

ORIGINAL ARTICLE

High Frequency of Ventilator Associated Pneumonia Nosocomial Co-Infection Caused by Methicillin Resistant *Staphylococcus aureus* and Carbapenem Resistant *Acinetobacter baumannii* in Intensive Care Unit

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ABSTRACT

Introduction: Ventilator Associated Pneumonia (VAP) is one of the most common nosocomial infections in the intensive care unit and leads to increase of mortality in affected patients. VAP infections occur mainly due to inadequate antibiotic treatment for multi drug-resistant (MDR) pathogens. The aim of this study was to identify the etiologic agents of VAP infections in intensive care unit and antibiotic susceptibility determination of isolated bacteria.

Methods: Overall, 179 respiratory secretions were collected from patients with VAP in vali-Asr hospital, Arak, Iran from October 2013 to November 2014. The samples were cultured in the laboratory culture media, their susceptibility tests were performed, and results were interpreted according to CLSI guidelines.

Presence of *mecA* and *Sa442* genes in *Staphylococcus aureus* and *oxA-51* like gene of *Acinetobacter baumannii* isolates separately were analyzed by PCR.

Results: Overall, 169 (90%) of respiratory specimens showed positive culture. The most common isolated pathogen was *A. baumannii* followed by *S. aureus*. Ninety five percent of *A. baumannii* were resistant to imipenem and 71% of *S. aureus* isolates were methicillin resistance *S. aureus* (MRSA).

Conclusion: The elevated prevalence of VAP nosocomial infection is alarming. Emergence of highly resistant isolates is another burden. Therefore, antibiotic prescription policy must be revised.

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INTRODUCTION

Ventilator- Associated Pneumonia (VAP) is defined by CDC, USA as a form of nosocomial pneumonia that occurs in a patient within 48 h or more after intubation with an endotracheal tube or tracheotomy tube, of no previous history [1, 2]. It is the second important nosocomial infection in intensive care units (ICU) and is considered as the most frequently infection in mechanically ventilated patient hospitalized in ICUs [3, 4].

VAP infection can cause increase in length of ICU and hospital stay, which results in the

increase healthcare costs and high mortality and morbidity in patients [1]. The mortality rate for VAP ranging is from 33-55% [3] and has been reported as high as 76% when the high-risk pathogens causing respiratory infections [5]. The predominant organisms responsible for VAP are *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterobacteriaceae* bacteria, and *Acinetobacter* species.

Emergence of antibiotic resistant bacteria is a major concern in hospital environment [6]. Therefore, the management of the VAP caused by multidrug-resistant (MDR) methicillin resistant *S. aureus* (MRSA), imipenem resistant *A. baumannii* and *P. aeruginosa*, extended-

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spectrum beta-lactamases. ESBL producing *Enterobacteriaceae* and vancomycin resistant *Enterococcus* is an ongoing challenge[6, 7].

Currently, it seems that the rate of VAP due to multiple drug resistant bacteria in Vali-asr hospital Arak, Iran has been increasing. Currently, the incidence of VAP infection is rising in this hospital and the presence of methicillin-resistant *S. aureus* and carbapenem resistant *A. baumannii* had been reported before in this hospital [8, 9].

Therefore, this study designed to identify the etiologic agents of VAP infections in ICU units and to detect the antibiotic resistant determinant of the isolated bacteria.

MATERIALS AND METHODS

In this cross sectional study from October 2013 to November 2014, 179 respiratory discharges were obtained from the patients admitted to the ICU of Valiasr Hospital, Arak, central Iran. Clinical diagnosis of VAP was made using a Modified Clinical Pulmonary Infection Score (CPIS) > 6.

The diagnosis was confirmed by performing a quantitative culture of the endotracheal aspirate and observing $\geq 10^5$ cfu/ ml[10]. Conventional microbiological techniques such as Catalase test, coagulase test,oxidative-fermentative (OF) test, Methyl Red (MR) and Voges-Proskauer (VP), Triple Sugar Iron(TSI), Sulfide indole motility (SIM), Simmons Citrate,urease test andLysine Iron Agar (LIA) were used for the isolation and identification of respiratory pathogens to species level. Antimicrobial susceptibility testing was performed on Mueller–Hinton agar (Merck, Germany) using the disk diffusion (Kirby–Bauer) technique, with zone size interpretation based on Clinical and Laboratory Standards Institute (CLSI) guidelines[11]. The antibiotics used were cefoxitin (FOX), ciprofloxacin (CIP), clindamycin (CLIN), erythromycin (ERY), fusidic acid (FUS), gentamicin (GM), tetracycline (TE), trimethoprim–sulphamethoxazole (SXT), mupirocin (MUP), imipenem (IPM), linezolid (LIN), vancomycin (VN), quinupristin/dalfopristin (QD), tigecycline (TGC), teicoplanin (TEC), chloramphenicol (C), rifampin (RIF) and netilmicin (NET). *S. aureus* ATCC 25923 and *S. aureus* ATCC 700698 were used as quality controls strains and *S. aureus* Isolates were confirmed as MRSA by oxacillin and cefoxitin susceptibility testing according to the CLSI guidelines.

Minimum inhibitory concentration (MIC) values for vancomycin (MRSA) and imipenem (*Acinetobacter*) were determined by the E-test

(bioMérieux, France), according to the manufacturer's instructions.

The production of extended-spectrum beta-lactamases (ESBL) was evidenced by combination disk test that performed with ceftazidime and ceftazidime/clavulanic acid disks[12]. The isolates, which showed intermediate or susceptible zones for imipenem, were evaluated by Modified Hodge test for carbapenemase production. Positive modified Hodge test isolates indicating carbapenemase production were subjected to imipenem (IMP)-EDTA[13] combined disc test for Metallo- Beta-Lactamases identification. In addition, all isolates were evaluated by AmpC disk test for detection of AmpCbetalactamasesresistant[14].

Genotypic investigation

Bacterial genomic DNA was extracted using QIAamp DNA mini kit (Qiagen, Germany) according to the manufacturer's protocol. All *S.aureus* isolates were tested for presence of Sa442 and mecA genes as an identification marker and to confirm MRSA isolates, respectively [13]. In addition, phenotypic characterization of *A.baumannii* was confirmed by investigation of OXA-51 gene [14].

RESULTS

One hundred and sixty one out of 179specimens showed positive indication for the presence of bacterial infection. The incidence of *A. baumannii*, *P. aeruginosa*, *S. aureus* Escherichia coli, Klebsiellapneumonia, *Enterobacteraerogenesa* as a single infecting agent was 56(35%), 13(9%), 21(13%), 4(2%), 11(7%), 3(2%) and 2(1%) respectively.

Dual agent infection caused by *A.baumannii* +*P. aeruginosa*, *A.baumannii*+*S. aureus* ,*S. aureus*+*P. aeruginosa* ,*K. pneumoniae*+*A. baumannii*, *A. baumannii*+*Citrobacterfrundeie*,*A.baumannii*+*entrobacter*, *Ecoli*+ *Klebsiella*, *Klebsiella*+*S. aureus*, *P.aeruginosa*+*Klebsiella* was 4(2%),21(13%),7(4%),7(4%)·1(0.6%),1(0.6%) ,1(0.6%), 2(1%) and 3(2%) respectively.

Triplex agent infection caused by *S. aureus*,*Klebsiella* ,*P. aeruginosa* - *S. aureus*,*Klebsiella* ,*A. baumannii* Each of was ,1(0.6%).

Out of 91 *A. baumanii* and 28 *P. aerogenosa* isolates 56 (5.61%) and 19(68%) were modified hodge test positive. Among them 39 (43%) and 8(28.5%) were MBL producers analyzed by MBL E-test. Among all of the *A. baumannii* isolates, 21 (23%) were AmpC β -lactamase producers.

The results of susceptibility testing are shown in Table 1, 2.

Table 1. The Frequency of resistance to antibiotics; (%) of Isolated Gram Positive and Gram Negative Bacteria

| Antibiotic | <i>P.aeruginosa</i> | <i>A. baumannii</i> | Antibiotic | <i>S. aureus</i> | <i>Enterococcus</i> |
|-----------------------|---------------------|---------------------|----------------|------------------|---------------------|
| Ceftriaxone | 26(93) | 80(88) | clindamycin | 21(100) | 2(100) |
| Cefixime | 27(96) | 90(99) | erythromycin | 21(100) | 2(100) |
| ciprofloxacin | 13(46) | 80(88) | ciprofloxacin | 19(90) | 1(50) |
| co-trimoxazole | 1(3.5) | 5(5.4) | Cefazolin | 15(71) | 2(100) |
| Norfloxacin | 18(64) | 78(86) | Cefoxitin | 15(71) | 2(100) |
| Ceftizoxime | 2(7) | 12(13) | Gentamicin | 10(48) | - |
| imipenem | 10(36) | 87(95) | co-Trimoxazole | 8(21) | 1(50) |
| Gentamicin | 13(46) | 87(95) | vancomycin | - | - |
| Amikacin | 7(25) | 87(95) | Ceftizoxime | 2(21) | - |
| | | | Cephalexin | 1(5) | - |

Table 2. The Frequency of resistance to antibiotics; (%) of in Isolated Positive ESBL

| Antibiotic | Positive ESBL | | |
|----------------------|-------------------|---------------------|----------------|
| | <i>Klebsiella</i> | <i>E. aerogenes</i> | <i>E. coli</i> |
| Ceftriaxone | 26(100) | 3(75) | 5(83) |
| Cefixime | 26(100) | 3(75) | 5(83) |
| Ceftazidime | 26(100) | 3(75) | 5(83) |
| Norfloxacin | 16(61) | - | 3(50) |
| ciprofloxacin | 13(50) | - | 2(33) |
| Amikacin | 7(27) | - | - |
| Gentamicin | 10(38) | - | 2(33) |
| imipenem | 1(4) | - | - |

The identity of all Methicillin-sensitive *S. aureus* (MSSA), Methicillin-resistant *S.aureus*(MRSA), *A.baumannii* isolates were confirmed by PCR based on the presence of Sa442, *mecA*, *oxa* -51-like genes respectively.

DISCUSSION

Due to high prevalence of VAP infection in our hospital, this study designed to address the etiological agents causing infection and to find out the antibiotic resistant determination of isolates.

VAP infections are considered as a serious concern in Valiasr Hospital, Arak as many others parts of the world. The frequency of VAP infection at our ICU was 90% which is higher than other studies in Iran (72%) and India (45%) [7],[8]. However, the overall incidence of VAP in ICUs ranges from 10-70% [7].

Different incidences of VAP in the literature depend on the definition, the type of hospital or ICU, the population studied and the level of antibiotic consumption that in our hospital were extremely high[6, 8].

Gram-negative bacilli such as *P. aeruginosa*, *Acinetobacter* spp., *Enterobacteriaceae* (from

55% to 85%) were the commonest respiratory pathogens followed by *S. aureus* (from 20% to 30%) and polymicrobial infections (from 40% to 60%)[6].

In another study, *S.aureus* (23.6%), *Klebsiella* spp (23.3%), *Acinetobacter* spp(20.7%), *P.aeruginosa* (18.2%), *E. coli* (7.7%) and *Enterobacter* were the most common causative organism for infection of endotracheal intubation [15]. *Acinetobacter*, *P. aeruginosa*, and *S. aureus* were the main causative microorganism of mechanical ventilation-associated pneumonia [15].

The commonest organism isolated from tracheal tubes of the patient in our study was *A. baumannii*. This result is in accordance with a recent study of VAP in Asian countries[15,20]

However, in Ahvaz, southern Iran, *Enterobacter* was found as the most prevalent bacteria, which is in contrast of our result[16].

In present study 34 ESBL-producing isolates were found, including *K. pneumonia* (100%), *Enterobacter* (75%), and *E. coli* (83%) were the most frequent ones. In another investigation, the highest rate of ESBL prevalence was 27.84% for *K. pneumonia*, 27.3% for *E. coli* and 23.9% for *Enterobacter*[17].

Our study showed that MBL was produced 43% and 28.5% of carbapenem non susceptible *A. baumannii* and *P. aeruginosa* isolated respectively. However in study previously performed in Iran among all of the *A. baumannii* isolated 45(71.4%) and 47(75%) were Ampc β -lactamase and MBL producers respectively[8]. This finding indicates that MBL producing *A. baumannii* could be due to determination of infection control measure in the hospital[8]. In Iran, MBL E-test method identified 8(19.5%) *P. aeruginosa* isolates as MBL producer[18].

According to the risk factors enhancing emergence for VAP due to MDR organisms in hospitalized patients are mainly include advanced age, immunosuppression, prior antibiotics use, especially broad-spectrum drugs such as third generation cephalosporin, fluoroquinolone, and/or imipenem, increased severity of illness, previous hospitalization or residence in a chronic care facility and using mechanical devices, the routine use of invasive techniques as well as ICU overcrowding and the increased susceptibility in this population of patients who usually suffer from severe illnesses, further increase the risk of infection with multidrug resistant microorganisms[7, 19]. It seems prior antibiotics usage and long stay in ICU could have been important role in prevalence of VAP due to MDR organisms in our hospital.

Conclusion: High prevalence of VAP infection demanding molecular epidemiology research for source tracing of MDR isolates. In addition, infection control measure in this hospital must be improved.

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