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RESEARCH ARTICLE

Bronchoalveolar lavage of ventilator-associated pneumonia patients for antibiotic resistance and susceptibility test

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Abstract

Background and Aims: Ventilator-associated pneumonia is highly prevalent nosocomial infection among patients under mechanical ventilation. Bronchoalveolar lavage (BAL) is effective in identifying the type of pathogen involved and determine the course of antibiotic. The aim of this study was to evaluate the prevalence of different pathogens involved in ventilator-associated pneumonia (VAP) and associated antibiotic resistance and sensitivity pattern.

Methods: In this descriptive cross-sectional study, patients admitted to the intensive care unit under mechanical ventilation at Shahid Madani Educational and Medical Center in Karaj during 2018 and 2020 were included. BAL samples were obtained from the patients. Demographic data, duration of hospitalization, duration of mechanical ventilation, and antibiotic susceptibility and resistance tests were recorded for all the patients.

Results: Among 335 patients included in the study, 215 (64.2%) were males. The mean age of the patients was 55.06 ± 14.90 years. The most common pathogens reported were *Acinetobacter baumannii* (40%), *Pseudomonas aeruginosa* (21.2%), and *Staphylococcus aureus* (13.4%). The mean age of the patients, gender, duration of mechanical ventilation, and duration of hospitalization were not associated with the type of pathogen, $P > .05$, respectively.

Conclusion: BAL of these patients indicated that various pathogens are responsible for VAP, and can vary from patient to patient. Antibiotic resistance and susceptibility pattern of these pathogens vary and therefore is important in determining the course of the treatment.

KEYWORDS

antibiotic resistance, Bronchoalveolar lavage, infection, mechanical ventilation, ventilator-associated pneumonia

1 | INTRODUCTION

Ventilator-associated pneumonia (VAP) is a hospital-acquired lung infection that occurs in patients who are mechanically ventilated for at least 48 hours, either through tracheostomy or endotracheal intubation.[1,2] It is divided early (within 4 days after mechanical ventilation) and late (from the fifth day onwards).[2] This disease is the second most common nosocomial infection in intensive care unit patients.[3,4] The most common pathogens are involved in VAP are bacteria, including *Staphylococcus aureus* gram-positive bacteria, but there are also resistant pathogens that can cause the disease, including Pseudomonas species.[5] Aerogenic and Acinetobacter, which are resistant to various antibiotics, are called MDR (multidrug resistant) species.[4,6]

There are several risk factors such as the duration of mechanical ventilation, underlying lung disease, sepsis, acute respiratory distress syndrome, neurological disease, trauma, previous use of antibiotics, and blood transfusion associated with VAP.[7] It is estimated that about 8% to 28% of patients who are mechanically ventilated develop VAP, which is 3 to 10 times more than patients who are not mechanically ventilated.[2,8] A meta-analysis showed that the mortality rate due to VAP in patients admitted to the intensive care unit is 32.5%.[7,9] Other studies have predicted that mortality from the disease depends on the correct or incorrect use of antibiotics where the prevalence of development of resistant pathogens in about 25% to 70%.[4,7]

Bronchoalveolar lavage (BAL) is a diagnostic method for collecting and obtaining cells (such as microorganisms and immune cells) and fluid from the bronchi and lung alveoli using a bronchoscope and administering physiological fluid (normal saline).[10,11] Diagnosis of pathogen using BAL has been indicated to be superior to clinical evaluation of VAP, since it can help physicians decide the specific antibiotic therapy required for the patient.[12]

The aim of this study is to evaluate pathogens involved in VAP among patients in our center, along with antibiotic resistance and sensitivity pattern of these pathogens.

2 | METHODS

In this descriptive cross-sectional study, 335 patients referred to Shahid Madani Educational and Medical Center in Karaj during 2018 and 2020 were randomly selected. In this study, positive culture was defined as the presence of more than or equal to 10 000 colony units per milliliter in the bronchoalveolar lavage specimen from patients with ventilator-dependent pneumonia. The inclusion criteria of the study were patients aged 18 years or above, hospitalized in the intensive care unit with mechanical ventilation for at least 48 hours, clinical diagnosis of ventilator-associated pneumonia (VAP), which includes body temperature more than 38°C or less than 36°C, infectious secretions of the trachea, white blood cell count greater than 10 000 or less than 4000, new pulmonary infiltration or progression in previous infiltration, and deterioration of respiratory status based arterial oxygen saturation. The exclusion criteria included patients with pulmonary Aspergillosis, pneumocystis pneumonia, mycobacterial infection, unknown pathogens or infection due to multiple pathogens, presence of more than 10 squamous cells in each microscope field with low magnification in sputum sample and more than 1% of bronchial cells in bronchoalveolar lavage sample, and those who could not be examined due to severe neutropenia or clotting. Immunodeficienct, cancer, and patients under antibiotics 24 hours prior to BAL were also excluded.

As per the standard protocol, bronchoalveolar lavage was performed by using a fibropeptic bronchoscope thrice, each time using 50 mL of sterile saline in diffuse, the middle lobe of the right lung, or lingula to collect the fluid samples.

Information regarding age, sex, reason for admission to the intensive care unit, and laboratory findings including response of bronchoalveolar lavage sample cultures and their microbiology and sensitivity and antibiotic resistance were collected and analyzed using SPSS v25. Chi-square, Fisher's exact test, one-way ANOVA test were used. *P* value less than 0.05 was considered significant.

This study was approved by the Research Ethics Board of Alborz University of Medical Sciences (IR.ABZUMS.REC.1399.158). https://ethics.research.ac.ir/ProposalCertificateEn.php?id=143106&Print=true&NoPrintHeader=true&NoPrintFooter=true&NoPrintPageBorder=true&LetterPrint=true.

3 | RESULTS

In the present study, 335 patients were studied. The mean age of patients was 55.06 ± 14.90 years (range: 18-98 years) (Figure 1). In terms of gender, 215 patients (64.2%) were male and 120 patients (35.8%) were female (Figure 2).

In terms of reasons for admission to the ICU, 68 patients (20.3%) were admitted due to multiple trauma, 98 patients (29.3%) due to head trauma, 123 patients (36.7%) due to chest trauma, and 46 patients (13.7%) were hospitalized for other reasons. The mean length of stay in the ICU was 15.17 ± 4.92 (range: 3-28 days). The average duration of mechanical ventilation was 12.71 ± 5.15 days (range: 2-26 days). In terms of pathogen of ventilator-induced pneumonia, *Acinetobacter baumannii* was reported in 134 patients (40%), *Klebsiella pneumoniae* in 43 patients (12.8%), *Enterobacteriaceae* in 21 patients (6.3%), *Pseudomonas aeruginosa* in 71 patients (21.2%), *Staphylococcus aureus* in 45 patients (13.4%), *Escherichia coli* was detected in 14 patients (4.2%), *Proteus mirabilis* in 3 patients (0.9%), and *Staphylococcus epidermis* in 4 patients (1.2%).

As can be seen in the tables, *Acinetobacter baumannii* was most susceptible to cholestin (85.8%) and tobramycin (32.1%), and the highest antibiotic resistance was observed to ciprofloxacin, cefotaxime, ceftriaxone, and cefixime (100%). The highest susceptibility of *Klebsiella pneumoniae* was seen to imipenem (90.7%), and the highest resistance to ceftazidime and levofloxacin (62.8%) was observed. *Enterobacteriaceae* was most susceptible to cefpime (61.9%) and highly resistant to ceftazidime (95.2%). *Pseudomonas aeruginosa* was mainly susceptible to cholestin (90.1%) and resistant to piperacillin (87.3%). *Staphylococcus aureus* showed highest susceptibility to vancomycin and linezolid (100%)

and the highest resistance to cefazolin (75.6%). The highest susceptibility of *Escherichia coli* to meropenem, imipenem, and amikacin (100%) and the highest resistance to ceftazidime and ciprofloxacin (92.9%) were observed. *Proteus mirabilis* showed highest susceptibility to meropenem, imipenem, and piperacillin (66.7%) and the highest resistance to ceftriaxone and ceftazidime (100%). The highest susceptibility of *Staphylococcus epidermis* to vancomycin and linezolid (100%) and the highest resistance to cefazolin and cloxacillin were observed, Table 1.

The mean age of patients in terms of pathogen was not significantly correlated, $P = .99$ (Table 2). The mean length of hospital stay in the ICU according to the pathogen. There was no significant correlation between the two variables, $P = .43$ (Table 2). The mean duration of mechanical ventilation in terms of pathogen was also not significantly different, $P = .403$ (Table 2). The gender frequency of patients was also not significantly different based on the pathogens, $P = .16$ (Table 3). The frequency of hospitalization of patients in terms of pathogen was also not significantly different, $P = .33$ (Table 4).

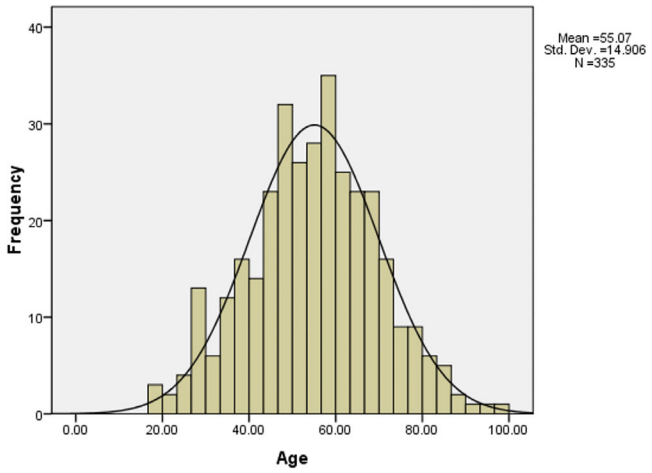

FIGURE 1 Age frequency of patients

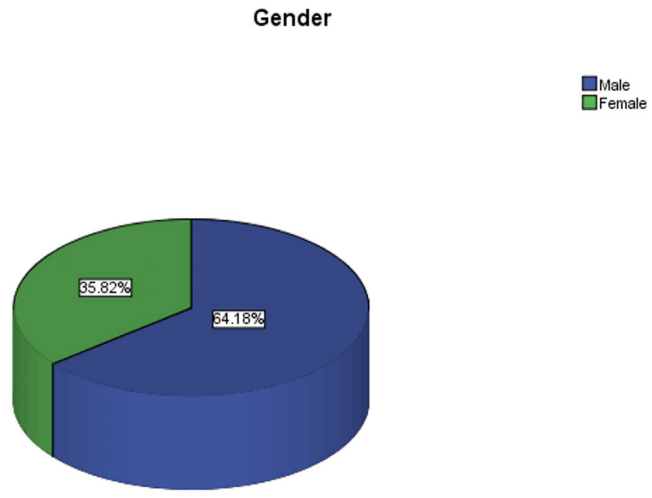

FIGURE 2 Frequency of patients by gender

4 | DISCUSSION

The aim of this study was to investigate the microbial pattern and antibiotic susceptibility pattern of BAL specimens in patients with VAP in patients admitted to the intensive care unit. The most common pathogens identified in these patients were *Acinetobacter baumannii* (40%), *Pseudomonas aeruginosa* (21.2%), *Staphylococcus aureus* (13.4%), and *Klebsiella pneumoniae* (12.8%).

In a study conducted by Kiai et al, in Isfahan, the most common pathogens causing VAP include *Acinetobacter baumannii* (40.4%), *Klebsiella pneumoniae* (31.8%), *Pseudomonas aeruginosa* (7.6%), and *Staphylococcus aureus* (8%).[13] The difference is due to the epidemiological pattern of VAP-causing bacteria in different geographical areas and medical centers. In another study conducted in Shiraz by Japoni et al, reported the most common VAP-causing pathogens, *Acinetobacter baumannii*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, respectively.[14] In another study conducted by Sanandaj, Afkhamzadeh et al, the most common pathogens causing VAP were *Klebsiella pneumoniae* (43.7%) and *Enterobacteriaceae* (31.2%), respectively. In the present study, the prevalence of *Enterobacteriaceae* was 6.3%.[14,15]

Regarding the pattern of antibiotic susceptibility, the highest susceptibility of *Acinetobacter baumannii* was observed to cholestin (85.8%) and tobramycin (32.1%) and the highest antibiotic resistance to ciprofloxacin, cefotaxime, and ceftriaxone was 100%.[16,17] Talebi et al conducted a study in Tehran and reported that *Acinetobacter baumannii* causing VAP was highly susceptible to cholestin and resistant to levofloxacin, cefotaxime, ceftriaxone, and cotrimoxazole,[18] which is consistent with the results of the present study. Our study showed the highest susceptibility of *Klebsiella pneumoniae* was to imipenem (90.7%) and the highest resistance to ceftazidime and levofloxacin (62.8%). In the study by Sadeghi et al, the highest susceptibility of *Klebsiella pneumoniae* was to imipenem, meropenem, and amikacin and the highest resistance to cefepime and ceftazidime was observed.[16] *Pseudomonas aeruginosa* showed highest susceptibility to cholestin and the highest resistance to piperacillin in our study. In the study by Japan et al, similar findings were reported. Discrepancies in these studies show the over-usage of antibiotic in particular center or region. Additionally, with overuse of antibiotics, increase in local temperature is also associated with antibiotic resistance,[19] which could explain variations in the data.

Our study did not find significant association between the pathogen type and age, gender, duration of hospitalization, and mechanical ventilation. Our study does not provide therapeutic outcomes and mortality in these patients. Further studies are therefore required in this aspect.

5 | CONCLUSION

The results of this study showed that the most common causes of ventilator-associated pneumonia (VAP) were *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, respectively. No correlation was found between patients' age, sex, length of ICU stay, and duration of ventilator with the pathogen type.

TABLE 1 Pathogens reported in patients and their antibiotic resistance and sensitivity pattern

	Pathogens															
	Acinetobacter baumannii (n = 134)		*Klebsiella pneumoniae* (n = 43)		*Enterobacteriaceae* (n = 21)		*Pseudomonas aeruginosa* (n = 71)		*Staphylococcus aureus* (n = 45)		*Escherichia coli* (n = 14)		*Proteus mirabilis* (n = 3)		*Staphylococcus epidermis* (n = 4)	
Antibiotics	Sensitivity	Resistance	Sensitivity	Resistance	Sensitivity	Resistance	Sensitivity	Resistance	Sensitivity	Resistance	Sensitivity	Resistance	Sensitivity	Resistance	Sensitivity	Resistance
Ciprofloxacin	0 (0%)	134 (100%)	28 (65.1%)	15 (34.9)	6 (28.6%)	15 (71.4%)	21 (29.6%)	50 (70.4%)	0	0	1 (7.1%)	13 (92.9%)	1 (33.3%)	2 (66.6%)	0	0
Doxycycline	40 (29.9%)	94 (70.1%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cotrimoxazole	2 (1.5%)	132 (98.5%)	20 (46.5%)	23 (53.5%)	5 (23.8%)	16 (76.2%)	0	0	29 (64.4%)	16 (35.6%)	0	14 (100%)	0	0	3 (75%)	1 (25%)
Ceftazidime	8 (6%)	126 (94%)	16 (37.2%)	27 (62.8%)	1 (4.8%)	20 (95.2%)	30 (42.3%)	41 (57.7%)	0	0	1 (7.1%)	13 (92.9%)	0	3 (100%)	0	0
Cefotaxime	0 (0%)	134 (100%)	24 (55.8%)	19 (44.2%)	3 (14.3%)	18 (85.7%)	0	0	0	0	0	14 (100%)	0	0	0	0
Meropenem	8 (6%)	126 (94%)	33 (76.7%)	10 (23.3%)	6 (28.6%)	15 (71.4%)	35 (49.3%)	36 (50.7%)	0	0	14 (100%)	0	2 (66.6%)	1 (33.3%)	0	0
Imipenem	8 (6%)	126 (94%)	39 (90.7%)	4 (9.3%)	11 (52.4%)	10 (47.6%)	41 (57.7)	30 (42.3%)	0	0	14 (100%)	0	2 (66.6%)	1 (33.3%)	0	0
Amikacin	5 (3.7%)	129 (96.3%)	31 (72.1%)	12 (27.9%)	12 (57.1%)	9 (42.9%)	29 (40.8%)	42 (59.2%)	0	0	14 (100%)	0	1 (33.3%)	2 (66.6%)	0	0
Tobramycin	43 (32.1%)	91 (67.9%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cefepime	9 (6.7%)	125 (93.3%)	22 (51.2%)	21 (48.8%)	13 (61.9%)	8 (31.8%)	12 (16.9%)	59 (83.1%)	0	0	9 (64.3%)	5 (35.7%)	1 (33.3%)	2 (66.6%)		
Colistin	115 (85.8%)	19 (14.2%)	0	0	0	0	64 (90.1%)	7 (9.9%)	0	0	0	0	0	0	0	0
Ampicillin	35 (26.1%)	99 (73.9%)	37 (89%)	6 (14%)	0	0	0	0	0	0	0	0	0	0	0	0
Levofloxacin	2 (1.5%)	132 (98.5%)	16 (37.2%)	27 (62.8%)	10 (47.6%)	11 (52.4%)	0	0	0	0	0	0	0	0	0	0
Piperacillin	5 (3.7%)	129 (96.3%)	0	0	0	0	9 (12.7%)	62 (87.3%)	0	0	12 (85.7%)	2 (14.3%)	2 (66.6%)	1 (33.3%)		
Gentamicin	12 (9%)	122 (91%)	26 (60.5%)	17 (39.5%)	5 (23.8%)	16 (76.2%)	27 (38%)	44 (62%)	0	0	4 (28.6%)	10 (71.4%)	1 (33.3%)	2 (66.6%)		
Ceftriaxone	0	134 (100%)	20 (46.5%)	23 (53.5%)	7 (33.3%)	14 (66.7%)	0	0	0	0	3 (21.4%)	11 (78.6%)	0	3 (100%)		
Cefixime	0	134 (100%)	0	0	0	0	0	0	0	0	0	0	0	0		
Cefazolin	0	0	0	0	0	0	0	0	11 (24.4%)	34 (75.6%)	0	0	0	0	0	4 (100%)
Cloxacillin	0	0	0	0	0	0	0	0	16 (35.6%)	29 (64.4%)	0	0	0	0	0	4 (100%)
Vancomycin	0	0	0	0	0	0	0	0	45 (100%)	0	0	0	0	0	4 (100%)	0
Linezolid	0	0	0	0	0	0	0	0	45 (100%)	0	0	0	0	0	4 (100%)	0 (0%)

TABLE 2 Comparison of mean age, length of ICU stay, and mechanical ventilation of patients by pathogen

		By age		Length of ICU stay		Duration of mechanical ventilation	
Pathogen	Number	Mean ± SD	*P* value	Mean ± SD	*P* value	Mean ± SD	*P* value
Acinetobacter baumannii	134 (40%)	55.15 (12.99)	.99	15.27 (5.00)	.43	12.89 (5.25)	.40
Klebsiella pneumoniae	43 (12.8%)	54.63 (17.24)		14.44 (4.61)		11.86 (4.78)	
Enterobacteriaceae	21 (6.3%)	54.14 (15.86)		13.29 (5.23)		10.67 (4.97)	
Pseudomonas aeruginosa	71 (21.2%)	54.73 (15.98)		15.59 (4.05)		12.99 (4.42)	
Staphylococcus aureus	45 (13.4%)	55.67 (15.82)		15.44 (6.15)		13.13 (6.39)	
E coli	14 (4.2%)	54.50 (14.07)		15.86 (4.47)		13.36 (4.62)	
Proteus Mirabilis	3 (0.9%)	56.33 (26.16)		15.27 (5.00)		16.67 (4.04)	
Staphylococcus epidermis	4 (1.2%)	62.00 (19.92)		14.44 (4.61)		11.75 (4.72)	
Total	335 (100%)	55.07 (14.91)		13.29 (5.23)		12.71 (5.15)	

TABLE 3 Comparison of the average duration of mechanical ventilation based on gender distribution and its correlation with the pathogen

Pathogen	Number	Male	Female	Mean ± SD	*P* value
Acinetobacter baumannii	134 (40%)	76 (35.3%)	58 (48.3%)	58 (48.3)	.16
Klebsiella pneumoniae	43 (12.8%)	29 (13.5%)	14 (11.7%)	14 (11.7)	
Enterobacteriaceae	21 (6.3%)	17 (7.9%)	4 (3.3%)	4 (3.3)	
Pseudomonas aeruginosa	71 (21.2%)	46 (21.4%)	25 (20.8%)	25 (20.8)	
Staphylococcus aureus	45 (13.4%)	32 (14.9%)	13 (10.8%)	13 (10.8)	
E coli	14 (4.2%)	10 (4.7%)	4 (3.3%)	4 (3.3)	
Proteus Mirabilis	3 (0.9%)	1 (0.5%)	2 (1.7%)	2 (1.7)	
Staphylococcus epidermis	4 (1.2%)	4 (1.9%)	0 (0%)	0 (0)	
Total	335 (100%)	215 (100%)	120 (100%)	120 (100)	

TABLE 4 Comparison of the average duration of patients' connection to mechanical ventilation according to the pathogen

Pathogen	Multiple trauma	Head trauma	Chest trauma	Other	*P* value
Acinetobacter baumannii	27 (39.7%)	40 (40.8%)	54 (43.9%)	13 (28.3%)	.326
Klebsiella pneumoniae	10 (14.7%)	9 (9.2%)	20 (16.3%)	4 (8.7%)	
Enterobacteriaceae	4 (5.9%)	2 (2%)	9 (7.3%)	6 (13%)	
Pseudomonas aeruginosa	15 (22.1%)	25 (25.5%)	20 (16.3%)	11 (23.9%)	
Staphylococcus aureus	5 (7.4%)	17 (17.3%)	17 (13.8%)	6 (13%)	
E coli	5 (7.4%)	2 (2%)	2 (1.6%)	5 (10.9%)	
Proteus Mirabilis	1 (1.5%)	1 (1%)	1 (0.8%)	0 (0%)	
Staphylococcus epidermis	1 (1.5%)	2 (2%)	0 (0%)	1 (2.2%)	
Total	68 (100%)	98 (100%)	123 (100%)	46 (100%)	

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None declared.

CONFLICT OF INTEREST

The authors deny any conflict of interest in any terms or by any means during the study.

TRANSPARENCY STATEMENT

Mojtaba Ahmadinejad affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

AUTHORS' CONTRIBUTION

Conceptualization: Mojtaba Ahmadinejad, Izadmehr Ahmadinejad.

Formal Analysis: Haleh Pak, Mahsa Rahimi.
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All authors have read and approved the final version of the manuscript.

Mojtaba Ahmadinejad had full access to all of the data in this study and takes complete responsibility for the integrity of the data and the accuracy of the data analysis.

HUMAN AND ANIMAL RIGHTS

No animals were used in this research. All human research procedures followed were in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national), and with the Helsinki Declaration of 1975, as revised in 2013. This study was approved by the Research Ethics Board of Alborz University of Medical Sciences.

CONSENT FOR PUBLICATION

Informed consent was obtained from each participant.

DATA AVAILABILITY STATEMENT

All relevant data and materials are provided within the manuscript.

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