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Short Communication

Polymicrobial ventilator associated pneumonia and antibiotic susceptibility of bacterial isolates in a university hospital, Tabriz, Iran

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Ventilator-associated pneumonia (VAP) is a common infection, developed in intensive care units (ICU). Early diagnosis and appropriate selection of antimicrobial therapy is important to reduce the mortality rate of these patients. Bronchoalveolar lavage (BAL) fluid was obtained from 27 patients, who fulfilled the criteria for ventilator-associated pneumonia. Samples underwent cytological and bacteriological analysis. Pathogens identified from culture of BAL and related antibiotic susceptibility was determined. Ventilator-associated pneumonia was determined in 39.70% of patients in the ICU. Out of enrolled patients, 92.59% had a polymicrobial infection. The most common form of poly microbial infection was with two different bacterial species which were isolated in 81.48% of patients. These isolates revealed a prominent susceptibility to Imipenem, Amikacin, Ciprofloxacin and Ceftazidime. According to the increasing rate of polymicrobial infections and bacterial drug resistance pattern, monotherapy in the treatment of VAP should be avoided. Based on our study, combination therapy with Imipenem or Ceftazidime accompanied by Amikacin or Ciprofloxacin can be recommended.

Key words: Ventilator-associated pneumonia, polymicrobial, infection, bacterial-resistance, bronchoalveolar lavage (BAL).

INTRODUCTION

Hospital-acquired pneumonia (HAP) is one of the most common nosocomial infections and the leading cause of death due to hospital-acquired infections (attributable to mortality 33-50%). Among HAP patients, ventilator-associated pneumonia (VAP) is the second most common nosocomial infection and has the highest morbidity and mortality.

VAP specifically refers to pneumonia developing in a patient on mechanical ventilator for more than 48 h after intubation or tracheostomy and its estimated incidence is

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10-20% (Jakribettu and Boloor, 2012; Diaz et al., 2010). In the United States, approximately five to ten HAP episodes per 1,000 hospital admissions occur or about 200 to 400 thousand HAP episodes per year, according to admission statistics from the US (Wilke and Grube, 2013). The crude intensive care units (ICUs) mortality rates for VAP range from 24 to 76%, and these patients are twice as likely to die as those patients on ventilator without pneumonia. Other than being an independent determinant of mortality; VAP is associated with longer ICU and hospital stays, prolonged mechanical ventilation, and higher costs (Behnia et al., 2014; Choudhuri, 2013).

The etiologic agents of VAP widely differ according to the population of patients in an intensive care unit, duration of hospital stay, and prior antimicrobial therapy (Golia et al., 2013; Wiener-Kronish and Dorr, 2008.

VAP was diagnosed in the presence of new and/or progressive pulmonary infiltrates on a chest radiograph, plus two or more of the following criteria: fever (≥ 38°C) or hypothermia (< 36°C), leucocytosis (WBC>11000/ L) or leukopenia (WBC<4000/L), purulent tracheobronchial secretions or a reduction of partial pressure of arterial oxygen (PaO_2)/fraction of inspired oxygen (FiO_2) of 15% or greater, in the past 48 hours (Mietto et al., 2013a). Accurate and timely diagnosis is important for initiating appropriate antibiotic therapy and avoiding further complications while avoiding unnecessary therapy and potential treatment-associated complications and cost (Jonker et al., 2012).

The treatment of ventilator-associated pneumonia (VAP) must be based on appropriate diagnosis, which can be done by microbiological examination of the samples obtained from the respiratory tract by bronchoscopic bronchoalveolar lavages (Medell et al., 2012a; Estella and Alvarez-Lerma, 2011). Studies have shown that use of bronchoscopic alveolar lavage (BAL) improves the outcomes of patients with suspected VAP (Su et al., 2012; Poddar, 2012).

As the type of etiologic agents and their susceptibility to antimicrobial medication widely differ according to the region and hospital; knowing the local microbial flora causing VAP and effective infection control practices are essential to improve clinical outcomes. The aim of this study was to identify the bacterial pathogens causing VAP in the intensive care unit (ICU) of Imam Khomeini Hospital and determine their antibiotic profile and further use data in treatment of VAP episodes.

MATERIALS AND METHODS

This study was approved by the ethic committee of Tabriz University of Medical Sciences, a written consent was obtained from all the patients or a legal next relative.

Patient recruitment

This prospective study was performed in the Department of Pulmonary and Intensive Care Medicine of Imam Khomeini Hospital in association with the bacterial laboratory of Microbiology Department. Sixty eight patients who were on mechanical ventilation for more than 48 h in the ICU were included in our study.

Our screening method to clinically diagnose VAP was recent or progressive pulmonary infiltrations on a chest radiograph plus two or more of the following criteria: Fever (≥ 38°C) or hypothermia (< 36°C); leucocytosis (WBC>11000/L) or leukopenia (WBC<4000/L).

Macroscopic purulent tracheobronchial secretion

Twenty seven patients of the study population matched the mentioned criteria. BAL fluid was obtained from all enrolled patients by bronchoscopy. Samples were transported to the laboratory immediately and underwent cytological and bacteriological analysis. Moreover, a proforma including the age, sex, underlying clinical condition, duration of hospitalization, previous immunosuppressive and anti-acid intake, other medical interventions, and duration of ventilator administration in the ICU was noted to evaluate the underlying factors in the incidence of VAP.

Bacterial analysis

Samples were homogenized by vortex and sterile glass beads for 1 min. 1/10, 1/100 and 1/1000 dilutions of homogenized BAL samples were obtained with sterile normal saline solution. The 0.01 micL of sample solution and 100 micL of diluted samples were plated on blood agar, MacConkey agar and chocolate agar. All the samples were incubated at appropriate temperatures. Identification on the isolated colonies was carried out according to standard microbiological tests. In addition, 100 micL of diluted samples which were incubated on Blood agar plates were processed to quantitative culture and colony count.

Cytological analysis

A sample of collected BAL fluid was centrifuged to separate the supernatants from the cell pellet. Sediments were subjected to Gimsa and Gram stain. Morphological features of microorganisms and infected cell count were obtained by means of staining.

Identification of pathogens and antibiotic susceptibility

The following criteria were considered to diagnose the pathogens causing VAP: A quantitative threshold of $>10^4$ colony-forming units (CFU)/mL for a specific bacterial specie on quantitative culture; Presence of intracellular bacteria within >2 % of cells.

For evaluation of antibiotic susceptibility, antibiotic sensitivity testing was performed on agar plates by Kirby-Bauer disc diffusion method.

Statistical analysis

The statistical analysis was performed using descriptive methods. The results were expressed as percentages for the analysis of various data. Calculations were performed using SPPS 12.0. Parametric data were presented as mean ± SD.

RESULTS

A total of 68 patients, who were on mechanical ventilation

Table 1. Demographic characteristics of the study population.

Patients	Value
Age	52.33
Male	19
Female	8
Duration of mechanical ventilation	23.10
Underlying disease	27
Immune -compromised	3
COPD	6
Total number of population	27

Table 2. Distribution of two bacterial species isolated from patients with VAP.

Organism	Number
Pseudomonas – Enterobacter	6
Pseudomonas – Serratia	5
Pseudomonas - Klebciella	4
Pseudomonas – E. coli	1
Pseudomonas - Citrobacter	1
Pseudomonas - S. aureus	1
Klebciella - Citrobacter	1
Acinetobacter- Citrobacter	1
Acinetobacter - E. coli	2
Total	22

in ICU, were included in this study. Twenty seven subjects fulfilled the clinical criteria for the diagnosis of VAP. Among them, 19 were male (70.37%) and 8 were female (29.62%). Their mean age was 52.33 years. Time duration of mechanical ventilation was shown to be median 23.10 days. 16 patients (59.25%) were detected with hyperthermia.Out of 27 patients, 7 cases (25.9%) had another underlying disease. COPD was the most common underlying problem, which accounted for 6 (22.2%) of them and diabetes mellitus was noted in one of the patients. Three patients (11.10%) suffered from immune-compromised state and 20 of the cases were using antacid or H2 blocker medication. Other medical interventions such as thoracostomy was done on 13 patients (48.14%) (Table 1).

VAP was determined in 39.70% of our study population. Twenty seven BAL fluid specimens were analysed. The majority, that is, 92.59% of the BAL fluid samples contained poly microbial infections. Mono microbial infections were diagnosed in 2 of the cases (7.41%), who did not have the history of antibiotic medication.

In poly microbial episodes of VAP, Gram-negative isolates were predominant with 96.44% frequency. Out of 25 poly microbial specimens, three different bacterial species were isolated in three patients (11.11%), and 2 different bacterial species were isolated in 22 patients (81.48%) (Tables 2 and 3).

Table 3. Distribution of three bacterial species isolated from patients with VAP.

Organisms	Number
Pseudomonas – Enterobacter - Citrobacter	1
Pseudomonas – Acinetobacter - Haemophilus	1
Pseudomonas – Klebciella - Ecoli	1
Total	3

Pseudomonas aerogionosa–Enterobacter isolates accounted for 27.27% of VAP cases followed by *P. aerogionosa–Serratia* isolates, which were responsible for 22.72% cases, and *P. aerogionosa-Klebciella* isolated from 18.18% patients.

Antibiotic sensitivity patterns of micro-organisms were detected against common antibiotics using the disc agar diffusion method. All mono microbial isolates showed significant sensitivity to Amikacin and Ciprofloxacin. The most predominant susceptibility to Amikaci, Ciprofloxaci, and Ceftriaxone was detected in the polymicrobial infections with three bacteria, and in two-microbial VAPs, the highest sensitivity to Imipenem, Amikacin, Ciprofloxacin and Ceftazidime was determined.

In this study, the most commonly identified bacteria was shown to be *P. aerogionosa*, accounting for 84% of VAP cases. *P. aerogionosa* strains showed the most predominant sensitivity to Ceftazidime and Ciprofloxacin (52.94%) followed by Amikacin (47.5%) and Imipenem (35.29%). On the other hand, 3 strains of *P. aeruginosa* (14.28%) were resistant to all the known antibiotics.

DISCUSSION

VAP is the most common infection seen in intensive care units (ICUs). It continues to complicate the course of 8 to 28% of patients receiving mechanical ventilation (Mietto et al., 2013b; Su et al., 2012). The incidence of VAP was reported to be 35.14 and 44.2% in earlier studies (Golia et al., 2013; Jakribettu and Boloor, 2012). In our study, it showed to be 39.70% among the mechanically ventilated patients.

The causative pathogens for VAP vary according to case mix, methodology of sampling and local resistance patterns. Poly microbial episodes are also common (Mietto et al., 2013b). In Charles et al. (2013) study, 72.2% of VAP patients had mono microbial and 27.8% had poly microbial infection. Combes et al. (2002) reported mono microbial infections in 52% and poly microbial infections in 48% of VAP patients. Our study results demonstrate the greater prevalence of poly microbial isolates, as compared to the mono microbial infection, which were 92.59 and 7.40%, respectively. These findings maybe due to the tracheobronchial toileting issue of the patients on ventilator.

The commonest causative pathogens of VAP may be Gram negative bacteria such as *P. aeruginosa* or Gram-positive bacteria such as *Staphylococcus aureus*, so the type of causative pathogens might vary depending on hospital and region. In our study, the most predominant organism responsible for infection was shown to be *P. aerogionosa*, accounting for 84% of VAP cases. The organisms implicated in VAP were similar in other such studies (Restrepo et al., 2013; Nakaviroj et al., 2014).

Analysis of antibiotic sensitivity pattern of organisms suggests that 42.61% of Gram-negative isolates were highly resistant to the commonly used drugs. Most strains of *P. aeruginosa* were resistant to Cotrimoxazole (76.47%), Gentamycin (70.58%) and Ceftriaxone (64.70%). The overall evaluation of Gram-negative isolates demonstrated that 52.25% of *P. aeruginosa* species, 66.6% of *Acinetobacter* strains and 42.61% of *Entrobacteria* isolates were resistant to Cotrimoxazole, Gentamycin and Ceftazidim. This correlates with the results of other similar studies, which was similar to studies done by Medell et al. (2012b) and Gupta et al. (2011).

Namiduru et al. (2004) retrospectively examined the microbiological sensitivities of 140 patients with VAP and determined that sulbactam and cefoperazone were the most appropriate antibiotics, despite the results of our study, which offers Imipenem, Amikacin and Ciprofloxacin and Ceftazidime as the best anti-microbial therapy against poly-microbial VAP infections.

In overall view, the incidence of drug resistant organisms is increasing; therefore, a targeted approach with an appropriate dose of the right antibiotic guided by evidence is one of the logical approaches for VAP. Moreover, preventive strategies are required to reduce the high incidence of VAP, such as prevention of aspiration, early extubation and sterilizing techniques for invasive procedures.

Conclusion

According to the results of our study, the incidence of poly-microbial infections is increasing and Gram-negative bacteria are the most predominate isolates of VAP patients. Timely and correct diagnosis of VAP is important for the appropriate antimicrobial treatment of patients. Hence, monotherapy with a specific type of antibiotic should be avoided. According to the susceptibility test results and drug resistant bacteria in our study, combination therapy with Imipenem or Ceftazidime accompanied by Amikacin or Ciprofloxacin is recommended.

Conflict of interests

The authors did not declare any conflict of interest.

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