

1 **Etiology and Drug Resistance Pattern of Ventilator Associated**
2 **Pneumonia in an Iranian 1000- bed Tertiary Care Hospital**

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4 H. Bahrami¹, M. Rahbar^{2,3}, N. Rahimifard⁴, H. H. Mehdipour, H. Rastegar⁴, H. R.
5 Ahmadi Ashtiani¹, F. bagheri¹, M. Saremi

6
7 1-Islamic Azad University, Pharmaceutical Sciences branch, Tehran, Iran.

8 2-Department of Microbiology, Reference Health Laboratories Research
9 Centre, Ministry of Health and Medical Education, Tehran, Iran.

10 3-Antimicrobial Resistance Research center, Iran University of Medical
11 sciences, Tehran, Iran

12 4-Department of pharmacology Food and Drug Laboratories Research
13 Centre (FDLRC); Ministry of Health and Medical Education (MOH). Tehran,
14 Iran

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16 ***Author's contributions*** ,

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

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43 1. Corresponding Author: Dr Mohammad Rahbar: Department of Microbiology, Iranian
44 Reference Health Laboratory Research Center, Ministry of Health and Medical Education.
45 Tehran, Iran

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 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. *Acinetobacter 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 multidrug 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.

Key Words: Ventilator Associated Pneumonia, Drug resistance

INTRODUCTION

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

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

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

122 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.

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 $>38.3^{\circ}\text{C}$ or (2) leukocytosis $>10,000$ cells/mm and/or (3) purulent tracheo-bronchial secretions and purulent endotracheal 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 Endotracheal 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 1 ml 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 0.01 ml 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 37°C 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.

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)

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.

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 cephalosprins 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 imipenem 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, MRSA 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.

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

233 needed in our country to compare the occurrence of VAP and community
234 acquired pneumonia. We also need cooperation with physicians for providing
235 clinical data and best qualified performance of laboratory practice.

236

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Table -1 prevalence of etiologic agents of VAP

Organism	No. of isolates (%)
<i>Staphylococcus aureus</i>	31 (24.60)
<i>Acinetobacter baumannii</i>	46 (36.50)
<i>Pseudomonas aeruginosa</i>	19 (15.07)
<i>Escherichia coli</i>	9 (7.14)
<i>Klebsiella pneumoniae</i>	9 (7.14)
<i>Stenotrophomonas maltophilia</i>	4 (3.17)
<i>Klebsiella planticola</i>	4 (3.17)
<i>Enterobacter cloacae</i>	1 (0.79)
<i>Burkholderia cepacia</i>	1 (0.79)
<i>Proteus mirabilis</i>	1 (0.79)
<i>Candida albicans</i>	1 (0.79)
Total	126 (100)

Table 2- Prevalence of Drug resistance among *Acinetobacterbaumannii* 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)

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)

REFERENCES

1. Goel V, Hogade SA, Karadesai S. Ventilator associated pneumonia in a medical intensive care unit: Microbial aetiology, susceptibility patterns of isolated microorganisms and outcome. Indian J Anaesth. 2012; 56:558-562.
2. Trouillet JL, Chastre J, Vuagnat A, Joly-Guillou ML, Combaux D, Dombret MC, Gibert C. Ventilator-associated pneumonia caused by potentially drug-resistant bacteria. Am J Respir Crit Care Med. 1998; 15:21-29.
3. Nair S, Sen N, Peter JV, Raj JP, Brahmadathan KN. Role of quantitative endotracheal aspirate and cultures as a surveillance and diagnostic tool for ventilator associated pneumonia: a pilot study. Indian Med Sci. 2008; 62:304-313.
4. Craven DE. Epidemiology of ventilator-associated pneumonia. Chest.

- 2000; 117:186S-187S.
5. Rea-Neto A, Youssef NC, Tuche F, Brunkhorst F, Ranieri VM, Reinhart K, Sakr Y. Diagnosis of ventilator-associated pneumonia: a systematic review of the literature. *Crit Care*. 2008; 12:R56.
 6. Ioanas M, Ferrer R, Angrill J, Ferrer M, Torres A. Microbial investigation in ventilator-associated pneumonia. *Eur Respir J*. 2001; 17:791-801.
 7. Wiener-Kronish JP, Dorr HJ. Ventilator-associated pneumonia: problems with diagnosis and therapy. *Best Pract Res Clin Anaesthesiol*. 2008; 22:437-449.
 8. Pugin J, Auckenthaler R, Mili N, Janssens JP, Lew PD, Suter PM. Diagnosis of ventilator-associated pneumonia by bacteriologic analysis of bronchoscopic and nonbronchoscopic "blind" bronchoalveolar lavage fluid. *Am Rev Respir Dis*. 1991; 143:1121-1129.
 9. Wood AY, Davit AJ 2nd, Ciraulo DL, Arp NW, Richart CM, Maxwell RA, Barker DE. A prospective assessment of diagnostic efficacy of blind protective bronchial brushings compared to bronchoscope-assisted lavage, bronchoscope-directed brushings, and blind endotracheal aspirates in ventilator-associated pneumonia. *J Trauma*. 2003 55:825-834.
 10. Perilla MJ, Ajello G, Bopp C, Elliott J, Facklam R, Knapp JS, Popovic T, Wells J, Dowell SF. 2003. Manual for the laboratory identification and antimicrobial susceptibility testing of bacterial pathogens of public health concern in the developing world. WHO/CDS/CSR/RMD.
 11. Bassetti M, Taramasso L, Giacobbe DR, Pelosi P. Management of ventilator-associated pneumonia: epidemiology, diagnosis and antimicrobial therapy. *Expert Rev Anti Infect Ther*. 2012; 10:585-596.
 12. Baselski V, Klutts JS. Point-Counterpoint: Quantitative cultures of bronchoscopically obtained specimens should be performed for optimal management in patients with ventilator associated pneumonia. *J Clin Microbiol*. 2013; 51:3740-3744.
 13. Porzecanski I, Bowton DL. Diagnosis and treatment of ventilator-associated pneumonia. *Chest*. 2006; 130:597-604.
 14. Martin-Loeches I, Deja M, Koulenti D, Dimopoulos G, Marsh B, Torres A, Niederman MS, Rello J; EU-VAP Study Investigators. Potentially resistant microorganisms in intubated patients with hospital-acquired pneumonia: the interaction of ecology, shock and risk factors *Intensive Care Med*. 2013; 39:672-681
 15. Ariza J. Impact of multidrug resistance on *Pseudomonas aeruginosa* ventilator-associated pneumonia outcome: predictors of early and crude mortality. *Eur J Clin Microbiol Infect Dis*. 2013; 32:413-420.
 16. Depuydt PO, Vandijck DM, Bekaert MA, Decruyenaere JM, Blot SI, Vogelaers DP, Benoit DD. Determinants and impact of multidrug antibiotic resistance in pathogens causing ventilator-associated pneumonia. *Crit Care*. 2008; 12:R142.
 17. Chung DR, Song JH, Kim SH, Thamlikitkul V, Huang SG, Wang H, So TM, Yasin RM, Hsueh PR, Carlos CC, Hsu LY, Buntaran L, Lalitha MK, Kim MJ, Choi JY, Kim SI, Ko KS, Kang CI, Peck KR; Asian Network for Surveillance of Resistant Pathogens Study Group. High prevalence of multidrug-resistant nonfermenters in hospital-acquired pneumonia in Asia. *Am J Respir Crit Care Med*. 2011; 184:1409-1417.

18. Rello J, Uldemolins M, Lisboa T, Koulenti D, Mañez R, Martin-Loeches I, De Waele JJ, Putensen C, Guven M, Deja M, Diaz E; EU-VAP/CAP Study Group. Determinants of prescription and choice of empirical therapy for hospital-acquired and ventilator-associated pneumonia. *Eur Respir J*. 2011; 37:1332-1339.
19. Japoni A, Vazin A, Davarpanah MA, Afkhami Ardakani M, Alborzi A, Japoni S, Rafaatpour N. Ventilator-associated pneumonia in Iranian intensive care units. *J Infect Dev Ctries*. 2011; 26:286-293.
20. Talaie H, Sabeti S, Mahdavinejad A, Barari B, Kamalbeik S. A survey of microorganisms and their sensitivity by E-test in ventilator-associated pneumonia at Toxicological-Intensive Care Unit of Loghman-Hakim Hospital. *Acta Biomed*. 2010; 81:210-216.
21. Afjeh SA, Sabzehei MK, Karimi A, Shiva F, Shamshiri AR. Surveillance of ventilator-associated pneumonia in a neonatal intensive care unit: characteristics, risk factors, and outcome. *Arch Iran Med*. 2012; 15:567-571.
22. Rahbar M, Hajia M. Detection and quantitation of the etiologic agents of ventilator-associated pneumonia in endotracheal tube aspirates from patients in Iran. *Infect Control Hosp Epidemiol*. 2006; 27:884-885.
23. Peña C, Gómez-Zorrilla S, Oriol I, Tubau F, Dominguez MA, Pujol M, Ariza J. Impact of multidrug resistance on *Pseudomonas aeruginosa* ventilator-associated pneumonia outcome: predictors of early and crude mortality. *Eur J Clin Microbiol Infect Dis*. 2013; 32:413-420.
24. Vila J, Pachón J. Therapeutic options for *Acinetobacter baumannii* infections: an update. *Expert Opin Pharmacother*. 2012; 13:2319-2336.
25. Rello J, Molano D, Villabon M, Reina R, Quispe R, Previgliano I, Afonso E, Restrepo MI. Differences in hospital-and ventilator-associated pneumonia due to *Staphylococcus aureus* (methicillin-susceptible and methicillin-resistant) between Europe and Latin America: A comparison of the EUVAP and LATINVAP study cohorts. *Med Intensive*. 2012. <http://dx.doi.org/10.1016/j.medin.2012.04.008>.