Journal of Medical Bacteriology

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

Ahmad Rastegar Lari [1], Leila Azimi [2], Mohammad Motamedifar [3], Abdolaziz Rastegar Lari [4*]*

[1] *Student Research Committee, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.*

[2] *Pediatric Infections Research Center, Research Institute for Children Health, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*

3 Shiraz HIV/Aids Research Center, Institute of Health & Bacteriology & Virology Department, Shiraz Medical School, Shiraz University of Medical Sciences, Shiraz, Iran.

4 Department of Microbiology, Iran University of Medical Sciences, Iran, Iran.

ARTICLE INFO

Article type:
Research Article

Article history:
Received: 13 Feb 2019
Revised: 11 Mar 2019
Accepted: 25 Apr 2019
Published: 13 Jun 2019

Keywords:
Acinetobacter baumannii, Carbapenemse, Ventilator association pneumonia.

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.

• ***Please cite this paper as:*** Rastegar Lari A, Azimi L, Motamedifar M, Rastegar Lari A. Antibiotic Resistance Patterns and Detection of *OXA-23* and *OXA-48* Genes in *Acinetobacter baumannii* Isolated from Ventilator Association Infections. *J Med Bacteriol.* 2019; **8** (3, 4): pp.18-22.

*Corresponding Authors:

Mohammad Motamedifar, Shiraz HIV/Aids Research Center, Institute of Health & Bacteriology & Virology Department, Shiraz Medical School, Shiraz University of Medical Sciences, Shiraz, Iran. *Tel*: +98-71-32304356, *E-mail:* motamedm@sums.ac.ir

Abdolaziz Rastegar Lari, Department of Microbiology, Iran University of Medical Sciences, Iran, Iran.

Tel: +98- 45-33512081, *E-mail:* Tel: +98-21-86703183, E-mail: azizlari@gmail.com

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 baumannii 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 carbapenemse 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/Sulbactam (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 1and 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 carbapenemse 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 alsoOXA-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	GATGTGTCATAGTATTCGTCGT
	TCACAACAACTAAAAGCACTGT
OXA-48-ike	CCAAGCATTTTTACCCGCATCKACC
	GYTTGACCATACGCTGRCTGCG

Conclusion

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

Acknowledgment

This study was financially supported by Shiraz University of Medical Sciences Grant No. 16906. This article is based on the thesis by Ahmad Rastegar Lari for MD degree under the supervision of Dr. M. Motamedifar.

Conflict of interest

No conflicts of interest were disclosed.

References

1. Garnacho-Montero J, Ortiz-Leyba C, Fernández-Hinojosa et al. *Acinetobacter baumannii* ventilator-associated pneumonia: epidemiological and clinical findings. *Intensive Care Med* 2005; **31**(5):649-55.
2. Azimi L, Talebi M, Khodaei F, et al. Comparison of multiple-locus variable-number tandem-repeat analysis with pulsed-field gel electrophoresis typing of carbapenemases producing *Acinetobacter baumannii* isolated from burn patients. *Burns* 2016; **42**(2):441-5.
3. Armin S, Fallah F, Azimi L, et al. Warning: spread of NDM-1 in two border towns of Iran. *Cell Mol Biol* (Noisy-le-grand) 2018; 30; **64**(10):125-9.
4. Tsakiridou E, Makris D, Daniil Z, et al. *Acinetobacter baumannii* infection in prior ICU bed occupants is an independent risk factor for subsequent cases of ventilator-associated pneumonia. *Biomed Res Int* 2014; **2014**:193516.
5. Azimi L, Talebi M, Pourshafie MR, et al. Characterization of Carbapenemases in extensively drug resistance *Acinetobacter baumannii* in a burn care center in Iran. *Int J Mol Cell Med* 2015; **4**(1):46-53.
6. Azimi L, Alaghehbandan R, Mohammadpoor M, et al. Identification of KPC-producing *Pseudomonas aeruginosa* and *Acinetobacter baumannii* in a burned infant: A Case Report. *J Med Bacteriol* 2012; **1**:46-9.
7. Babapour E, Haddadi A, Mirnejad R, et al. Biofilm formation in clinical isolates of nosocomial *Acinetobacter baumannii* and its relationship with multidrug resistance. *Asian Pac J Trop Biomed* 2016; **6**(6):528-33.
8. Ardebili A, Talebi M, Azimi L, et al. Effect of efflux pump inhibitor carbonyl cyanide 3-Chlorophenylhydrazone on the minimum inhibitory concentration of ciprofloxacin in *Acinetobacter baumannii* clinical isolates. *Jundishapur J Microbiol* 2014; **7**(1):e8691
9. Nowak P, Paluchowska P, Budak A. Distribution of *blaOXA* genes among carbapenem-resistant *Acinetobacter baumannii* nosocomial strains in Poland. *New Microbiol* 2012; **35**(3):317-25.
10. Kooti S, Motamedifar M, Sarvari J. Antibiotic resistance profile and distribution of Oxacillinase genes among clinical isolates of *Acinetobacter baumannii* in Shiraz teaching Hospitals, 2012–2013. *Jundishapur J Microbiol* 2015; **8**(8):e20215.
11. Clinical Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: nineteenth informational supplement M100–S21. CLSI, Wayne, PA. 2018.
12. Lee K, Lim Y S, Yong D, et al. Evaluation of the Hodge test and the imipenem-EDTA double-disk synergy test for differentiating metallo-b-lactamase-producing isolates of *Pseudomonas* spp. and *Acinetobacter* spp. *J Clin Microbiol* 2003; **41**:4623-9.
13. Voets GM, Fluit AC, Scharringa J, et al. A set of multiplex PCRs for genotypic detection of extended-spectrum β-lactamases, carbapenemases, plasmid mediated *AmpC* β-lactamases and *OXA* β-

lactamases. *Int J Antimicrob Agents* 2011; **37**(4):356-9.
14. Fallah F, Abdolghafoorian H, Sadat Sajadi Nia R, et al. Antimicrobial resistance patterns of *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* isolated from patients with nosocomial infections admitted to Tehran Hospitals. *Arch Pediatr Infect Dis* 2015; **3**(4):e32554
15. Royer S, Faria AL, Seki LM, et al. Spread of multidrug-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa* clones in patients with ventilator-associated pneumonia in an adult intensive care unit at a university hospital. *Braz J Infect Dis* 2015; **19**(4):350-7.
16. Owlia P, Azimi L, Gholami A, et al. ESBL- and MBL-mediated resistance in *Acinetobacter baumannii*: a global threat to burn patients. *Infez Med* 2012; **20**(3):182-7.
17. Medina-Presentado JC, Seija V, Vignoli R, et al. Polyclonal endemicity of *Acinetobacter baumannii* in ventilated patients in an intensive care unit in Uruguay. *Int J Infect Dis* 2013; **17**(6):e422-7.
18. Phu VD, Nadjm B, Duy NHA, et al. Ventilator-associated respiratory infection in a resource-restricted setting: impact and etiology. *J Intensive Care* 2017; **19**;5:69.
19. Khurana S, Mathur P, Kumar S, et al. Incidence of ventilator-associated pneumonia and impact of multidrug-resistant infections on patient's outcome: Experience at an Apex Trauma Centre in North India. *Indian J Med Microbiol* 2017; **35**(4):504-10.
20. Bagheri-Nesami M, Rezai MS, Ahangarkani F, et al. Multidrug and co-resistance patterns of non-fermenting Gram-negative bacilli involved in ventilator-associated pneumonia carrying class 1 integron in the North of Iran. *Germs* 2017; **7**(3):123-31
21. Mohammadi F, Goudarzi H, Hashemi A , et al. Detection of ISAbaI in *Acinetobacter baumannii* strains carrying *OXA* genes isolated from Iranian burn patients. *Arch Pediatr Infect Dis* 2017; **5**(2):e39307.
22. Nowak J, Zander E, Stefanik D, et al. High incidence of pandrug-resistant *Acinetobacter baumannii* isolates collected from patients with ventilator-associated pneumonia in Greece, Italy and Spain as part of the MagicBullet clinical trial. *J Antimicrob Chemother* 2017; **72**(12):3277-82.