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Characteristics of Bacterial Isolates from Patients with Ventilator-Associated Pneumonia and the Hospital Environment in Urmia, Iran

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Background: Ventilator-associated pneumonia is a nosocomial infection involving hospitalized patients using mechanical ventilation. The endotracheal tube is a place for colonizing microbial populations and subsequent biofilm formation, leading to relative drug resistance. Also, the hospital environment may worsen the infection. This study aims to identify the microbial cause of the infection and evaluate its characteristics.

Materials and Methods: Sampling was done using endotracheal tube suction among hospitalized patients and from different places in intensive care units in selected hospitals in Urmia, Iran. All specimens were identified by microbial and biochemical tests. Then, the antibiotic susceptibility tests were performed using disk diffusion and minimum inhibitory and bacteriocidal concentrations for selected antibiotics. Finally, the biofilm formation rates of all isolates were evaluated.

Results: The majority of clinical bacterial isolates were Gram-negative bacilli, including *A. baumannii*. *Bacillus spp.* and *Staphylococcus aureus* were the most isolated bacteria from environmental samples. The antibiotic susceptibility testing results showed that most Gram-negative bacilli were resistant to common antibiotics and were extensively-drug resistant. The intermediate rate was higher than resistance to colistin, but evidence showed the efficiency of colistin against Gram-negative bacilli. Most of the isolates were strong biofilm producers, and this was correlated with the extensively drug-resistant pattern of bacteria.

Conclusion: The evidence shows that Ventilator-associated pneumonia is a critical infection. According to the findings, there is a high rate of biofilm formation and consequent drug resistance among bacterial causes of Ventilator-associated pneumonia, which needs new insights for preventing and controlling this healthcare problem.

Keywords: Biofilms; Intensive care units; Antimicrobial sensitivity tests; Nosocomial infections; Ventilator-associated pneumonia

INTRODUCTION

The term “pneumonia” refers to the infection of the lower respiratory tract, including the lungs and bronchi. It is one of the most common infections in the elderly and is mainly classified into three clinical subsets: community-acquired pneumonia (CAP), hospital-acquired pneumonia (HAP), known as nosocomial pneumonia, and pneumonia in immunocompromised patients (1, 2).

HAP is defined as a type of pneumonia that occurs in hospitalized patients, with no signs of pneumonia before their hospitalization. Ventilator-associated pneumonia (VAP) is a subset of HAP, which is related to hospitalized patients mainly in intensive care units (ICUs) and being intubated for at least 48-72 hours (2-4). VAP accounts for about half of the HAPs, with the highest rate, up to about 40%, of early onset in the first five days of mechanical ventilation. It is also the second most prevalent HAP in intubated patients hospitalized in ICUs. Due to some related complications in adults, such as immunodeficiency and cardiopulmonary diseases, higher rates of mortality and morbidity exist in the elderly than in younger ages (4-6).

The clinical signs and symptoms for the diagnosis of VAP are variable in infected patients. Fever, purulent secretions and sputum from the endotracheal tube (ETT), infiltration on chest radiography, leukocytosis or leukopenia, are all extracted from physical examination, chest radiography, and laboratory test results (7, 8).

Mechanical ventilation is established through the ETT, which is the direct route for the transmission of pathogens to the lower respiratory tract (4, 9). Most of the bacteria colonized in ETT and causing VAP are drug-resistant pathogens, including *Acinetobacter spp., Klebsiella pneumoniae, Pseudomonas aeruginosa*, and other types of organisms such as *Streptococcus spp., Staphylococcus spp., Hemophilus influenza, Escherichia coli, Proteus spp. Enterobacter spp.*, etc. Some other species, including *Corynebacterium spp., Neisseria spp.,* and Coagulase-negative *Staphylococcus spp.,* are also obtained from ETT secretions (4, 6). Some of the pathogens of VAP, as mentioned above, belong to "ESKAPE" groups (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter spp.*), which are the six most life-threatening and drug-resistant pathogens (10). In addition, many of these organisms have the capacity to form biofilm after tracheal intubation, leading to a further increase in their antibiotic resistance and complexity in treatment (9, 11).

In this study, we aim to detect VAP and its related risk factors, identify the causative pathogens, evaluate the antimicrobial resistance patterns in related organisms, assess their rate of biofilm formation, and evaluate the role of the hospital environment in causing VAP. Collecting these data may contribute to a better understanding of the major causes of infection, the microbiological characteristics of the causative pathogens, and effective antibacterial treatment options, potentially informing the development of new infection-control protocols.

MATERIALS AND METHODS

Sampling and study population

This cross-sectional study was carried out on adult patients hospitalized in the ICU and to be ventilated for at least 72 hours, in Taleghani and Imam Khomeini hospitals in Urmia, Iran. The sampling was carried out during five months (January 2023- May 2023), from 43 patients according to the inclusion criteria. The inclusion criteria were one of the clinical signs as follows: fever, cough, purulent ETT secretions, leukopenia, leukocytosis, sputum, or pulmonary secretions. The children or patients with a cause of hospitalization of pneumonia or any visible pulmonary infections in chest X-rays were excluded from the study.

Endotracheal aspirates were obtained using ETT-suction for clinical sampling, and the environmental samples were collected with sterile swabs soaked in sterile normal saline. Sampling was performed from the places near patients' beds (Sink, drug trolley, ventilator, etc.). It should be noted that for each patient, an environmental sample was also taken. All of the samples were placed in brain heart infusion broth (Condalab, Spain) and were immediately transferred to the research microbiology laboratory of Urmia University of Medical Sciences. After 2 hours of incubation at 37 °C, all clinical and environmental specimens were cultured on Eosin Methylene Blue agar, Blood Agar, and Chocolate Agar and incubated overnight at 37 °C. For further analysis, all positive clinical and environmental cultures were streaked in Trypticase Soy Broth (TSB) + 16% glycerol and frozen at -20 °C.

Bacterial isolates identification

First, the Gram stain was performed on all of the isolates. Then, based on the results, further differential microbial tests were conducted, which are described as follows (12, 13):

For Gram-negative bacteria: Oxidase, Catalase, TSI (Triple Sugar Iron Agar test), SIM (SH_2, Indole, Motility test), MR/VP (Methyl-Red/Vogues-Proskauer test), Citrate utilization, Urea utilization, O/F (Oxidative/Fermentative test), the analysis of pigmentation, and the analysis of growth at 42 °C were performed.

For Gram-positive bacteria: Oxidase, Catalase, Coagulase, the analysis of susceptibility to Optochin and Bacitracin, Bile esculin, and NaCl 6.5% were carried out.

Antimicrobial susceptibility testing (AST)

Evaluating the antibiotic resistance pattern of the common pathogens (gram-negative bacilli and gram-positive cocci) was performed according to Clinical and Laboratory Standards Institute 2023 guidelines (CLSI 2023) (14). The bacterial suspension was prepared to an equal of 0.5 McFarland standard ($1.5x10^8$ cells/ml, Wavelength: 625nm, Optical density: 0.08 - 0.13). Disk diffusion of the Gram-negative bacilli was performed on Mueller-Hinton Agar (MHA) (Condalab, Spain) for Cefepime (30 µg), Piperacillin/Tazobactam (100/10 µg), Meropenem (10 µg), Amikacin (30 µg), and Ciprofloxacin (5 µg) (Condalab, Spain). Additional Trimethoprim/Sulfamethoxazole (1.25/23.75 µg) (Condalab, Spain) was also used for fermentative Gram-negative bacilli, including *Enterobacteriaceae*. The disk diffusion for *Staphylococcus spp.* was performed for Ciprofloxacin (5 µg), Trimethoprim/Sulfamethoxazole (1.25/23.75 µg), Linezolid (30 µg), and Tetracycline (30 µg) (Condalab, Spain). Cefoxitin 30 µg (Condalab, Spain) was used for the screening of Methicillin-resistant *S. aureus* (MRSA). Lastly, the incubation at 35 °C for 24 hours was conducted.

Microdilution MIC (Minimum Inhibitory Concentration) test was done for colistin sulfate on Gram-negative bacilli. Bacterial suspensions equal to the dilution of 1:100 of a 0.5 McFarland standard were prepared, and the colistin solution (with a total concentration of 1024 µg/ml as the stock suspension) was prepared by dissolving the antibiotic powder in sterile distilled water. The bacterial inoculum was prepared in Mueller-Hinton broth (MHB) using a 96-well microplate with the antibiotic serial dilution from 256 µg/ml to 0.125 µg/ml, and finally incubated overnight at 35 °C. For the quality control of AST tests, *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 were used for non-fermenters and *Enterobacteriaceae*, respectively.

According to AST, the bacterial isolates are divided into three resistance categories: Multidrug resistance (MDR), Extensively drug-resistant (XDR), and Pan-drug resistance (PDR) (15, 16). Gram-negative bacilli are defined as:

MDR: Resistance to at least three antibiotics from different classes: Cephalosporines (Cefepime), Fluoroquinolone (Ciprofloxacin), and Aminoglycosides (Amikacin)

XDR: MDR pattern + resistance to Carbapenems (Meropenem).

PDR: XDR pattern + resistance to Polymyxin B and polymyxin E (colistin) (resistance to all tested classes of antibiotics)

Lastly, the minimum bactericidal concentration (MBC) of colistin sulfate was also evaluated. In order of MBC analysis, four concentrations greater than MIC were cultured on MHA, and after an overnight incubation at 35 °C, the first concentration with negative growth was considered the MBC.

Biofilm formation assay

The biofilm formation test was carried out using a microtiter plate colorimetric assay according to Jabalameli et al. and O'Toole procedures (17, 18). First, all isolates were suspended from fresh Mueller-Hinton agar culture into Trypticase Soy broth supplemented with 1% glucose (TSB+1% Glu), and their concentration was set equal to 0.5 McFarland standard. After an overnight incubation at 37 °C, a 1:100 dilution for each suspension was prepared with fresh TSB+1% Glu, then 200 µl of each dilution was transferred into a well of a 96-well flat-bottom microplate

(three repeats considered for each isolate) (Figure 1). After another overnight incubation at 37 °C, all contents of the microplate were evacuated and washed two times with distilled water. After resting the microplate for hours to dry, 150 µl of 99% methanol was added to each well for 15 minutes to fix the biofilms. Then the contents of the microplate were evacuated, and after drying at ambient temperature, 200 µl of 0.01% Crystal violet was added to each well, and the microplate was incubated for 15-20 minutes. Then the contents were evacuated and washed again. The plate was left upside down for 24 hours to completely dry. Eventually, 200 µl 33% acetic acid was loaded into wells, and after 15 minutes, the optical density (O.D) of wells was read at 545 nm wavelength using a microplate reader (Awareness Technology Inc, USA). In each microplate, three wells were considered blank wells and only contained 200 µl 33% acetic acid as a negative control. A positive control was not required in this method of biofilm assay.

The results were reported using the following formulas:

$O.D_C$: The average mean of O.D of negative controls + 3 (SD of negative controls)

$O.D_S$: The average mean of the O.D. of each isolate

Non-biofilm former: $O.D_S \leq O.D_C$

Weak biofilm former: $O.D_C \leq O.D_S \leq 2\ O.D_C$

Moderate biofilm former: $2\ O.D_C \leq O.D_S \leq 4\ O.D_C$

Strong biofilm former: $4\ O.D_C \leq O.D_S$

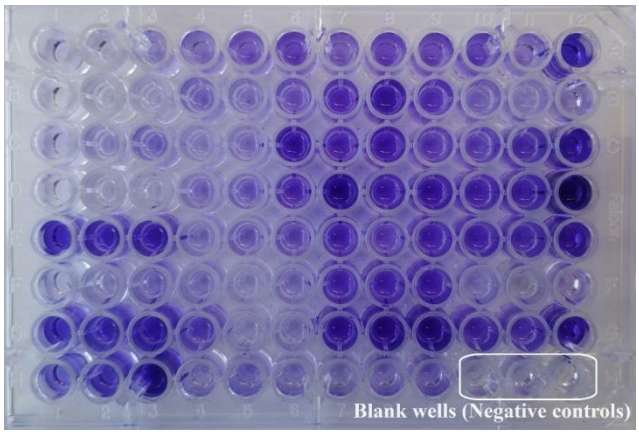

Figure 1. Biofilm formation assay. In each row of the microplate, four isolates with three repetitions were cultured. Therefore, a total of 31 isolates with 3 blank wells were specified in the depicted microplate.

Statistical analysis

The number and the frequency of qualitative variables and the mean ± SD (Standard deviation) for quantitative variables were assessed. Statistical analysis and data processing were performed via the Chi-square test and independent t-test for all study groups using SPSS version 26 (IBM Corp., USA). Also, statistical significance was determined as *P*-value ≤ 0.05.

RESULTS

Bacterial isolation and identification

Among the 43 non-duplicate patients included in the study, 40 (93%) were VAP-positive, including 26 (65%) males and 14 (35%) females. The mean age of VAP patients was estimated as 65 years old, with a mean duration of ventilation of 12 days. The ventilated patients had different clinical signs, including Dyspnea, Edema, COPD+ (Chronic obstructive pulmonary disease), Tachypnea, Asthma, Hemoptysis, Lethargy, ARDS+ (acute respiratory distress syndrome), Hypertension, Bradycardia, Convulsion, Hemiplegia, and high CRP (C-reactive protein) and ESR (Erythrocyte sedimentation rate) with at least one period of antibiotic therapy. Also, drug abusers were assigned the most predisposing condition among these patients. Out of environmental sampling, 25 (58.1%) were culture positive. Bacterial isolates of 40 clinical samples were identified as 18 (45%) *A. baumannii,* 10 (25%) *Klebsiella pneumoniae*, three (7.5%) *Proteus mirabilis*, two (5%) *Pseudomonas aeruginosa*, two (5%) *Escherichia coli*, two (5%) *Corynebacterium spp.,* and three (7.5%) other *Enterobacterial spp*. Out of 25 environmental samples, seven (28%) *Bacillus spp*. (gram-positive bacilli), six (24%) *Staphylococcus aureus,* three (12%) *A. baumannii,* three (12%) *Klebsiella pneumoniae,* one (4%) *Micrococcus spp.*, one (4%) *Enterococcus spp.*, and four (16%) were other uncommon *Enterobacterial* spp. We did not find any relationship between the source of bacterial isolates and biofilm production (p = 0.093)

Antimicrobial susceptibility testing (AST)

The antibiotic resistance patterns are described in Table 1, Table 2, and Table 3. Out of clinical Gram-negative

bacilli, seven isolates (18.4%) were MDR, 18 isolates (47.3%) were XDR, and 10 isolates (26.3%) were PDR. Out of nine environmental Gram-negative bacilli, 0 isolates (0%) were MDR, six isolates (66.6%) were XDR, and two isolates (22.2%) were PDR. Amikacin was the most effective against Gram-negative bacilli. The MIC results showed that 18 (47.37%) and 20 (52.63%) of clinical Gram-negative bacilli were resistant (MIC≥4 µg/ml) and intermediate (MIC≤2 µg/ml) to colistin, respectively. The MIC results for nine environmental Gram-negative bacilli were three (33.33%) resistant and six (66.66%) intermediate isolates for colistin. Also, the MBC results of 37 clinical and environmental isolates (75.51%) were equal to their MIC results, illustrating the great efficiency of the drug against tested isolates. Further MIC details are described in Table 4. The analysis of the correlation of the drug resistance between clinical and environmental isolates could not be computed because the data were statistically constant.

Biofilm formation assay

Among the 40 clinical isolates, 29 (72.5%) were biofilm producers; among them, *P. aeruginosa* (100%), *P. mirabilis* (100%), and *Acinetobacter spp.* (94.4 %) had the highest rate of biofilm formation, followed by *K. pneumoniae* (60%). Also, among the 25 environmental isolates, 24 (96%) were biofilm producers, and only one *S. aureus* isolate was a non-biofilm producer among all of the environmental bacteria. The highest rate of Strong biofilm producers among environmental isolates was *Acinetobacter Spp.* (100%), *Enterococcus Spp.* (100%), and some other unknown *Enterobacterials* (75%). More details of biofilm formation among the tested isolates are depicted in Figure 2 and Figure 3.

According to Table 5, there is no significant correlation between biofilm formation and drug resistance in high drug-resistant Gram-negative bacilli (*P*-value > 0.05).

Table 1. AST results for non-fermentative Gram-negative bacilli (*A. baumannii and P. aeruginosa*)

Antibiotic	Clinical isolates (n=20)				Environmental isolates (n=3)			
	Sensitive	Sensitive dose-dependent	Intermediate	Resistant	Sensitive	Sensitive dose-dependent	Intermediate	Resistant
Piperacillin/tazobactam	3	-	-	17	-	-	-	3
Amikacin	6	-	-	14	-	-	-	3
Ciprofloxacin	3	-	-	17	-	-	-	3
Cefepime	5	-	-	15	-	-	-	3
Meropenem	2	-	-	18	-	-	-	3

Table 2. AST results for fermentative Gram-negative bacilli (Enterobacteria)

Antibiotic	Clinical isolates (n=18)				Environmental isolates (n=6)			
	Sensitive	Sensitive dose-dependent	Intermediate	Resistant	Sensitive	Sensitive dose dependent	Intermediate	Resistant
Piperacillin/tazobactam	3	2	0	13	0	1	0	4
Amikacin	7	0	2	9	2	0	1	2
Ciprofloxacin	1	0	2	15	1	0	0	4
Cefepime	5	2	0	11	1	0	0	4
Trimethoprim/Sulfamethoxazole	4	0	1	13	3	0	0	2
Meropenem	6	0	2	10	0	0	0	5

Table 3. AST results for gram-positive cocci (*S. aureus*)

Antibiotic	Environmental isolates (n=6)			
	Sensitive	Sensitive dose-dependent	Intermediate	Resistant
Linezolid	5	-	-	1
Tetracycline	3	-	-	3
Ciprofloxacin	1	-	-	5
Trimethoprim/Sulfamethoxazole	3	-	-	3
Cefoxitin	5 (83.33%) MRSA			

Table 4. MIC results for all Gram-negative bacilli tested

Concentrations (µg/ml)	≥256	128	64	32	16	8	4	2	1	0.5	0.25	0.125
Number of isolates	3	1	1	2	2	2	7	12	14	3	0	0
Frequency (%)	6.3	2.1	2.1	4.2	4.2	4.2	14.8	25.5	29.7	6.3	0	0
Total MIC 50*/90†	1/32 µg/ml											

* MIC 50: The MIC concentration in which the growth of 50% of all tested isolates was inhibited

† MIC 90: The MIC concentration in which the growth of 90% of all tested isolates was inhibited

Table 5. The correlation between Biofilm formation and AST patterns of Gram-negative bacilli

Biofilm rate / AST pattern	Non-producer	Weak producer	Moderate producer	Strong producer	*P*-value
Sensitive (n=4)	1 (25%)	2 (50%)	0 (0%)	1 (25%)	0.093
MDR (n=7)	1 (14.3%)	2 (28.6%)	1 (14.3%)	3 (42.9%)	
XDR (n=24)	5 (20.8%)	2 (8.3%)	3 (12.5%)	14 (58.3)	
PDR (n=12)	2 (16.7%)	0 (0%)	3 (25%)	7 (58.3%)	
Total (n=47)	9	6	7	25	-

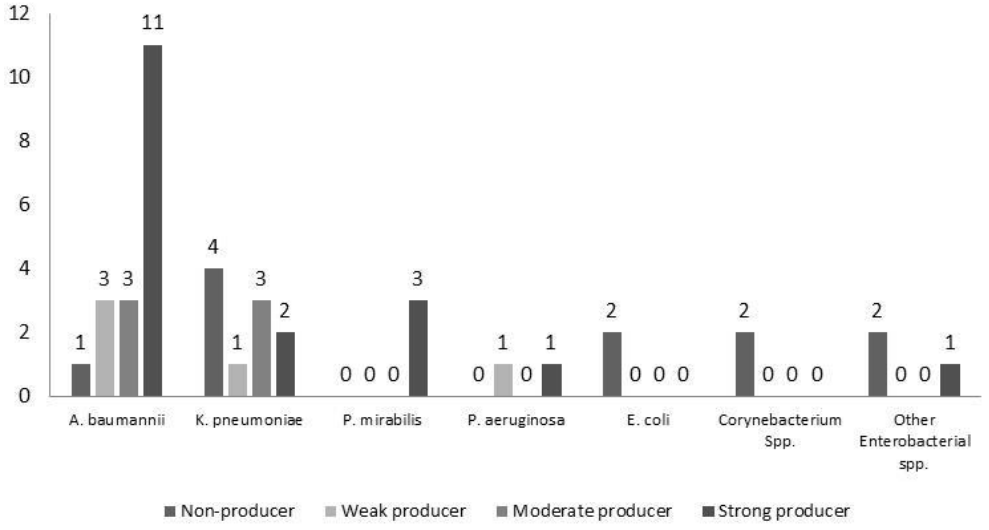

Figure 2. Biofilm formation rate in clinical isolates

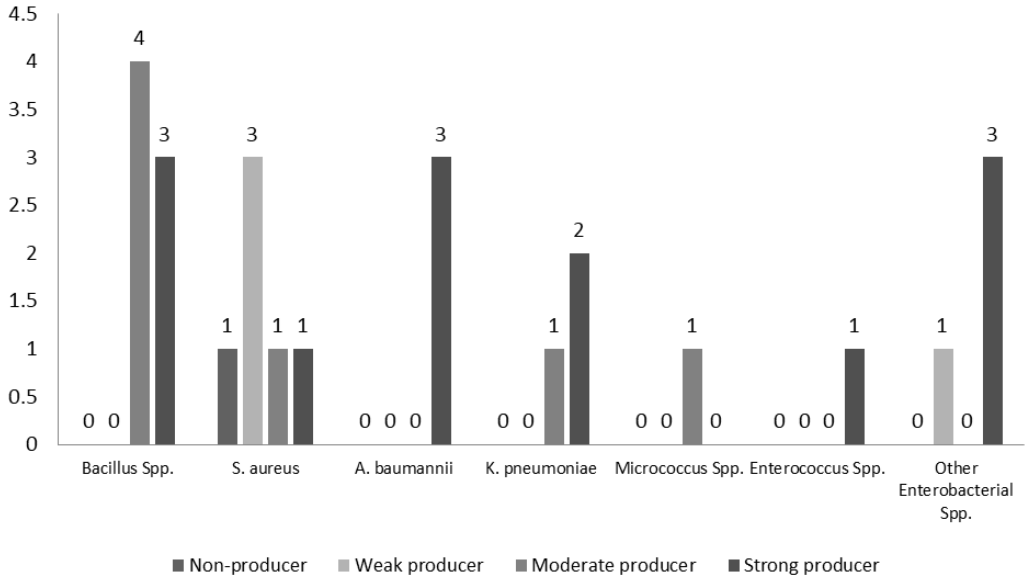

Figure 3. Biofilm formation rate in environmental isolates

DISCUSSION

Ventilator-associated pneumonia is one of the pulmonary and hospital-acquired infections caused by different microbial organisms and causes a high mortality rate among patients hospitalized in ICU wards (1). In this study, we aimed to investigate the etiology of VAP in two major infectious disease hospitals in the city of Urmia, Iran. Also, the impact of the hospital environment on the facilitation of infection was investigated. First, the main risk factors resulting from this research were the use of the ventilator, addiction, and related drug poisoning. Also, aging can be effective in causing this infection. Gram-negative bacteria were taken from VAP patients, including *A. baumannii*, *K. pneumoniae*, *P. aeruginosa*, and *E. coli,* as the most isolated bacteria. These organisms were also isolated in previous studies (19-33), while we did not isolate *S. aureus* in any clinical samples. Furthermore, according to the report of Charles et al., we isolated *Proteus mirabilis* from clinical specimens, and this may be included as a rare etiologic agent of VAP (34). Also, we have isolated *Corynebacterium* species from tracheal samples, and based on the Dargahi et al., Clariot et al., and Nhan et al. studies (24, 35, 36), it is possible to isolate *Corynebacterium* species from VAP patients. This bacterium would be *Corynebacterium macginleyi,* as mentioned in Kebbe and Mador's study (37).

As in many previous studies (19, 21, 24, 25, 32), *A. baumannii* is one of the most prevalent isolated pathogens with high drug resistance and a high rate of biofilm formation, found in clinical samples and hospital environments. It also has a propensity for transmission to the lower respiratory system through ETT in ventilated patients, which makes this opportunistic bacterium very dangerous for hospitalized, critically ill patients and those with immunodeficiencies. *K. pneumoniae* is an encapsulated gram-negative bacterium with an affinity for the respiratory tract system and can cause many lethal infections (26, 27, 30). As mentioned in an earlier study, *K. pneumoniae* is the most common pathogen isolated in pediatric VAP around the world (30). Due to its capsular coating, this bacterium can be a biofilm producer, and this may lead to an increase in the frequency of multidrug-resistant *K. pneumoniae* (MDRKp) in many regions (26, 30). Nevertheless, compared to the mentioned studies (24, 25), *P. aeruginosa* was less frequent in our research. All these gram-negative bacteria are considered pathogenic and

virulent due to high biofilm formation and consequent high drug resistance. It seems they need novel consideration for infection control management in HAPs.

Among environmental isolates, *Bacillus spp*., *S. aureus, A. baumannii*, and *K. pneumoniae* were the most common microorganisms obtained from environmental samples, respectively. In contrast to earlier studies (30, 31, 33), analysis of the results of our study showed no relationship between clinical and environmental bacteria, therefor we can declare that hospital environment contamination had few effects on infection transmission to the hospitalized patients. Thus, it seems that the strict controls of hospital environment pollution applied in the studied hospitals may lead to fewer hospital-acquired infections.

Among the antibiotics tested for Gram-negative bacilli, Amikacin was the best and the most effective treatment option. Similar to the study by Tehrani et al. (32), most of *A. baumannii* and *P. aeruginosa* isolates were resistant to meropenem, piperacillin/tazobactam, and ciprofloxacin. For tested Gram-positive cocci (*S. aureus*), Linezolid was the most effective antibiotic, as also mentioned in the Dargahi et al. study (24), and Ciprofloxacin was not an impressive antibacterial drug against *S. aureus*. In contrast to previous studies (19-21, 24) and similar to the study by Tehrani et al. (32), no *S. aureus* was found in our clinical samples. Unfortunately, the majority of *S. aureus* isolated from the environmental samples in the present study were MRSA and could be transmitted to hospitalized patients. The MIC results for colistin in our study were classified into resistance and intermediate categories according to CLSI 2023 guidelines, unlike in other studies (19, 22-24, 32), which reported the sensitivity of pathogens to colistin. In the present study, the frequency of Gram-negative bacilli with an intermediate pattern to colistin was more than the resistant ones, and most of the tested isolates had a MIC of 1 µg/ml.

All ventilated patients may be at a high risk of acquiring infection with biofilm-producing pathogens. Due to the hard structure of biofilm formed on ETT as a solid route, many antibiotics and other antimicrobial agents could not be effective against these bacteria; thus, it would be the reason for increasing the rate of drug resistance. Similar to the study of Khoshnood et al. (29), our results illustrated that the strongest biofilm-forming bacteria showed an XDR antimicrobial pattern. The antimicrobial pattern of these bacteria may come from the impermeability of the biofilm for most antibiotic classes (Cephalosporines, Fluoroquinolone, Aminoglycosides, and Carbapenems) except colistin, or possess specific resistance genes, including bla_{OXA}-like genes, *VIM, SPM, IMP*, *NDM*, etc. (22, 28, 32). Unfortunately, following the high frequency of the XDR pattern, the PDR pattern was common among clinical and environmentally isolated bacteria. This indicates an alarm for the only treatment option for XDR isolates (colistin), which may become ineffective. Probably, these organisms are also carrying colistin resistance genes such as *MCR* (38). It is recommended that these genes be screened in PDR organisms to verify this hypothesis. Also, most of the MRSAs in this study were weak biofilm producers, in accordance with Mazloomirad's study (19). This may be because of carrying the *mecA* gene responsible for Methicillin resistance in *S. aureus* (MRSA). According to the evidence in our study, unlike previous research, there was no significant correlation between biofilm formation and high levels of drug resistance (19, 23, 26, 28, 29).

This study showed the main etiologic factors of VAP, and the rate of antibiotic resistance and biofilm formation of related pathogens. Due to a lack of some equipment, we could not identify some of the pathogens further than genus, including some of *Bacillus Spp., Corynebacterium Spp, Micrococcus Spp.,* and *Enterococcus Spp.* Also, their antibiotic susceptibility profile was not carried out. Nevertheless, the high rate of antibiotic resistance and biofilm formation showed there is an urgent need for new protocols to control the infection; for example, some anti-microbial and anti-biofilm structures to be used for ETT or new antimicrobial agents to replace recent antibiotics. On the other hand, it is recommended to decrease the time of

hospitalization in ICU wards, or, if possible, reduce the time of intubation and reintubation of the patients.

Finally, we suggest performing the proper antimicrobial susceptibility testing for all uncommon pathogens isolated from the hospital environment, including *Bacillus spp.* and *Micrococcus spp.* It's better to analyze the presence of some uncommon resistance genes, such as *MCR,* in drug-resistant isolates for confirmation of in vitro ASTs and presentation of infection control policies. Also, evaluating the distribution of these highly drug-resistant pathogens in a certain area by molecular typing methods would be impressive and helpful.

CONCLUSION

Out of different clinical isolates of VAP specimens, Gram-negative bacilli, including *A. baumannii, K. pneumoniae, P. mirabilis,* and *P. aeruginosa,* were isolated. Also, *Bacillus spp.* and *S. aureus* were the most frequently isolated environmental bacteria, which indicates no transmission of hospital microbial population to hospitalized patients. The majority of isolates were classified into XDR resistance patterns, showing resistance to common antibiotics except for colistin. The XDR pattern of most isolates was related to strong biofilm formation. Also, AST results of *S. aureus* isolates showed that most of them were MRSA. PCR confirmatory tests for resistance genes may be helpful to verify the mentioned in-vitro tests.

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Ethical approvement

All of the procedures began after ethical approval from the research ethics committee of Urmia University of Medical Sciences with the ethics code (IR.UMSU.REC.1401.301).

Conflict of interest

The authors declare no financial conflicts in this study.

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