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Etiology and Drug Resistance Pattern of Ventilator Associated Pneumonia in an Iranian 1000- Bed Tertiary Care Hospital

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Authors' contributions

This work was carried out in contribution of all authors. Author HB designed the study and wrote the protocol. Authors MR and NR wrote the first draft of the manuscript. Authors HR, HRAA, FB and MS conducted the sampling and analysis. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim: The aim of this study was to determine etiology and drug resistance pattern of most frequency isolates of microorganisms responsible for VAP in an Iranian 1000-bed tertiary care hospital in Tehran Iran.
Place and Duration: This study was conducted in microbiology laboratory of Milad Hospital in Tehran, Iran from November 2010 to December 2011.
Methodology: Ventilator Associated Pneumonia (VAP) was defined as any lower respiratory tract infection that developed 48 hours after mechanical ventilation. Tracheal aspirate specimens were collected and processed according standard microbiological procedures. Bacterial identification and susceptibility testing were performed using disk

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diffusion standard procedures as recommended by CLSI.
Results: One hundred and one patients developed at least one episode of nosocomial pneumonia were subject of our study. Of 101 patients 61 patients were male and 40 female patients. The mean time for hospitalization in ICUs and ventilation duration were 16 and 9, 5 days respectively. Old age, History of previous use of antibiotics and duration of ventilation times were the most important risk factors for VAP. In total 126 microorganisms were isolated from VAP cases. *Acinetobacer baumannii* with 46 (36.5%) isolates was the predominant organism followed by *Staphylococcus aureus* with 31 (24. 60% and *Pseudomonas aeruginosa* with 19 (15%) isolates. Other isolated organisms included *Klebsiella pneumoniae* and *E. coli*. The majority isolated organism included *Acinetobacter baumannii* and *Pseudomonas aeruginosa* were resistant to many antibiotics including the third generation of cephalosporins and nearly 50% isolates were resistant to amikacin. Colistin was the most effective antibiotic against multidurug resistant (MDR) isolates. We found a high rate of methicillin resistant Staphylococcus aureus (93.54%). All isolates of *S. aureus* were susceptible to vancomycin.
Conclusion: Our study revealed that *A. baumannii, S. aureus* and *P. aeruginosa* were the major etiological agents of VAP in our hospital. The majority isolates were resistant to routinely used antibiotics including the third generation of cephalosporins. We also observed a high rate of MRSA among our isolates.

Keywords: Ventilator associated pneumonia; drug resistance.

1. INTRODUCTION

Ventilator-associated pneumonia (VAP) is defined as pneumonia occurring more than 48 hours after patients have been intubated and received mechanical ventilation [1]. The incidence of nosocomial pneumonia in ventilated patients especially in intensive care units (ICUs) is very high and ranging from 7% to more than 40%. Such nosocomial infections prolong hospital stay and causes patients' mortality [2,3]. In the ICUs, the risk of mortality appears to be two to 10 folds higher in patients with nosocomial pneumonia than those patients without. In addition, investigators have reported that nosocomial pneumonia increased the duration of hospitalized patients twofold to threefold compare to patients without nosocomial pneumonia [4]. In spite of remarkable progress in diagnosis and treatment of ventilator associated pneumonia over the recent years, conflicts persist over his optimal methods for diagnosis of VAP applying conventional laboratory methods is critical for identifying specific etiologic agents, for establishing appropriate treatment protocols [3,4,5].

There are still not well accepted gold standards for diagnosis of VAP, but rather there are some diagnosis methods with different sensitivity and specificity [6,7]. Nonbronchoscopic methods, such as blinded bronchoalveolar lavage (BAL) or quantitative endotracheal aspiration, along clinical signs, are more specific than only clinical diagnosis. Other methods such as bronchoscopic methods for obtaining BAL or a protected specimen brushing (SPS) has a higher specificity also than a clinical diagnosis [8,9]. However, regardless of diagnostic method used, the American Thoracic Society Consensus group surest empirically therapy, based on the severity of the patients disease and the stage of onset, using antibiotics to cover common pathogens and patient's specific risk factors [3,4].

The bacteriologic diagnosis of VAP is still a controversial issue. This challenges in microbiology due to differentiation of between organisms responsible for the infection and colonizing normal flora. As motioned above there are many techniques for specimen collection to determine the true etiological agents. Also, some techniques such as bronchoscopic are recommended as a gold standard method; however some researchers have argued against routine use of these techniques and have suggested empiric therapy or less invasive techniques such a tracheal aspiration that is more cost effective approaches in clinical practice [4-9].

The aim of this study was to determine etiologic agents of VAP in patients hospitalized in an Iranian 1000-bed tertiary care hospital. Our other objective was a detection antimicrobial susceptibility pattern of isolated microorganism to improve antibiotic strategy policy in our hospital.

2. MATEREIALS AND METHODS

This prospective study was performed in ICUs of Milad hospital. Milad Hospital is a 1000-bed non-teaching social security hospital. This hospital has an infection control system supervised by the infection control committee of the hospital.

VAP was defined as any lower respiratory tract infections that developed after 2 days of mechanical ventilation. The criteria for clinical suspicion of nosocomial pneumonia included being new or present lung opacity on chest X-ray in addition two of the following items: 1-Fever >38.3ºC or (2) leukocytosis >10,000 cells/mm and/or (3) purulent tracheo-bronchial secretions and purulent endothracheal aspirate [12]. Patients in whom VAP was suspected, a deep tracheal aspiration with quantitative culture performed within 6 hours after the development of a new pulmonary infiltrate. Briefly Endothracheal aspiration performed in aseptic conditions using sterile suction catheters and traps. The presence of epithelial cells of >10% was indication of the specimen contamination whilst <10% neutrophils suggested that the diagnosis of pneumonia was less likely. Quantitative analysis of ETA was done according to gram stain smear interpretation. Depending on the number of organisms seen on direct smear and colony count suspected microorganisms were identified using conventional microbiological methods. After the tracheal aspiration collection, it was transferred into vials containing 1ml of sterile lactate Ringers solution. The vial was vigorously agitated for at least 60s to suspend all the materials from the aspirate. Specimens were immediately sent to the microbiology laboratory for quantitative cultures. Aliquots of 0.01ml of specimen were taken from the original suspension and inoculated in Blood agar, Chocolate agar, MacConkey agar, and Sabouraud Dextrose Agar. All culture plates were incubated at 37ºC for 24 hours Chocolate agar plate was incubated at 10% CO_2. All microorganisms were identified by conventional microbiological methods such as gram-staining and biochemical reactions [10]. Bacterial counts of 10^5 CFU/ml or greater were used as the cutoff point for the diagnosis of VAP [11,12].

Microorganisms susceptibility testing was performed using disk diffusion methods as guideline recommended by Clinical laboratory standard institute [13]. E-test MIC method was used for colistin and vancomycin. Results of susceptibility testing expressed as percentage of susceptible, intermediate or resistant. Demographic data of patients including age, sex history of previous antimicrobial therapy, mechanical ventilation duration were abstracted from the patient's file.

3. RESULTS AND DISCUSSION

In our study a total of one hundred and one VAP patients were involved. Out of the 101 patients, 61 were male and 40 were female. The mean time of staying in hospital and having ventilation were 16 and 9.6 days respectively.

The previous use of antibiotics and duration of ventilation were the major predisposing risk factors for development of VAP.

A total of 126 microorganisms were isolated from specimens of VAP patients, in which *Acinetobacter baumannii* with 46 (36.5%) isolates was the main causative agent followed by *Staphylococcus aureus* with 31 (24.6%) isolates (Table-1). *Pseudomonas aeruginosa* was accounted 19 (15.7%) isolates. Other isolated organisms included *Klebsiella pneumoniae, E. coli* and other miscellaneous gram negative organisms. We have also an isolate of *Candida albicans*. The majority of the microorganisms including *Acinetobacter baumannii* and *Pseudomonas aeruginosa* were resistant to many antibiotics including the third generation of cephalosporins and aminoglycosides (Tables 2 and 3). About 50% isolates were resistant to amikacin. Colistin was the most effective antibiotic against multidrug resistant (MDR) isolates. We found a high rate of methicillin resistant *Staphylococcus aureus* (MRSA). In our study 93.54% of the isolates were MRSA. All isolates of *S. aureus* were susceptible to vancomycin (Table 4)

Table 1. Prevalence of etiologic agents of VAP

Organism	No. of isolates (%)
Staphylococus aureus	31 (24 .60)
Acinetobacter baumannii	46 (36.50)
Pseudomonas aeruginosa	19 (15.07)
Escherichia coli	9 (7.14)
Klebsiella pneumoniae	9 (7.14)
Stenotrophomonas maltophilia	4 (3.17)
Klebsiella planticola	4 (3.17)
Enterobacter cloacae	1 (0.79)
Burkholderia cepacia	1 (0.79)
Proteus mirabilis	1 (0.79)
Candida albicans	1 (0.79)
Total	126 (100)

Ventilator–associated pneumonia continues to be the most common nosocomial infections in ICU hospitalized patients and accounted nearly one third of the total nosocomial infections Patients with VAP have worse outcomes and longer hospital stays and a mortality rate of 15% to 50%. In addition ICU in-patients with VAP increased by a mean of 6.1 days and a high excess cost [14]. A wide variety of pathogenic organisms are etiologic agents of VAP In the management of VAP, specific antimicrobial therapy should be directed at the pathogen involved in each patient. Use of microbiology methods is an attempt to determine etiologic agent of

VAP cases [10]. The distal airways become colonized a few hours after intubation and nearly 10 species of microorganisms were recovered in ventilated patients. Because of their peculiar antibiotic resistance patterns methicillin resistant *S. aureus*, *P. aeruginosa* and *Acinetobacter baumannii* should be considered in the initial decision in the choice of antimicrobial therapy. Many studies have shown that these three organisms are the leading cause of death owing to VAP [14-18]. In Many studies predominant gram-negative microorganisms responsible for VAP were *A. baumannii, P. aeruginosa* and *K. pneumonia and* accounted more than 75% of isolates. Among gram–positive bacteria, *Staphylococcus aureus* was the leading causative agent of VAP [19-22]. In a study from Brazil the microbiological profile associated with VAP was *A. baumannii* (28%). *P. aeruginosa* (19%) and *S. aureus* (20%) [23], which was similar to our results in the present study.

Table 2. Prevalence of Drug resistance among *Acinetobacter baumannii* isolates from the VAPs

Antibiotic	No. of isolates (%)		
	Susceptible	Intermediate	Resistant
Cefotaxim	0 (0.00)	0 (0.00)	46 (100)
Ceftizoxim	0 (0.00)	0 (0.00)	46 (100)
Ceftazidim	0 (0.00)	0 (0.00)	46 (100)
Ceftriaxone	0 (0.00)	0 (0.00)	46 (100)
Imipenem	5 (10.86)	2 (4.34)	39 (84.78)
Tobramycin	2 (4.34)	8 (17.39)	36 (78.26)
Amikacin	6 (13.4)	4 (8.69)	36 (78.26)
Gentamycin	7 (15.21)	8 (17.39)	31 (67.39)
Ciprofloxacin	0 (0.00)	1 (2.17)	45 (97.82)
Tetracycline	2 (4.34)	1 (2.17)	43 (93.47)
Colistin	46 (0.00)	0 (0.00)	0 (0.00)

The prevalence of multidrug-resistant (MDR) organisms has increased over the past decade and a significant rise in these isolates in VAP has been observed. MDR seems to cause major early mortality and an adequate therapy is essential to treat VAP [24]. In our study the majority of the *P. aeruginosa* and *A. baumannii* isolates were MDR resistant to the third generation of cephlosprins was prevalent; and also 84.78% of the isolates were resistant to imipenem. With the increase in resistance to carbapenems, colistin has been extensively used; however some data suggest that the doses recommended are insufficient before a steady state reached, implying that the administration of a loading dose on initiation of treatment may be beneficial. Combinations of antibacterial agents such as impenem plus sulbactam or imipenem plus colistin have been successfully used to treat VAP [25]. In our study colistin was the most effective antibiotic against *A. baumannii* isolates. Among the gram-positive cocci, MRSAs were an important causative agent of VAP. Treatment of VAP caused by MRSA associated with poor outcomes in comparison with MSSA. In our study nearly all isolates were MRSA and vancomycin was the most effective antibiotic against MRSA isolates.

Table 3. Drug resistant patterns of *P. aeruginosa* isolated from the VAPs

Antibiotic	No. of isolates (%)		
	Susceptible	Intermediate	Resistance
Cefotaxim	0 (0.00)	0 (0.00)	19 (100)
Ceftizoxime	0 (0.00)	0 (0.00)	19 (100)
Ceftazidime	2 (10.53)	2 (10.53)	15 (78.94)
Tobramycin	2 (10.53)	2 (10.53)	15 (78.94)
Imipenem	8 (42.10)	3 (15.78)	8 (42.10)
Amikacin	4 (21.05)	1 (5.26)	15 (78.94)
Ciprofloxacin	7 (36.74)	7 (36.74)	5 (36.21)
Gentamicin	4 (21.05)	4 (21.05)	11 (57.89)
Ceftriaxone	0 (0.00)	0 (0.00)	19 (100)
Tetracycline	0 (0.00)	0 (0.00)	19 (100)
Colistin	19 (100)	0 (0.00)	0 (0.00)

Table 4. Prevalence of Drug resistance among *S. aureus* isolates from the VAPs

Antibiotic	No. of isolates (%)		
	Susceptible	Intermediate	Resistant
Penicillin	0 (0.00)	0 (0.00)	31 (100)
Oxacillin	2 (6.45)	0 (0.00)	29 (93.54)
Vancomycin	31 (100)	0 (0.00)	0 (0.00)
Azithromycin	1 (3.23)	1 (3.23)	29 (93.54)
Tetracycline	1 (3.23)	0 (0.00)	30 (96.77)
Clidamycin	0 (0.00)	1 (3.23)	30 (96.77)
Rifampin	6 (19.35)	0 (0.00)	25 (80.64)
Ciprofloxacin	1 (3.23)	1 (3.23)	29 (93.54)
Co-trimoxazole	6 (19.35)	0 (0.00)	25 (80.64)
Erythromycin	2 (6.45)	2 (6.45)	27 (87.09)
Gentamycin	3 (9.67)	2 (6.45)	26 (90.32)
Chloramphenicol	29 (93.54)	1 (3.23)	1 (3.23)

Our study had some limitation and did not allow a complete analysis of all risk factors responsible in VAP and MDR organisms. Also more studies are needed in our country to compare the occurrence of VAP and community acquired pneumonia. We also need cooperation with physicians for providing clinical data and best qualified performance of laboratory practice.

4. CONCLUSION

Our study revealed that *A. baumannii, S. aureus* and *P. aeruginosa* were the major etiological agents of VAP in our hospital. The majority isolates were resistant to routinely used antibiotics including the third generation of cephalosporins. We also observed a high rate of MRSA among our isolates.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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