Original Article

Prevalence of Gram-negative Bacteria Isolated from Patients with Ventilator-Associated Pneumonia in Intensive Care Units of Imam Khomeini Hospital, Ahwaz, Iran

Seyed Hamid Borsi¹, Maryam Haddadzadeh Shoushtari¹, Hanieh Raji¹, Faramarz Ghalavand*¹

¹ Air pollution and Respiratory Disease Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Received: 23 March 2018; Accepted: 17 June 2018

Abstract

Background: Ventilator-associated pneumonia (VAP) is a common nosocomial infection among hospitalized patients who have undergone intubation and mechanical ventilation for more than 48 hours. Patients admitted to the intensive care unit (ICU) are at risk of developing life-threatening VAP due to specific conditions, especially with Gram-negative pathogens with advanced drug resistance. Hereby, the control of these agents and its monitoring is of particular importance. In this study, the pattern of antibiotic resistance of Gram-negative bacteria isolated from tracheal culture of patients with VAP investigated in ICU of Imam Khomeini Hospital of Ahwaz.

Materials and Methods: In this cross-sectional study, tracheal samples were collected during April 2016 to April 2017 from patients who were on mechanical ventilation in ICU of Imam Khomeini Hospital in Ahwaz, Khuzestan province, southwest of Iran. After isolation, bacterial strains were identified using biochemical tests. Then, antimicrobial resistance pattern of these isolates investigated using standard disc diffusion according to clinical and laboratory standards institute 2016 (CLSI 2016) guidelines.

Results: A total of 111 bacterial isolates were identified which were as following; Acinetobacter baumannii, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Stenotrophomonas maltophilia, and Serratia marcescens, with prevalence of 54%, 19.8%, 14.4%, 6.4%, 4.5%, and 0.9%, respectively. Antibiotic susceptibility test of isolates showed that almost all isolates had high resistance to treatment antibiotics and were multi-drug resistance (MDR). The A. baumannii isolates were resistant to ciprofloxacin and piperacillin-tazobactam, but ampicillin-tazobactam had a good effect.

Conclusion: The results of this study showed that patients admitted to ICU due to their conditions of treatment are more likely to develop VAP by Gram-negative pathogens. The empirical treatment of VAP due to predominant bacterial causes and emerging drug resistance has become more challenging. It requires to use of multidrug regimens for routine clinical practice. It should be noted that in order to appropriate antimicrobial therapy, precise and correct diagnosis is very important.

Keywords: Acinetobacter baumannii, Antibiotic resistance, Enterobacteria, ICU, ventilator-associated pneumonia

*Corresponding Author: Faramarz Ghalavand, Air pollution and Respiratory Disease Research Center, Joundishapur University of Medical Sciences, Ahvaz, Iran Email: ghalavandfaramarz@yahoo.com
Introduction

Ventilator-associated pneumonia (VAP) is the most common nosocomial infection diagnosed in the intensive care units (ICUs), affecting about 20% of patients in ICU and up to 30% of mechanically ventilated patients. The mortality rate of VAP has been estimated to be 25 to 50%, and can reach up to 75% in some specific settings or when lung infection is caused by high-risk pathogens, which is higher than those of other nosocomial infections. In addition, VAP is associated with significant morbidity, including prolonged mechanical ventilation, prolonged ICU stays, and consequently increased costs of hospitalization. Ventilator-associated pneumonia is defined as pneumonia that develops 48 h more or longer after mechanical ventilation is given by means of an endotracheal tube or tracheostomy, caused by microbial pathogens not present or incubating at the time mechanical ventilation was commenced. It may be of two types: first, early-onset VAP that is defined as VAP that happens within the first four days of ventilation, and second, late-onset VAP which is defined as VAP that develops longer than four days after commencement of mechanical ventilation.

Delays in diagnosis of VAP and thus delay in starting appropriate antibiotic therapy may be result in worse outcomes in the patients. On the other hand, an inappropriate diagnosis may lead to unnecessary treatment and following complications and risks associated with therapy. Therefore, early and accurate diagnosis of the causative agent and antibiotic susceptibility profile of the agent is fundamental in the management of patients with VAP. The most frequently isolated causative bacterial agents are Gram-negative bacilli, which show a significant decline in sensitivities to generally prescribed antimicrobial agents. It has been reported that Gram-negative bacilli, such as Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, and Acinetobacter species, account for more than 60% of VAP cases. Of note, isolated Acinetobacter strains are practically resistant to all commonly used antibiotics. However, some authors have reported that some Gram-positive bacteria have become increasingly more common, with Staphylococcus aureus being the predominant isolate.

As the kind of causative the pathogens and the antimicrobial susceptibility profile of them may vary depending on geographical region and hospital, data of microbial surveillance are essential for each area. Accordingly, this study was conducted to investigate the prevalence and pattern of antibiotic resistance of Gram-negative bacteria isolated from tracheal culture of patients with VAP was investigated in intensive care units of Imam Khomeini Hospital of Ahwaz in 2017.

Methods

Ethics statement: The ethics committee of School of Medicine, Ahvaz Jundishapur University of Medical Sciences was approved this study (IR.AJUMS.REC.1396.615).

Patients, Isolation and Identification: This cross sectional study was performed in intensive care medicine of Imam Khomeini Hospital of Ahwaz, Khuzestan province, southwest of Iran, with collaboration of Bacterial laboratory. Patients who were on mechanical ventilation for at least 5 days in ICU, who had CPIS values including fever greater than 38°C, leukocytosis, oxygenation or progressive radiographic infiltrate, were included in our study. Exclusion criteria included extrapulmonary infection sources, surgical history and any previous antibiotic therapy <48 h before the study.

Bronchoalveolar lavage (BAL) fluid and endotracheal aspiration were obtained from all enrolled patients by bronchoscopy. Samples are immediately transported to microbiology laboratory for bacteriological analysis. Standard conventional biochemical tests for identification of the isolates were performed on colonies from primary cultures. In addition, our study was included demographical information such as age, sex, underlying clinical condition, duration of hospitalization, and Duration of intubation was noted for evaluation of underlying factors for incidence of VAP.
**Antimicrobial Susceptibility Testing:** Standard disc diffusion method which is recommended by clinical laboratory and standard institute 2016 (CLSI 2016) was carried on evaluate antimicrobial susceptibility of the isolate microorganisms. Accordingly, susceptibility of the isolates to following antibiotics: amikacin (30 μg/disk), trimethoprim/sulfamethoxazole (1.25/23.75 μg/disk), cefepime (30 μg/disk), ceftriaxone (30 μg/disk), piperacillin/tazobactam (100/10 μg), ciprofloxacin (5 mg), meropenem (10 μg/disk), ceftazidime (30 μg/disk) and ampicillin-sulbactam (10/10 μg) (Mast Co., Darmstadt, Germany) were examined. In addition, minimum inhibitory concentrations (MICs) were determined by the E-test method according to the manufacturer’s guidelines for colistin against A. baumannii, K. pneumoniae, and P. aeruginosa (Liofilchem Co., Roseto, Italy). The MIC was read where inhibition of growth intersected the E-test strip. When small colonies grew within the zone of inhibition or a haze of growth occurred around MIC end points, the highest MIC intersec was recorded. For standard strain, E. coli ATCC 25922 was used.

**Statistical analysis:** SPSS version 22.0 (IBM Corp., Armonk, NY, USA) was applied for statistical analysis. Chi-square test was used for the comparison between the categorical variables. A p value <0.05 was considered to be statistically significant.

**Results**

In the present study, from April 2016 to April 2017, 111 bacterial isolates were collected from patients who were on mechanical ventilation in ICU of Imam Khomeini Hospital in Ahwaz. Among them 50.4% were male and 49.6% were female, which is not significantly different. Their mean age was 56 years. Most bacterial isolates were related internal ICU patients and internal surgical ICU.

Different bacterial species were isolated. The bacterial strains were identified as following: Acinetobacter spp., P. aeruginosa, K. pneumoniae, E. coli, Stenotrophomonas maltophilia, and Serratia marcescens, with prevalence of 54% (n=60), 19.8% (n=22), 14.4% (n=16), 6.4% (n=7), 4.5% (n=5), and 0.9% (n=1), respectively.

All of A. baumannii isolates were resistant to ciprofloxacin, Piperacillin/Tazobactam and Meropenem antibiotics. Antibiotic susceptibility to A. baumannii isolates indicates that isolates causing VAP infection have high resistance to common antibiotics. The most active antibiotic against tested A. baumannii isolates were ampicillin-sulbactam and trimethoprim-sulfamethoxazole, with susceptibility rate of 9% and 4%, respectively. In this study, all of A. baumannii isolates were classified as multidrug resistant (MDR) strains (resistant to at least 3 different classes of antibiotics). The results of antimicrobial susceptibility test of A. baumannii isolates from VAP patients is indicated in Table 1.

The present study indicated that all of P. aeruginosa isolates were resistant to ampicillin-sulbactam. High resistance rates were detected for trimethoprim-sulfamethoxazole (86.4%), ceftriaxone (72.2%), meropenem (72.2%), cefepime (63.6%), and amikacin (59.1%). Table 2 illustrates the results of antimicrobial susceptibility testing of P. aeruginosa isolates.

Our results indicated that K. pneumoniae isolates show the highest resistance to ampicillin-sulbactam, cefepime, ceftriaxone (whit resistance rate of 81.3%). The most active antibiotics were amikacin and levofloxacin (Table 3). In addition, the antibiotics susceptibility results showed that all of S. maltophilia isolates were resistant to cefepime, ceftriaxone, ciprofloxacin, piperacillin/tazobactam, meropenem, levofloxacin, and ampicillin-sulbactam. Only two isolates (28.6%) were susceptible to amikacin and trimethoprim-sulfamethoxazole.

**Discussion**

Ventilator-associated pneumonia causative pathogens generally are antibiotic resistant strains in many cases, it is commonly problematic to choose appropriate antibiotics. Furthermore, the mortality rate has been described to increase if an early antibiotic treatment is not provided to patients with VAP. Therefore, in order to start suitable empirical antibiotic therapy clinicians, need to identify predominant causative agents of VAP in ICU or any specific clinical setting and their antimicrobial susceptibility profile. Although, establishing a diagnosis of VAP, based on pathology or histology plus culture of the lung tissue, is considered the best available gold standard, but it has a considerable degree of uncertainty. The risk factors for VAP include age, severity of illness or...
injury, previous hospitalization prior to admission to the intensive care unit, mechanical ventilation time or tracheal intubation, and hospital stay in ICU, back rest, illness. Underlying conditions, chronic heart disease, neurological damage, trauma, heart and internal surgery, previous use of corticosteroids, or previous antibiotic therapy\textsuperscript{16,17}. VAP diagnosis is based on clinical criteria, chest X-ray and microbiological tests. Identification of each of the potential bacterial VAPs and the exact identification of each one to select the best antibiotic treatment and to reduce the duration of hospitalization in the ICU is essential\textsuperscript{18,19}.

In the present study, \textit{A. baumannii} was shown to be the most common causative pathogen of VAP at ICU, and \textit{P. aeruginosa}, \textit{K. pneumonia}, \textit{S. maltophilia} and
Prevalence of Gram-Negative Bacteria Isolated from Patients with Ventilator-Associated … Raji et al.

**Table 3**: The results of antimicrobial susceptibility testing of *K. pneumoniae* isolates from VAP patients.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Resistance. NO (%)</th>
<th>Intermediate NO (%)</th>
<th>Susceptible. NO (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin (AMK)</td>
<td>7 (43.8)</td>
<td>0</td>
<td>9 (56.3)</td>
</tr>
<tr>
<td>Cefepime (FEP)</td>
<td>13 (81.3)</td>
<td>0</td>
<td>3 (18.8)</td>
</tr>
<tr>
<td>Ceftriaxone (CRO)</td>
<td>13 (81.3)</td>
<td>1 (6.3)</td>
<td>1 (6.3)</td>
</tr>
<tr>
<td>Ciprofloxacin (CIP)</td>
<td>12 (75)</td>
<td>3 (18.8)</td>
<td>1 (6.3)</td>
</tr>
<tr>
<td>Piperacillin/Tazobactam (TZP)</td>
<td>11 (68.8)</td>
<td>0</td>
<td>5 (31.3)</td>
</tr>
<tr>
<td>Trimethoprim-Sulfamethoxazole (SXT)</td>
<td>10 (62.5)</td>
<td>2 (12.5)</td>
<td>4 (25)</td>
</tr>
<tr>
<td>Meropenem (MEM)</td>
<td>11 (68.8)</td>
<td>1 (6.3)</td>
<td>4 (25)</td>
</tr>
<tr>
<td>Levofloxacin (LVX)</td>
<td>8 (50)</td>
<td>1 (6.3)</td>
<td>7 (43.8)</td>
</tr>
<tr>
<td>Ampicillin-Sulbactam (SAM)</td>
<td>13 (81.3)</td>
<td>0</td>
<td>3 (18.8)</td>
</tr>
</tbody>
</table>

*S. marcescens* followed *A. baumannii* in that order. This finding is similar to the result reported by Hashemian *et al.* in Iran. Unfortunately, these bacterial strains are well known by their very high resistance to multiple antimicrobial, and except appropriate antibiotic therapy is administered early, mortality rate is significantly high. Resistance is mediated by several mechanisms such as multiple efflux pumps expressed intrinsically or up regulated by mutation. Increase in incidence of MDR isolates of *Acinetobacter* spp., *P. aeruginosa* and *K. pneumoniae* was observed in many parts of word, and some the isolates are susceptible only to colistin. In an investigation in Greece, which provided original data on the prevalence of Gram-negative bacteria in patients admitted to ICU, *A. baumannii*, *P. aeruginosa* and *K. pneumoniae* were the most common causative agents of VAP. In another study, it has been notified that VAP associated with *P. aeruginosa* which are resistant to carbapenems and fluoroquinolones antibiotics show a significant increasing.

VAP causative pathogens are varied based on case mix, methodology of sampling and local resistance profiles. According to the study conducted by Charles *et al.* in India, 72.2% of VAP patients had mono microbial and 27.8% had poly-microbial infection. Moreover, they reported that half of isolates associated with late-onset VAP were MDR, while 22% isolates obtained from patients with early-onset VAP were MDR.

In the present study, results of antimicrobial susceptibility testing of the isolates illustrate the highly resistant to the commonly used drugs. Among *A. baumannii* strains, except for ampicillin-sulbactam, more than 95% of isolates were resistant to the tested antibiotics. In addition, all isolates showed MDR phenotypes. In a study conducted by Kazemi *et al.* it has been shown that resistance rates of *A. baumannii* isolates against rifampicin, gentamicin, meropenem, piperacillin, ceftazidime, and colistin were 46%, 67%, 100%, 98%, 96%, and 0%, respectively.

Our results indicated that all of *K. pneumoniae* isolates, which contained 14.4% of the isolates, were MDR and resistant to at least four antibiotic classes. Moreover, amikacin, levofloxacin and piperacillin/tazobactam showed the best activity on *K. pneumoniae* isolates, and other antibiotics had no significant effect on these isolates from VAP patients. Yadegarynia *et al.*, conducted a study on 62 *K. pneumonia* isolates from clinical samples of ICU and general wards during one year. The least resistance
was related to colistin (4.8%) and amikacin (14.5%), respectively, and the most resistance was observed against ciprofloxacin (66.1%), ceftriaxone (62.9%) and gentamicin (59.7%), respectively. Resistance to imipenem was observed among 38.7% of the isolates.

**Conclusion**

It has been reported that mortality of VAP associated with Gram-negative bacteria is high. Data from the available literature confirm that there is a direct association between antimicrobial resistance profiles and mortality in ICU patients. The results of this study showed that patients admitted to ICU due to their conditions of treatment are more likely to develop VAP by Gram-negative pathogens. In the present study we focused on Gram-negative bacteria isolated from patients in ICU and it founded that the major of them were drug resistant microorganism, including A. baumannii, P. aeruginosa and K. pneumoniae. It should be noted that precise and correct diagnosis to appropriate antimicrobial therapy are important.

**Acknowledgment**

None.

**Conflict of Interest**

The authors declare that there is no conflict of interests.

**References**

23. Zilberberg MD, Shorr AF. Prevalence of multidrug-resistant pseudomonas aeruginosa and carbapenem-resistant enterobacteriaceae among specimens from hospitalized patients with...
24. Paramythiotou E, Routsi C. Association between infections caused by multidrug-resistant gram-negative bacteria and mortality in critically ill patients. World Journal of Critical Care Medicine. 05/0410/03/received12/30/revised03/07/accepted 2016;5(2):111-120.