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ORIGINAL ARTICLE

Ventilator associated pneumonia: microbiology and identification of antimicrobial resistance pattern by disk-diffusion and E.test methods

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ABSTRACT

Background: ventilator- associated pneumonia (VAP) continues to complicate the course of 8-28 % patients receiving mechanical ventilation. The mortality rate for VAP is high, ranges from 24-50% and can reach 76% in some specific settings or when lung infection is caused by high risk pathogens. The etiologic agents widely differ according to the population of patients in an intensive care unit, duration of hospital stay, and prior antimicrobial therapy. Because appropriate antimicrobial treatment of patients with VAP significantly improves outcome, more rapid identification of infected patients and selection of antimicrobial agents represent important clinical goals. Our goal was determination of the VAP incidence, identification of common pathogenic causes and determination of antimicrobial resistance pattern by disk-diffusion and E.Test methods.

Materials and methods: In an observational study we evaluated the microbiology and antimicrobial resistance pattern of VAP in medical Intensive Care Units (ICUs) of 2 teaching hospitals from January 2005 to January 2006. Diagnostic criteria for VAP were the radiographic appearance of a new or progressive and persistent pulmonary infiltrate in conjunction with at least 2 of the following criteria: purulent respiratory secretions, temperature > 38.5 ^{oc} or < 35^{oc}, leukocyte count >10,000 mm^3 or < 1,500/mm^3. Mini–BAL was planned for all of suspicious cases. For each causative pathogen, antibiotic susceptibility was determined by disk-diffusion and E.Test methods.

Results: Among 114 patients under mechanical ventilation, 6 patients (5.3%) had a VAP episode, 3 patients in each hospital. In VAP patients the mean days of hospitalization were 26.3 days (± 20.92) and ICU stay were 22.8 days (± 21.53). There was a significant statistical correlation between use of H2 blockers and VAP (P< 0.05). Pseudomonas aeruginosa and Klebsiella pneumonia were isolated by mini-BAL (>$10^{4cfu/ml}$) in 6 patients (each pathogen in 3 cases). Antimicrobial susceptibility pattern were determined by disk-diffusion and E.Test methods and all of pathogens except one (pseudomonas aeruginosa) were resistant to ceftriaxone and ceftazidime.

Conclusion: This study showed the increasing incidence of resistance to third and fourth generations of cephalosporins among gram negative bacilli that has occurred in parallel with increasing use of these drugs in our ICUs. More judiciouse use of antibiotics will be necessary to limit this trend.

Keywords: *Ventilator associated pneumonia, Incidence, Antimicrobial susceptibility pattern.*
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INTRODUCTION

Ventilator-associated pneumonia (VAP), one form of nosocomial pneumonia specifically refers to pneumonia developing in mechanically ventilated or intubated patients more than 48 hours after intubation (1). Mechanical ventilation can increase the risk of VAP 7-21 times and 10-25% of patients undergoing mechanical ventilation develop pneumonia. One study demonstrated 17.5% of

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patients develop VAP 9±5.9 days after admission to Intensive Care Unit (ICU) and the cumulative risk increased over time, but the daily hazard rate decreased after 5 days. Several risk factors for VAP have been mentioned such as primary admitting diagnosis of burns; trauma; CNS, respiratory and cardiac disease; mechanical ventilation in the previous 24 hours and paralytic agents (2).

Another study showed that the mean interval between intubation, admission to the ICU, hospital admission, and the identification of VAP was 3.3 days, 4.5 days, and 5.4 days, respectively. Identified independent risk factors for the development of VAP were male gender, trauma, and intermediate deciles of underlying illness severity. Hospital mortality did not differ significantly between cases and matched control subjects. Nevertheless, patients with VAP had a significantly longer duration of mechanical ventilation, and hospital stay (3). Chastre and Fagon concluded in their study about VAP that in contrast to infections of more frequently involved organs (e.g. UTI) which mortality rate is low, the mortality rate for VAP can reach 76% in some specific settings (4).

Microorganism responsible for VAP may differ according to the population of patients in the ICU, the duration of hospital and ICU stays, and the specific diagnostic methods used. The high rate of respiratory infections due to gram negative bacilli in this setting has been documented (5-7).

Resistance rate of gram negative bacilli to penicillins, cephalosporins, Fluoroquinolones and aminoglycosides varies between hospitals, but is generally increasing. Van Eldere studied about pseudomonas aeruginosa susceptibility patterns in nosocomial infections. Of the fluoroquinolones, ciprofloxacin showed the least resistance (24%), of the aminoglycosides, amikacin was the most potent antibiotic (10.5% resistance), of the beta-lactam antibiotics meropenem was the most active (9.5 % resistance), and piperacillin/tazobactam had 17.5 % resistance (8). During several years we confronted nosocomial infections (including VAP) by resistant gram negative bacilli especially pseudomonas and klebsiella in our teaching hospitals (Firoozgar and rasoul-e-Akram). The purpose of this prospective study was to identify the incidence of VAP in 114 consecutive patients under mechanical Ventilation from January 2005 through January 2006 in two university hospital ICUs with particular attention given to etiology and antimicrobial resistance pattern identified by disc-diffusion and E. Test methods.

PATIENTS and METHODS

In an observational study we evaluated the microbiology and antimicrobial resistance pattern of VAP in medical ICUs of 2 teaching hospitals (Firoozgar and Rasoul-e–Akram hospitals) from January 2005 to January 2006.

According to standardized procedures, we abstracted the following information prospectively from each patient more than 48 hours under mechanical ventilation (114 patients) : demographic data (age, sex), cause of admission, comorbidities (COPD, CHF, Renal failure), length of ICU stay period, duration of ventilation therapy, prior antibiotics, H2-blocker and Glucocorticoid use, and occurrence of VAP. Exclusion criteria were: 1) pneumonia being the cause of admission, 2) intubation without mechanical ventilation in ICU, 3) patients who were suffering from chest trauma. Diagnostic criteria for VAP were the radiographic appearance of a new or progressive and persistent pulmonary infiltrate in conjunction with at least two of the following criteria: purulent respiratory secretions, temperature > 38.5^{oc} or < 35 ^{oc}, leukocyte count > 10,000 mm^3 or <1,500 /mm^3. Establishment of etiologic diagnosis required isolation of bacteria in significant quantity from a sample of lower respiratory tract secretions (mini-BAL >$10^{4cfu/ml}$). Only the first episode of

documented VAP was taken into account. Acquired specimens were transferred to the microbiological laboratory. By using a modified twofold dilution scheme we determined the quantitative culture in cfu/ml. The media and incubation conditions were appropriate for the cultivation of the most probable respiratory pathogens. For each causative pathogen, antibiotic susceptibility was routinely determined by a disk–diffusion and E.Test methods (ceftrixone, ceftazidime, Amikacine, cefepime, Imipenem). Statistical analysis was performed using SPSS ver. 11.5 software. Differences between groups with VAP and non-VAP were calculated by using the t-test and X^2. P. values less than 0.05 were considered significant. Frequency, mean, and SD were applied to report statistical information.

RESULTS

During the study period, 114 patients were under mechanical ventilation (33 patients in Firoozgar and 81 patients in Rasoul-e-Akram hospital). Among them only 6 patients (5.3%) met VAP criteria, 3 patients in each hospital (3.7% in Rasoul-e-Akram hospital and 9.1% in Firoozgar hospital). There were 59 women (51.8%) and 55 men (48.2%), the mean age of patients (±SD) was 63 years (±16.8). Of the 6 patients with VAP, 3 patients were men. The mean days of staying in hospital (±SD) were 18.1 days (±11.65) and staying in ICU were 12.6 days (±10.07). In 6 patients with VAP the mean days of staying in hospital (±SD) were 26.3 days (±20.92) and staying in ICU were 22.8 days (±21.53). Of the 114 patients, 9 patients (8%) had abnormal chest X Ray (including 6 patients with VAP) like consolidation, pleural effusion, cavitation and pneumothorax.

Eighty nine of 114 cases (78.1%) died (all of VAP patients). The causes of death in non VAP patients were underlying diseases and in VAP patients was uncontrolled sepsis. Risk factors associated with VAP were shown in Table 1.

Table 1. Medication and comorbidities as risk factors in 114 ICU patients.

Risk factors	OR	95%CI	P
prior use of Antibiotics	<1	0.9-2.1	NS
prior use of H2 blockers	2.5	1.6-6.4	<0.05
prior use of GC	<1	0.8-3.2	NS
COPD	<1	0.5-2.8	NS
CHF	<1	0.4-2.6	NS
Renal failure	<1	0.5-1.8	NS

NS: Not Signifigant

Pseudomonas aeruginosa in 2 cases, and klebsiella pneumonia in one case in Firoozgar hospital, and klebsiella in 2 cases and pseudomonas in one case in Rasoul-e-Akram hospital were isolated. We showed antimicrobial sensitivity pattern (Reference amount) and antimicrobial susceptibility pattern of 6 pathogens by E. Test method in table 2 and 3 respectively. All of 6 pathogens were resistant to third generation cephalosporins by disk- diffusion method.

Table 2. Antimicrobial sensitivity pattern (Reference amount microg/ml).

Antibiotics	sensitive	Intermediate	Resistance
Cefepime	≤ 8	16	≥ 32
Ceftriaxone	≤ 8	16-32	≥ 64
Cefazidime	≤ 8	16	≥ 32
Imipenem	≤ 4	8	≥ 16
Amikacin	≤ 16	32	≥ 64

Table 3. Antimicrobial susceptibility pattern by E. Test method.

	Pathogen					
	1	2	3	4	5	6
Cefepime	0.75(S)	48 (R)	64 (R)	36 (R)	96 (R)	6 (S)
Ceftriaxone	6(S)	96 (R)	128 (R)	48 (R)	96 (R)	128 (R)
Cefazidime	1(S)	64 (R)	64 (R)	48 (R)	64 (R)	96 (R)
Imipenem	2(S)	24 (R)	1.5 (S)	3 (S)	8 (I)	1 (S)
Amikacin	1.5(S)	32 (I)	8 (S)	32 (I)	98 (S)	8 (S)

DISCUSSION

The prevalence of nosocomial infection is higher in ICUs than in general hospital wards.

Catheter-related blood stream infections, ventilator-associated pneumonias, and surgical site infections cause the majority of these infections, which result in a considerable increase of morbidity, mortality, and cost. Overall length of stay, stay in the ICU, and duration of mechanical ventilation are prolonged among surviving patients.

All physicians who treat VAP in an ICU face a dilemma. Numerous data suggest that inadequate empirical antimicrobial therapy is associated with increased mortality. Conversely, it is also clear that excessive use of antibiotics leads to emergence of resistant pathogens (9-13). There are some suggestive strategies in order to minimize the risk of emergence of resistant strains: restriction of the use of some antibiotics specifically third generation cephalosporins, circulation of antibiotics and, finally use of broad spectrum antibiotics in initial empiric therapy followed by a subsequent spectrum reduction (14). The latter strategy employing an initial antimicrobial treatment based on updated data of local epidemiological conditions (incidence and susceptibility of organisms), followed by de-escalation after identification of causative bacteria and determination of antimicrobial susceptibility is now advocated and or performed by numerous authors (15-18).

According to these suggestions we decided to identify the incidence of VAP in our ICUs and antimicrobial susceptibility pattern. Of 114 patients under intubation 6 patients (5.2%) suffered from VAP. The low number of VAP in comparison to other studies is probably due to short duration of study. Chester and colleague reported that VAP can complicate the course of 8-28% of patients receiving mechanical ventilation (4). Craven and colleagues studied about nosocomial pneumonia in 233 ICU patients requiring mechanical ventilation and reported that 21% of the patients suffered from VAP (19).

In our study all of 6 patients with VAP used H2 blockers (P. Value <0.05), like another study that reported H2 blockers are risk factors for VAP (20). According to meta-analyses of the efficacy of stress ulcer prophylaxis in ICU patients, respiratory tract infections were significantly less frequent in patients treated with sucralfate than in those receiving antacids or H2 blockers(4).

P. values for other variables were not significant. We think it is due to the small number of VAP patients and we need a large study to evaluate other risk factors. Unlike community-acquired pneumonia, it may be difficult to determine whether pneumonia has developed in a hospitalized ventilator- dependent patient and there is conflict data about diagnosis of VAP. Rouby and colleagues reported that mini-BAL identified 77% of causative microorganisms of VAP (20). Torres and colleagues assessed the accuracy of clinical parameters for the diagnosis of VAP, as well as the diagnostic value of several invasive techniques, such as protected specimen brush (PSB), BAL, fiberoptic bronchial aspirates (FBAS), and percutaneus lung needle aspiration (PLNA). They compared the results of these techniques with the histopathology of immediate postmortem pulmonary biopsies, considered as the gold standard reference test. They found the following sensitivities for PSB, BAL FBAS, and PLNA: 36, 50, 44 and 25%, and the related specificities were 50, 45, 48, and 79% respectively. They reported that the sensitivities and specificities of different invasive techniques are much lower than those reported in clinical studies (21). In another study authors reported nonbronchoscopic techniques (blind bronchial sampling and mini-BAL) is preferable to BAL and protected specimen brush for the diagnosis of VAP (22). Kollef and colleagues showed that mini-BAL is a safe and technically simple procedure for obtaining quantitative lower airway cultures in patients requiring mechanical ventilation. Quantitative culture results obtained by mini-BAL were similar to those obtained by the protected specimen brush technique (23). Shorr did a meta analysis about invasive approaches to the diagnosis of VAP and

reported invasive strategies do not alter mortality but affect antibiotic use and prescribing (24), but fagor showed an invasive management strategy was significantly associated with fewer death at 14 days, earlier attenuation of organ dysfunction, and less antibiotic use in patients suspected of having VAP, compared with a noninvasive strategy (25). In a pilot study in Spain, the impact of bronchoscopy was to lead to more frequent antibiotic changes with no change in mortality (26). Unfortunately, we don't have any research about this conflict data in Iran and we think further studies with larger population samples are warranted to confirm these data in our country.

The results of many epidemiologic investigations have clearly demonstrated a direct relationship between the use of antimicrobial agents and increased resistance of Enterobacteriaceae and other pathogens. The authors of several studies of VAP have reported increased rates of multi-resistant bacteria (27). Many P.aeruginosa and A.baumannii strains have become class I cephalosporinase producers and are resistant to piperacillin, Aztreonam, and ceftazidime. K.pneumoniae and other Enterobacteriaceae strains are also increasingly being recognized as producers of transferable extended spectrum B-lactamase, which confer resistance to third-generation cephalosporins.

Unfortunately irrational use of third generation cephabsporins in Iran has led to emergence of multi resistant pathogens specifically in ICUs. In our study we isolated Pseudomonas aeruginosa and Klebsiella pneumoniae in 6 patients. Among these only 1 pathogen was susceptible to ceftriaxone and ceftazidime.

We suggest that fourth generation of cephalosporins can not be used in treatment of VAP because Klebsialla pneumonia in 3 patients was resistant to cefepime. According to antimicrobial susceptibility patterns Imipenem and Amikacin are rational choices.

We had similar results in disc-diffusion and E. Test methods. The cost of E. Test method is higher than disc-diffusion, so we may rely on results of disc-diffusion to identify antimicrobial susceptibility pattern, albeit only for gram negative bacilli.

These data suggest that the initial selection of antibiotics for the empiric treatment of VAP in our ICUs should be broad enough to cover all likely pathogens, including antibiotic-resistant bacteria.

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REFERENCES

1. Kullef MH. The prevention of ventilator-Associated pneumonia. N Engl J Med 1999;340:627-34.

2. Cook DJ, walter SD, cook RJ, Griffith LE, Guyatt GH, Leasa D, et al, editors. Incidence of ank risk factors for ventilator- associated pneumonia in critically ill patients. Ann Intern Med 1998;129(6): 433-40.

3. Rello J, Ollendor f DA, Oster G, Vera-Lionch M, Bellm L, Redman R, et al. Epidmiology and outcomes of ventilator- associated pneumonia in a large US data base. Chest 2002;122(6):2115-21.

4. Chastre J, Fagon JY. Ventilator- associated pneumonia. Am J Respir Crit Care Med 2002;165(7):867-903.

5. Fagon JY, chastre J, Domart Y, Trouillet JL, Pierre J, Darne C, et al. Nosocomial pneumonia in patients receiving continuous mechanical ventilation. Prospective analysis of 52 episodes with use of a protected specimen brush and quantitative culture teachiques. Am Rev Respir Dis 1989;139:877-84.

6. Torres A, Aznar R, Gatell JM, Jimenze P, Gonzalez J, Ferre A, et al. Incidence, risk, and prognosis factors of nosocomial pneumonia in mechanically ventilated patients. Am Rev Respir Dis 1990;142:593-98.

7. Rello J, Ausina V, Ricart M, Castella J, prats G. Impact of previous antimicrobial therapy on the etiology and outcome of ventilator- associated pneumonia. Chest 1993;104:1230-35.

8. van Eldere J. Multicentre surueillance of pseudomonas aeruginosa susceptibility patterns in nosocomial infections. J Antimicrob chemother 2003;51(2):347-52.

9. Namias, samiian L, Nino D, shirazi E, O'Neill K, kett DH. Incidence and susceptibility of pathogenic bacteria vary between intensive care units within a single hospital: implications for empiric antibiotic strategies. J Trauma 2000;49:638-45.

10. Luna CM, Vujacich, Niederman MS, Vay C, Gherardi C, Matera J, et al. Impact of BAL data on the therapy and outcome of ventilator-associated pneumonia. Chest 1997;111:676-85.

11. Kollef MH, ward S. The influence of mini-BAL cultures on patients outcomes: implications for the antibiotic management of ventilator-associated pneumonia. Chest 1998;114:412-20.

12. Moine P, Timsit JF, de Lassence A, Troche G, Fosse JP, Albertic C, et al. Mortality associated with Late-onset pneumonia in the intensive care unit: results of a multi-center cohort study. Int care med 2002;28:154-63.

13. Iregui M, ward S, Sherman G, Fraser VJ, kollef MH. Clinical importance of delays in the initiation of appropriate antibiotic treatment for ventilator–associated pneumonia. Chest 2002;122:262-68.

14. Paterson DL, Rice LB. Empirical antibiotic choice for the seriously ill patient: are minimization of selection of resistant organisms and maximization of individual outcome mutually exclusive? Clin Infect Dis 2003;36:1006-12.

15. Kollef MH. Hospital- optimizing antibiotic therapy in the intensive care unit setting. crit care 2001;5:184-95.

16. Kollef MH. Hospital- acquired pneumonia and de-escalation of antimicrobial treatment. Crit care med 2001;29:1473-75.

17. Hoffken G, Niederman MS. Nosocomial pneumonia: the importance of a de-escalating strategy for antibiotic treatment of pneumonia in the ICU. Chest 2002;122:2183-96.

18. Leone M, Bourgoin A, Combon S, Dubuc M, Albarese J, Martin C. Empirical antimicrobial therapy of septic shock patients: adequacy and impact on the outcome. Crit care med 2003;31:462-67.

19. Craven DE, Kunches LM, Kilinsky V, Lichtenberg DA, Make BJ, McCabe WR. Risk factors for pneumonia and fatality in patients receiving continuous mechanical ventilation. Am Rev Respir Dis 1986;133(5):792-96.

20. Rouby JJ, Martin De Lassale E, poete P, Nicolas MH, Bodin L, Jarlier V. Nosocomial bronchopneumonia in the critically ill. Histologic amd bacteriologic and aspects. Am Rev Respir Dis 1992;146(4):1059-66.

21. Torres A, el-Ebiary M, padro L, Gonzalez J, de la Bellacasa JP, Ramirez J. Validation of different techniques for the diagnosis of ventilator-associated pneumonia. Comparison with immediate postmortem pulmonary biopsy. Am J Respir crit care med 1994;149(2 pt 1):324-31.

22. Papzian L,Thomas P, Garbe L, Guignon I , Thirion X, charrel J, et al. Bronchoscopic or blind sampling techniques for the diagnosis of ventilator-associated pneumonia. Am J Respir crit care Med 1995;152:1982-91.

23. Kollef MH, Bock KR, Richards RD, Hearns ML. The safety and diagnostic accuracy of mini bronchoalveolar lavage in patients with suspected ventilator-associated pneumonia. Ann Intern Med 1995;15:743-48.

24. Shorr F, sherner JH, Jackson WL, Kollef MH. Invasive approaches to the diagnosis of ventilator-associated pneumonia: a meta-analysis. Crit care Med 2005;33(1):46-53.

25. Fagon JY, chastre J, Wolff M, Gervais C, Parer-Aubas S, Stephan F, et al. Invasive and noninvasive strategies for management of suspected ventilator-associated pneumonia. A randomized trial. Am Intern Med 2000;132(8):621-30

26. Sanchez-Nieto JM, Torres A, Garcia-Cordoba F, El-Ebiary M, carrilo A, Ruiz J, et al. Impact of invasive and noninvasive quantitative calture sampling on outcome of ventilator-associated pneumonia: a pilot study. Am J Respir Crit Care 1998;157(2):371-76.

27. No authors listed. National Nosocomial Infections Surveillance (NNIS) System report, data summary from January 1990-May 1999, issued June 1999. Am J Infect Control 1999;27(6):520-32.