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Research Article

Microorganisms Causing Ventilator-Associated Pneumonia and Their Antibiotic Susceptibility

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Abstract

Objectives: The aim of this study was to determine the microorganisms causing ventilator-associated pneumonia (VAP) and investigate their antibiotic susceptibility.

Methods: Patients diagnosed with VAP in the adult intensive care units (ICUs) between January 2015 and December 2018 were included in the study. VITEK 2 (bioMérieux, Marcy l'Etoile, France) automated microbiology system was used to identify microorganisms and to determine their antibiotic susceptibility.

Results: Average VAP rates was found 26.51 per 1000 ventilator-days. A single microorganism was isolated in 104 of a total of 105 patients while two microorganisms were isolated in 1 patient. Of the isolated microorganisms, 94.3% (n=100) were Gram-negative bacteria and 5.7% (n=6) were Gram-positive bacteria. When the distribution of all microorganisms is examined in order of frequency, 62.2% were found to be *Acinetobacter spp*., 17.9% *Pseudomonas spp*., 6.6% *Klebsiella pneumoniae*, 4.7% *Staphylococcus aureus*, 3.7% *Serratia marcescens*, 2.8% *Escherichia coli*, 0.9% *Enterococcus faecium*, and 0.9% *Stenotrophomonas maltophilia*. The most effective antibiotics against *Acinetobacter spp*. were found to be colistin (96.9%), tigecycline (95%), amikacin (15%) and gentamicin (5%), whereas those the most effective against *Pseudomonas spp*. were found to be colistin (94.1%), ceftazidime (57.8%), gentamicin (55.5%), ciprofloxacin (50%), amikacin(50%), and piperacillin/tazobactam (42.1%).

Conclusion: *Acinetobacter spp*. was the most common agent in VAP. The fact that *Acinetobacter spp*., which is resistant to carbapenems, quinolones, piperacillin-tazobactam and cephalosporins, was the most common agent in VAP, can significantly affect the mortality rate of the infection.

Keywords: Antibiotic susceptibility, bacteria, intensive care unit, microorganism, ventilator-associated pneumonia

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Ventilator-associated pneumonia is a type of nosocomial pneumonia that occurs in patients receiving mechanical ventilation.[1,2] VAP is a severe infection with high mortality in ICUs. It differs from other nosocomial infections with high mortality, extended hospital stay and increased hospital costs.[3]

Orofarengeal colonization, elimination of the effectiveness of the upper respiratory tract and other defence systems due to the endotracheal tube, decreased cough reflex, deterioration of ciliary functions, decreased macrophage function, hypoxia, uremia, malnutrition, ventilation and perfusion imbalance, insufficient endotracheal aspira

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tion and devices used in ventilator therapy play a role in the pathogenesis of VAP in ICUs.[4–6] Other pathways of infection are hematogenous spread, inhalation of infected aerosols, and exogenous spread from extra-pulmonary infection focus.[7]

Because of the low sensitivity and specificity of clinical or radiological findings, Gram staining and culture of the lower respiratory tract samplings constitute the most critical part of the diagnosis of VAP.[8] Empirical treatment should be started in patients who are considered to have VAP because obtaining the results of culture take time.[9,10] To reduce mortality and morbidity, to determine empirical antibiotic selection and to determine appropriate antibiotic use, it is a must to identify the causative microorganisms and know the profiles of antibiotic resistance. In this study, it was aimed to determine the microorganisms and antibiotic susceptibility isolated in patients diagnosed with VAP in ICUs.

Methods

The study included patients diagnosed with VAP in adult ICUs of Erzincan Binali Yildirim University Faculty of Medicine Hospital between January 2015 and December 2018. The adult ICUs of the Faculty of Medicine have 25 beds (17 beds in step 3, 8 beds in step 2). All the beds have a mechanical ventilator and closed aspiration module. In ICUs with the central ventilation system, particle measure-ments are performed intermittently. Each bedside has alco-hol-based hand antiseptics.

The data of the patients diagnosed with VAP were obtained from electronic records and patient files in the infection surveillance system associated with the National Health Service. The diagnosis of VAP was determined according to the VAP criteria of the Center for Disease Control and Prevention (CDC). Accordingly, VAP was defined as pneumonia developed 48 hours after intubation in the patient with invasive mechanical ventilation support and without pneumonia during intubation. Patients who were diagnosed with VAP according to clinical, microbiological and radiological criteria and had significant growth in their endotracheal aspirate (ETA) samplings were included in the study. At least one of the following was present in the diagnosis of VAP: a new or gradual infiltration; new or progressive infiltration, consolidation, cavitation in lung radiography; high temperature (>38 °C) not due to any known cause; leukopenia [<4.000 white blood cells (WBC)/mm^3] or leukocytosis (≥12.000 WBC/mm^3); altered mental status in an elderly person aged ≥70 which could not be attributed to another reason. Besides, new purulent sputum, a change in the properties of sputum, an increase in the amount of respiratory secretion, new onset or worsening cough, dyspnea, or tachypnea, rale or bronchial breathing sounds or a worsening gas were used in the diagnosis of VAP.

The culture results of endotracheal aspirate (ETA) samplings of patients in the ICUs were investigated to determine the causative microorganism in patients diagnosed with VAP. Specially designed catheters were used to collect the ETA samples. Samples obtained by aspiration of saline from the endotracheal tube under sterile conditions were delivered to the microbiology laboratory under appropriate conditions. ETA sample were simultaneously inoculated onto 5% sheep blood agar, Eosin Methylene Blue (EMB) and chocolate agar media following quantitative culture techniques. The plates were incubated at 37 °C for 18-24 hours, and ≥10^4 cfu/ml were considered positive. VITEK 2 (bioMérieux, Marcy l'Etoile, France) automated microbiology system was used to identify microorganisms and to determine their antibiotic susceptibility. The criteria of the "Clinical and Laboratory Standards Institute" (CLSI) was used between 2015 and 2016, and the criteria of the "European Committee on Antimicrobial Susceptibility Testing" (EUCAST) were used between 2017 and 2018 to determine the antibiotic susceptibility.[11,12]

If the same microorganism isolated in a patient's ETA samples which were sent at different times, these samples were excluded from the study. The age, sex and underlying systemic diseases [hypertension (HT), diabetes mellitus (DM), congestive heart disease (CHF), chronic obstructive lung disease (COPD), chronic renal failure (CRF), cerebrovascular disease (CVD), etc.] of all patients included in the study were recorded. The study was approved by the local Ethics Committee.

The results were expressed as mean±standard deviation and median (minimum-maximum) for continuous variable and as "n" and percentage for categorical variables. For the analysis of the data, IBM SPSS Statistics for Windows Version 19.0 (IBM Corp., Armonk, NY, USA) package program was used.

Results

During the four-year study, a total of 105 patients were diagnosed with VAP. 41.9% of the patients were female (n=44) and 58.1% were male (n=61). The patients were in the 17-97 age range, and the mean age was 71.4 years. All patients underwent mechanical ventilation. Average VAP rates was found 26.51 per 1000 ventilator-days.

Eleven patients (10.6%) had an additional infection with VAP. In addition, in 89 patients (84.7%) who were diagnosed with VAP, underlying chronic diseases were detected. Of the patients who were diagnosed with VAP, 40.9%

(n=43) had COPD, 33.3% (n=35) had HT, 20.9% (n=22) had CVD, 17.1% (n=18) had DM, 12.4% (n=13) had CHD, and 7.6% (n=8) had CRF.

A single microorganism was isolated in 104 of 105 patients while two microorganisms were isolated in 1 patient. Of the isolated microorganisms, 94.3% (n=100) were Gram-negative bacteria and 5.7% (n=6) were Gram-positive bacteria. When the distribution of all microorganisms is examined in order of frequency, 62.2% were found to be *Acinetobacter spp*., 17.9% *Pseudomonas spp*., 6.6% *Klebsiella pneumoniae* (*K. pneumoniae*), 4.7% *Staphylococcus aureus* (*S. aureus*), 3.7% *Serratia marcescens*, 2.8% *Escherichia coli*, 0.9% *Enterococcus faecium* and 0.9% *Stenotrophomonas maltophilia*.

The most effective antibiotics against *Acinetobacter spp*. were found to be colistin (96.9%), tigecycline (95%), amikacin (15%) and gentamicin (5%), whereas those the most effective against *Pseudomonas spp*. were found to be colistin (94.1%), ceftazidime (57.8%), gentamicin (55.5%), ciprofloxacin (50%), amikacin(50%), and piperacillin/tazobactam (42.1%). On the other hand, the most effective antibiotic against Enterobacteriaceae was tigecycline (85-100%) and carbapenems (57.1-100%) (Table 1). Of a total of five *S. aureus*, four were MRSA while one was MSSA. The only isolated *Enterococcus faecium* was penicillin-resistant and vancomycin-sensitive.

Discussion

VAP, which develops in patients undergoing mechanical ventilation, is a common nosocomial infection with a high rate of mortality in ICUs.[13] This infection, which usually occurs approximately 48-72 hours after mechanical ven-tilation in hospitals, should be identified and treated very quickly.[14,15] For the detection of infection, appropriate samples should be taken under appropriate conditions, and microorganism should be isolated.[16] One of the most appropriate methods is the ETA sample taken under sterile conditions. In this study, ETA samples taken under sterile conditions were studied by the quantitative culture method, and thus the risk of contamination was minimized.

One of the most critical steps in designing the treatment of VAP is the determination of the agent microorganism. Studies have shown that the most commonly isolated microorganisms in VAP are Gram-negative bacteria. Previous studies highlighted the role of *Pseudomonas aeruginosa (P. aeruginosa)* and *Enterobacteriaceae spp*., whereas more recent studies have primarily reported *Acinetobacter spp*.[13,17]

In a ten year surveillance study of VAP, Kanafani et al. reported the most commonly isolated bacteria as Acinetobac-ter baumannii (*A. baumannii*) (32.6%), *P. aeruginosa* (16.5%), *Escherichia coli* (12.4%), *K. pneumoniae* (8.3%) and other bacteria (30.2%).[17] In another study, El-saed et al.[18] reported the most common pathogens as *Acinetobacter spp*. (26.5%), *P. aeruginosa* (21.7%), *S. aureus* (15.3%), *Klebsiella spp*. (6.8%), *Haemophilus spp*. (6.1%) and *Enterobacter spp*. (5%).

Studies in Turkey have also reported similar findings. Binici et al. investigated the frequency, risk factors and agents of VAP and reported that *Acinetobacter spp*. (31%) and Pseudomonas spp. (20.6%) were the most commonly isolated agents.[13]

In a multicenter study conducted in Turkey similarly reported the agents causing VAP as 29.2% *Acinetobacter spp*., 26.7% *Pseudomonas spp*., 24.2% *S. aureus*, 14.9% En-

Table1. Antibiotic susceptibility rates of isolated microorganisms (%)

Antibiotics	*Acinetobacter spp.* (n=66)	*Pseudomonas spp.* (n=19)	*Klebsiella pneumoniae* (n=7)	*Serratia marcescens* (n=4)	*Escherichia coli* (n=3)
Tigecycline	95	-	85.7	100	100
Colistin	96.9	94.1	71.4	-	100
Trimetoprim/Sulfametoksazol	49.1	-	57.1	100	0
Amikacin	15	50	85.7	100	100
Gentamicin	5	55.5	57.1	100	66.6
Ciprofloxacin	0	50	42.8	100	33.3
Levofloxacin	0	35.2	50	100	-
Meropenem	0	33.3	57.1	100	66.6
İmipenem	0	25	100	-	0
Piperacillin/Tazobactam	0	42.1	28.5	100	66.6
Ceftazidime	0	57.8	28.5	100	0
Cefepime	0	38.8	28.5	100	50
Ceftriaxone	-	-	25	100	0
Ampicillin	0	0	0	0	0

terobacteriaceae, 3% Candida spp. and other microorganisms.[19] On the other hand, some researchers reported less *A. baumannii* than *P. aeruginosa* and *K. pneumoniae* as the agents causing VAP. Ergül et al.[20] reported that 96% of the patients diagnosed with VAP had Gram-negative bacteria and that 32% of these bacteria were *P. aeruginosa*, 24%were *K. pneumoniae*, and 22% were *A. baumannii*.

Similar to the findings reported from many other countries, the present study found *Acinetobacter spp*. and *Pseudomonas spp*. as the most common agents. However, the fact that this study found *Acinetobacter spp*. as 62.2% is an important difference. *Acinetobacter spp*., which is more resistant than many other bacteria, is the most common pathogen associated with VAP. Another important difference of our study is that *Serratia marcescens* was detected only in 4% of the VAP. Since the Serratia species cause epidemics in hospitals, this bacteria should not be ignored in VAP.

Because early and appropriate treatment is effective in reducing mortality in VAP, the diagnosis should be made as soon as possible. Once appropriate samples have been collected to identify the agent, appropriate empirical antimicrobial therapy should be initiated.[21] However, resistant microorganisms occur in ICUs due to long term antibiotic use.[22,23] In particular, carbapenem resistance of A. baumannii and *P. aeruginosa*, expanded-spectrum β-lactamase production and carbapenem resistance of Enterobacteriaceae and methicillin resistance of *S. aureus* lead to problems.[22, 24]

Acinetobacter spp, *Pseudomonas spp*. and *Klebsiella spp*. have high carbapenem resistance in recent years.[8] Carbapenems, the broadest-spectrum β-lactam antibiotics, are used in strains with high resistance. The main resistance mechanisms against these antibiotics are the production of carbapenemase, the modification of penicillin binding proteins, and loss of porin.[25]

In this study, isolated all of *Acinetobacter spp* strains were found resistant to carbapenems, ciprofloxacin, levofloxacin and cephalosprins. Tigecycline, colistin and amikacin were found to be the most effective antibiotics against these strains. Tartar et al. determined the carbapenem resistance of Acinetobacter isolates as 97.7% and colistin resistance as 2.9%. In another study, Ergül et al. reported that all of the Acinetobacter strains were susceptible to colistin, some were resistant to piperacillin-tazobactam (50%), but all strains were resistant to amikacin and meropenem. The same researchers reported the antibiotic susceptibilities of Pseudomonas strains as follows: 100% against amikacin, 87.5% against ciprofloxacin, 87.5% against colistin, 87.3% against gentamycin, 50% against ceftazidime and 31.3% against cefepime. In our study, antibiotic susceptibilities of *Pseudomonas spp*. were found to be colistin (94.1%), ceftazidime (57.8%), gentamicin (55.5%), ciprofloxacin (50%), amikacin (50%), and piperacillin/tazobactam (42.1%).

Conclusion

In conclusion, in our study, *Acinetobacter spp*. was found to be the most common agent in VAP, similar to the studies performed in many countries. However, the present study found higher levels of *Acinetobacter spp*. than many other studies. The fact that *Acinetobacter spp*, which is more resistant than many other bacteria, is a more common agent in VAP, can significantly affect the mortality rate of this infection.

Disclosures

Ethics Committee Approval: The study was approved by the Ethics Committee of Erzincan Binali Yildirim University (Approval No: 05.03.2019–01/03).

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Conflict of Interest: None declared.

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