gram-positive bacteria, especially *S.aureus* and *coagulase-negative staphylococci*,

pose significant treatment challenges[21]. *S.aureus* is an important agent in nosocomial infection and community-acquired pneumonia, and methicillin resistance, which limits the efficacy of β-lactam antibiotics, is becoming increasingly common in nosocomial isolates[22]. There are, thus, problems with therapy, as methicillin-resistant strains could also be resistant to most other antibiotic groups[23]. The high resistance of *S.aureus* to oxacillin (71.4%) and ciprofloxacin (85.7%) found in our study emphasizes the importance of surveillance and infection control measures to prevent the spread of MRSA[24]. However, the lack of resistance to vancomycin, teicoplanin, and linezolid offers treatment options for these infections[25].

The Limitations of Study

This study has several limitations that should be acknowledged. First, its retrospective design and reliance on electronic health records may introduce selection bias or incomplete data, particularly regarding clinical outcomes such as mortality rates, treatment durations, or longitudinal follow-up. Second, the study's single-center nature limits our findings' generalizability to other ICUs with differing patient demographics, antibiotic stewardship protocols, or regional resistance patterns. Third, excluding polymicrobial cultures and contaminated samples, while necessary to ensure analytical clarity, may overlook clinically relevant co-infections or complex microbiological interactions encountered in real-world ICU settings. Additionally, certain antibiotics (e.g., colistin, tigecycline) could not be evaluated due to methodological constraints (e.g., EUCAST criteria), which may affect the interpretation of susceptibility profiles for critically important therapeutic agents.

Our results provide valuable information about VAP agents' epidemiology and resistance profiles in intensive care units. These data are critical for guiding empirical antibiotic therapy and implementing effective infection control measures. Furthermore, the high prevalence of multidrug-resistant (MDR) pathogens in this study's endotracheal aspirates from intensive care unit patients emphasizes the need for strict surveillance, appropriate antimicrobial stewardship and effective infection control practices. Additional multicentre studies are warranted to monitor antimicrobial resistance trends and develop strategies to combat these challenging pathogens.

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