Antibiotic Resistance Patterns and Detection of OXA-23 and OXA-48 Genes in Acinetobacter baumannii Isolated from Ventilator Associated Pneumonia

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ABSTRACT

Background: Acinetobacter baumannii is one of the important causes of nosocomial infection worldwide. Patients who use ventilator are in high risk of ventilator association pneumonia (VAP) caused by nosocomial pathogens such as A. baumannii. Carbapenem is one of the last lines of antibiotic therapy in MDR A. baumannii infections. Then, carbapenem resistant strains are a very important challenge for physicians. OXA types carbapenemase enzymes are important mechanisms to carbapenem resistance in A. baumannii. The aim of this study was to determine oxa-23 and oxa-48 producing A. baumannii isolated from VAP.

Methods: In this cross-sectional study, 51 sputum specimens from VAP in hospitalized patients in Hazrat-E-Rasul Hospital, Tehran, Iran were used. Antibiotic susceptibility testing was done after identification according to CLSI 2018. DNA extraction was done by boiling assay and oxa-23 and oxa-48 genes were detected by PCR. Thirty-two (63%) A. baumannii were confirmed according to microbiological and biochemical tests.

Results: The highest resistance was observed against Piperacillin, Cefotaxime, Ciprofloxacin and Ceftazidime with 97% antibiotic resistance, and ampicillin/Sulbactam was the most effective antibiotic (78% sensitivity). Generally, 31 isolates of A. baumannii harbored the oxa-23 gene and none of them had oxa-48. The high prevalence of MDR A. baumannii in VAP is a great problem, especially for the nosocomial infection committee in hospitals.

Conclusion: Rapid detection of MDR and carbapenemase producing strains can be the first step in preventing their spread in hospitals.

Introduction

Hospitalized patients are in high risk of infection by nosocomial pathogens and are ready to accept hospital health care association infection (HCAI), especially those using auxiliary medical equipment (1-3). HCAI can increase the mortality and morbidity, especially in immune suppressed patients (2). Patients who use ventilator are in high risk of nosocomial infection such as Ventilator Associated Pneumonia (VAP) (1, 4).

Acinetobacter baumanii is one of the common infectious agents in VAP and may lead to long term hospitalization; also, it can impose high costs on the health care system and patients (1, 4). Regularly, Multi-drug resistant (MDR) strains of A. baumannii cause VAP because the MDR isolates of this gram-negative bacterium survives in the environment of the hospitals and can cause nosocomial infection (4-6). A. baumannii can survive in the hospital environment and it can be transmitted to patients and so, causes HCAI (5, 6). The resistance of A. baumannii is associated to different mechanisms such as efflux pumps and various antibiotic hydrolyzing enzymes (7). Production of carbapenemase is one of the important mechanisms that can be a reason for appearance of MDR strains (5, 6, 8). Strains of A. baumannii produce different types of carbapenemase such as metallo beta-lactamase, groupA carbapenemase and different types of oxacillin-hydrolyzing enzymes (OXA). OXA-23 is one of the most common OXA enzymes in A. baumannii which have been widely reported, worldwide (5, 6, 8, 9). Also, OXA-48 is one of the OXA types in this bacterium with high carbapenemase activity (5).

Therefore, considering lack of enough data on the prevalence of the above genes, the aim of this study was to identify OXA-23 and OXA-48 in MDR A. baumannii isolated from VAP in hospitalized patients in Hazrat-E-Rasul Hospital, Tehran, Iran.

Materials and Methods

Bacterial strains

In this cross-sectional study, 51 sputum specimens from patients with VAP in hospitalized patients in Hazrat-E-Rasul Hospital, Tehran, Iran were collected from November 2010 November to 2011. Bacteria were identified by conventional biochemical and microbiological methods such as TSI, SIM, oxidase (10). Identification of A. baumannii was confirmed using blaOXA-51-like PCR assay by specific primers (10).

Antibiotic susceptibility testing

Antibiotic susceptibility testing was done after identification according to CLSI 2018 (11) guideline against: cefepime (30 µg), imipenem (10 µg), piperacillin (100 µg), aztreonam (30 µg), trimethoprim/Sulfamethoxazole (1.25/23.75 µg), ampicillin/Sublactam (10-10 µg), tobramycin (10 µg), cefotaxime (30 µg), amoxicillin/Clavulanic acid (20-10 µg), tetracycline (30 µg), piperacillin/Tazobactam (100-10 µg), amikacin (30 µg), ciprofloxacin (5 µg), ceftazidime (30 µg), gentamicin (10 µg). Antibiotics disks used in this study were purchased from MAST Company (Mast Diagnostics, UK). P. aeruginosa ATCC 27853 was used as the control strain in the antibiotic susceptibility testing (11).

OXA-23 and OXA-48 detection

DNA extraction was performed using boiling method and the extracted genomic material was preserved at -80 °C up to the setup of the PCR assays. OXA-23 and OXA-48 genes were identified by specific primers (Table 1). The PCR products were loaded on 1% agarose and visualized by a gel document system. PCR conditions are as follows: initial denaturation at 94 °C for one minute and 30 cycles of denaturation at 94 °C for 30 seconds, annealing at 55 °C for one minute, extension at 72 °C for one minutes, and final extension at 72 °C for 7
minutes (11, 12). Internal positive controls for OXA-23 and OXA-48 genes were used. The PCR products were run in 1.5% agarose gel electrophoresis, stained with ethidium bromide (CinnaGene, Iran) and visualized under UV gel trans-illuminator.

Result

In this study, 32 (63%) A. baumannii were identified and confirmed according to microbiological and biochemical tests. Piperacillin, Cefotaxime, Ciprofloxacin and Ceftazidime with 97% resistance showed the highest antibiotic resistant frequency and ampicillin/Sulbactam was the most effective antibiotic (78% sensitivity). Antibiotic susceptibility frequency is shown in Table 2. All of the carbapenem resistant strains include oxa-23 gene and none of them had oxa-48 according to PCR results (Figures 1 and 2).

Discussion

A. baumannii is considered as an opportunistic hospital pathogen in the whole world (5, 8, 14). Overuse and maybe misuse of antibiotics can cause appearance of MDR and XDR strains of this gram-negative bacterium (5, 6). Long survival of A. baumannii in hospital environment can cause circulation and spread of this pathogen in the hospital that can cause nosocomial infection such as VAP (4, 15). Some of antibiotic resistant genes like carbapenemas are located on mobile genetic elements such as plasmids (5). These transferable genes can easily and rapidly spread in the bacteria and increase the morbidity and mortality, especially in high risk patients such as ventilated ones (5, 16). A. baumannii colonization on the ventilator can cause pneumonia and threatens the life of the patient (4, 15). In this study, 63% of the patients were colonized by A. baumannii. Medina-Presentado et al. worked on isolated A. baumannii from ventilated patients and reported 35% colonized patients with this bacterium (17). A. baumannii was isolated in 43.8% patients who used ventilator in Vietnam (18) and 54% in India (19). These rates are lower than the results of the current study and it can be due to different rates of the presence of dominant A. baumannii in intensive care units in two hospitals. Isolated A. baumannii from VAP showed a high rate of resistance to the third generation of cephalosporin and also ciprofloxacin and 75% resistance to carbapenem in this study. In Vietnam, 84% isolated A. baumannii was carbapenem resistant (18). In Bagheri-Nesami et al.’s study in Iran, a high rate of resistance to ceftazidime and ciprofloxacin was observed like the results of the current study (20). OXA-23 is one of the important mechanisms for carbapenem resistance in A. baumannii. In this regard, 97% of the isolated strains carried OXA-23 gene. The most detected carbapenemase isolated from A. baumannii was OXA-23 in the studies conducted by Mohamadi et al. (21) and Azimi et al in Iran (5), Nowak et al. in Greece (22), Royer et al. in Brazil (15), like ours. The results of the current study and other studies showed a high rate of antibiotic resistance, and also OXA-23 genes in A. baumannii isolated from VAP. These results can be very important in ventilated patients and can increase the morbidity and mortality.

| Table 1. Representing list of primers used and their sequence. |
|--------------------|-----------------|
| Primers    | Nucleotide Sequences (5-3)                          |
| OXA-23     | GATGTTGCTAGTAGTATTCCGTCGT                           |
|            | TCACAACACTAAAGCAGCTGT                                 |
| OXA-48-ike | CCAAGCATTTTTTACCCGCATCKACC                           |
|            | GYTGACCATACGCCTGRCG                                   |
Conclusion

The nosocomial infection committee in the hospitals should control the A. baumannii colonization in ICUs and especially in ventilated patients.

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Conflict of interest

No conflicts of interest were disclosed.

References


