Iranian Journal of Microbiology
Volume 5 Number 4 (December 2013) 315-322

Genotyping of carbapenem resistant *Acinetobacter baumannii* isolated from tracheal tube discharge of hospitalized patients in intensive care units, Ahvaz, Iran

Saeed Shoja[1], Mojtaba Moosavian[1*], Amir Peymani[2], Mohammad Amin Tabatabaiefar[3], Soodabeh Rostami[1], Nasim Ebrahimi[1]

[1]Department of Microbiology, School of Medicine, Infectious & Tropical Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. [2]Department of Microbiology, Qazvin University of Medical Sciences, Qazvin, Iran.[3]Genetic Department, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

Received: June 2013, **Accepted:** August 2013.

ABSTRACT

Background and Objectives: Carbapenem resistant *A. baumannii* is an emerging cause of nosocomial infections. The aims of this study were identification of the most prevalent of carbapenem resistant genes, molecular typing and antimicrobial evaluation of *A.baumannii* in intensive care units.

Materials and Methods: Two hundred and six *A. baumannii* were isolated from tracheal tube discharge of hospitalized patients at different intensive care units in Ahvaz, Iran. Antimicrobial susceptibility test was done on all isolates. Multiplex and singleplex PCR were performed for detection of $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-51-like}}$, $bla_{\text{OXA-58-like}}$, bla_{VIM}, bla_{IMP}, bla_{SPM} and bla_{NDM} genes. Genetic relationship of all isolates was determined by REP-PCR method.

Results: Out of 206 examined isolates, 198 (96.1%) isolates were resistant to imipenem and meropenem. However 3.9% isolates were sensitive to these antibiotics. The $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-24-like}}$ genes were detected in 85% and 8.7% of strains, respectively. No $bla_{\text{OXA-58- like}}$, bla_{IMP}, bla_{VIM}, bla_{SPM} and bla_{NDM} were detected. REP-PCR results showed that isolates were belonged to five genotypes: Genotype A was the most prevalent (P- value < 0.001): it was observed in 75 of 206 strains (36.4%). Genotype B, and C were found in 28.6% and 27.7%, respectively. The rate of other genotypes was as follows: D (2.4%), E (1%).

Conclusion: Based on the obtained results, the rate of carbapenem resistance was high among of *A. baumannii* which was isolated from intensive care units patients and oxacillinase genes were the most prevalent carbapenem resistant genes. These results revealed that three clones, A, B and C of *A.baumannii* are common in our hospitals.

Keywords: *Acinetobacter baumannii*, Carbapenem resistant, Intensive Care Units

INTRODUCTION

Acinetobacter baumannii has been recognized over the last decades as a major pathogen that responsible for severe infections, especially in the Intensive Care Units (ICU) patients (1, 2). Ventilator-associated pneumonia (VAP) occurs in critically ill patients who admitted to ICU and *A.baumannii* frequently isolated in respiratory specimens of these patients (2, 3). VAP complications are increased mortality rate, prolonged hospitalization and high medical costs (4, 5). Although, previously, most of *A.baumannii* isolates were sensitive to carbapenems, and imipenem was the gold standard treatment for *A.baumannii* pneumonia

* Corresponding author: Mojtaba Moosavian
Address: Dept.of Microbiology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
Tel: +98-611-3330074
Fax: +98-611-3332036
E-mail: moosavian_m@yahoo.com

(1, 5), widespread use of carbapenems has led to an emergence of resistant strains (6). Nowadays, carbapenem resistant *A. baumannii* (CRAB) has been reported worldwide, and it has became a global problem (7). In addition, an important concern is nosocomial infection causing by multidrug resistant *A. baumannii* (MDRAB) isolates (8). Due to increasing resistance, selection of an appropriate therapeutic option is difficult (9). The main mechanisms of carbapenem resistance in *A. baumannii,* is the production of carbapenem-hydrolyzing enzymes, mostly OXA types carbapenemases such as $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-58-like}}$ and $bla_{\text{OXA-143-like}}$ and less common by metallo-β-lactamases (7, 10). In order to control and prevention of spread of resistant isolates, it is necessary to use a molecular method for typing of MDRAB *A. baumannii* (8). Because no study has been performed independently solely on *A. baumannii* tracheal isolates in Ahvaz, this study was planned with the following objectives: (i) to study the antimicrobial susceptibility pattern (ii) to identify the most prevalent OXA types and metallo-β-lactamase genes and (iii) To characterize clonal relationship among *A. baumannii* isolates collected from the tracheal tube discharge in Ahvaz, south west of Iran using repetitive sequence-based polymerase chain reaction (REP-PCR).

MATERIALS AND METHODS

Bacterial isolates. This study was conducted at the 8 ICUs of 2 teaching hospitals, Imam Khomeyni and Golestan, associated to Ahvaz Jundishapur University of Medical Sciences from March 2010 to November 2012. A total of 206 *A. baumannii* isolates were collected from a tracheal tube discharge of ICUs patients which were colonized or infected by these bacteria. The patients were hospitalized in ICU sections such as: ICU internal, surgery, A & B, neurosurgical ICU (NSICU), general 1, general 2, cardiovascular ICU (CVICU). Initial identifications of *A. baumannii* were performed using standard laboratory tests (11).

Antimicrobial susceptibility test. All isolates were subjected to antimicrobial susceptibility testing by standard disk diffusion method according to Clinical and Laboratory Standard Institute (CLSI) guidelines (12). The tested antimicrobial agents were as follows: imipenem 10 µg, meropenem 10 µg, polymyxin B 300 unit, gentamicin 10 µg, ceftriaxone 30 µg, colistin 10 µg, piperacillin 100 µg, piperacillin-tazobactam 100/10 µg, cefepime 30 µg tobramycin 10 µg, tigecyclin 15 µg, amikacin 30 µg, tetracycline 30 µg, ciprofloxacin 5 µg, trimethoprim-sulfamethoxazole 1.25/23.75 µg, ceftazidime 30 µg, rifampin 5 µg, aztreonam 30 µg and ampicillin-sulbactam (10/10 µg), (MAST, Group Ltd, Merseyside, UK). Reference strains include *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used for quality control testing (12). The results of disk diffusion were interpreted by CLSI. The US Food and Drug Administration-approved criteria for enterobacteriaceae used for tigecycline breakpoint (13).

Extraction of DNA. DNA was extracted by phenol–chloroform method with some modification. After growth of bacteria on Mueller-Hinton's agar plates, 2 or 3 colonies were suspended in 180 µl of distilled water. Then, 20 µl of proteinase K (1 mg/ml) was added and after adding 200µl of buffer solution (0.5% SDS, 0.005 M EDTA and 0.01 M Tric-Cl, pH 7.8) mixture was incubated at 55°C for 2 hours. Then 200 µl of phenol and 200 µl of chloroform was added and gently mixed. After centrifugation at 11000 rpm for 5 minutes at 4°C, the supernatant was collected and transferred to a new tube carefully. To precipitate of DNA, 50µl of ammonium acetate 7.5 M and 200 µl of absolute ethanol were added. DNA was removed and transferred to a new tube. After washing three times with 70% ethanol, DNA was placed at 30 °C overnight. Dried DNA was dissolved in 100µl of TE buffer (14). The DNA purity of extracted was measured by photobiometer (Eppendorf, Germany) in 260/280 nm UV long waves.

Detection of $bla_{\text{OXA-51-like}}$. To confirm the identity of *A. baumannii*, $bla_{\text{OXA-51-like}}$ gene was sought by PCR assay using specific primers listed in Table 1 (15). To amplify this gene, each reaction was carried out in a final volume of 25 µl containing 1x PCR buffer, 1U Taq polymerase, 1.5 mM $MgCl_2$, 200 µM of dNTP, 10 pmol of each primer (Eurofins MWG Operon, Germany) and 1 µl of extracted DNA. Amplification conditions were programmed in Mastercycler Eppendorf (Eppendorf, Germany) as follows: Initial denaturation at 94°C for 3 min; 35 cycles of 94°C for 45 s, annealing 57°Cfor 45 s, extension 72°C for 1min and final extension 72°C for 5 min. PCR products

Table 1. Primers sequences used in this study.

Primer name	Sequence (5'-3')	Amplicon size (bp)	Reference
$bla_{\text{OXA-51- like}}$ - F	TAATGCTTTGATCGGCCTTG		
$bla_{\text{OXA-51- like}}$ -R	TGGATTGCACTTCATCTTGG	353	
$bla_{\text{OXA-23- like}}$-F	GATCGGATTGGAGAACCAGA		
$bla_{\text{OXA-23- like}}$-R	ATTTCTGACCGCATTTCCAT	501	
$bla_{\text{OXA-24- like}}$-F	GGTTAGTTGGCCCCCTTAAA		(16)
$bla_{\text{OXA-24- like}}$-R	AGTTGAGCGAAAAGGGGATT	249	
$bla_{\text{OXA-58- like}}$F	AAGTATTGGGGCTTGTGCTG		
$bla_{\text{OXA-58- like}}$-R	CCCCTCTGCGCTCTACATAC	599	
bla_{IMP}-F	TCGTTTGAAGAAGTTAACGG		
bla_{IMP}-R	ATGTAAGTTTCAAGAGTGATGC	568	
bla_{VIM}-F	GGTGTTTGGTCGCATATCGCAA		(18)
bla_{VIM}-R	ATTCAGCCAGATCGGCATCGGC	502	
bla_{NDM}- F	GGTTTGGCGATCTGGTTTTC		
bla_{NDM}-R	CGGAATGGCTCATCACGATC	624	
bla_{SPM}-F	AAAATCTGGGTACGCAAACG		(17)
bla_{SPM}-R	ACATTATCCGCTGGAACAGG	271	
REP-1	IIIGCGCCGICATCAGGC		
REP-2	ACGTCTTATCAGGCCTAC		(19)

were separated on 1.5% agarose gel (CinnaGen, Iran) by electrophoresis, stained with ethidium bromide (CinnaGen, Iran) and then visualized under UV gel documentation system. *A. baumannii* NCTC 12156 (ATCC 19606) was used as positive control. A negative control was included in each PCR reaction, containing all components except the DNA template which was replaced by distilled water (15, 16).

Multiplex PCR for detection of oxacillinase genes. $bla_{\text{OXA-23- like}}$, $bla_{\text{OXA-24- like}}$ and $bla_{\text{OXA-58-like}}$ were sought for all isolates by multiplex PCR according to protocol as described previously (15). Each PCR reaction was performed in a final volume of 25 µl with 1x PCR buffer, 1U Taq polymerase, 2 mM $MgCl_2$, 200 µM of dNTP (CinnaGen, Iran), 0.2 µM of each primer (TAG, Copenhagen A/S Denmark) and 1 µl of template DNA. PCR conditions were programmed in Mastercycler Eppendorf (Eppendorf, Germany) as follows: Initial denaturation at 94°C for 5 min; followed by 30cycles at 94°C for 30 s, 53°C for 40 s and 72°C for 50s and final extension at 72°C for 6 min. PCR products were separated with electrophoresis on 1.5% agarose gel (CinnaGen, Iran) and after staining with ethidium bromide, visualized under UV gel documentation system. *A. baumannii* reference strains including NCTC 13304, NCTC 13302, NCTC 13305 used as positive control for $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$ and $bla_{\text{OXA-58-like}}$ respectively (15).

Detection of metallo ß-lactamase genes. The presence of different metallo ß-lactamase encoding genes such as: bla_{IMP}, bla_{VIM}, bla_{SPM} and bla_{NDM} were investigated by PCR with specific primers that were listed in (Table 1) and protocol from two previous studies (16, 17).

REP-PCR. In order to determine genetic relationship of carbapenem resistant *A.baumannii*, REP-PCR was carried out on all isolates with specific primers previously described (18) and are listed in (Table 1). Amplification condition was performed based on the method of Bou *et al*. with some modifications (14). Each reaction mixture was done in volume of 25 µl with 1x PCR buffer, 3.5 mM of $MgCl_2$, 300 µM of dNTP, 3% DMSO (Cinna Gen, Iran), 0.5 µM of each primer (TAG, Copenhagen A/S Denmark), and 1U of Taq polymerase and 1 µl of genomic DNA. PCR conditions were as follows: 94°C for 10 min; 30 cycles of 94°C for 1min, annealing temperatures 45°C for 1 min and 72°C for 2 min and 72°C for 16

Table 2. The results of antibiogram test for *A.baumannii* isolates.

Antibiotic	Sensitive (%)	Intermediate (%)	Resistant (%)
Imipenem 10 µg	8 (3.9)	-	198 (96.1)
Meropenem 10 µg	8 (3.9)	-	198 (96.1)
Ceftazidime 30 µg	7 (3.4)	1 (0.5)	198 (96.1)
Cefepime 30 µg	8 (3.9)	-	198 (96.1)
Ceftriaxone 30 µg	1 (0.5)	7 (3.4)	198 (96.1)
Colistin 10 µg	206 (100)	0	0
Piperacillin 100 µg	6 (2.9)	1 (0.5)	199 (96.6)
Piperacillin-tazobactam 100/10 µg	8 (3.9)	0	198 (96.1)
Polymyxin B 300 unit	206 (100)	0	0
Gentamicin 10 µg	33 (16)	2 (1)	171 (83)
Tobramycin 10 µg	44(21.4)	0	162 (78.6)
Amikacin 30 µg	13 (6.3)	10 (4.9)	183 (88.8)
Tetracycline 30 µg	15 (7.3)	16 (7.8)	175 (85)
Ampicillin-sulbactam 10/10 µg	34 (16.5)	29 (14.1)	143 (69.4)
Ciprofloxacin 5 µg	7 (3.4)	0	199 (96.6)
Trimethoprim-sulfamethoxazole 1.25/23.75 µg	16 (7.8)	5 (2.4)	185 (89.8)
Rifampin 5 µg	0	4 (1.9)	202 (98.1)
Aztreonam 30 µg	0	3 (1.5)	203 (98.5)
Tigecycline 15 µg	13 (6.3)	66 (32)	127 (61.7)

min. Products were separated with electrophoresis on 1.2% agarose gel (Cinna Gen, Iran); after staining with ethidium bromide, they were visualized under UV gel documentation system; then they were photographed and compared together with visual inspection (14). All fingerprints were observed by one observer. Fingerprints were interpreted according to previous studies (18, 19).

Statistical analysis. The results were analyzed using the SPSS version 16 to obtain frequencies and comparison among clones. Non parametric Chi-square test was used (X^2= 156.6, df = 5). A *P*-value < 0.001 was considered statistically significant.

RESULTS

Bacterial isolates and antimicrobial susceptibility pattern. The rates of isolation from different wards were as follows: ICU Internal (41.3%), Surgery (14.1%), B (13.1%), Neurosurgical Intensive Care Unit NICU (9.2%), A (7.8%), General 1 (5.3%), General 2 (7.8%), Cardiovascular Intensive Care Unit (CVICU) (1.5%). All 206 studied isolates contained $bla_{\text{Oxa-51-like}}$ gene and were known as *A. baumannii*. All isolates (100%) were susceptible to colistin and polymyxin B. Maximum resistance rate was observed for aztreonam and rifampin with 98.5% and 98.1%, respectively. The rate of resistance to antimicrobial agents is shown in Table 2.

Detection of Oxacillinase and Metallo-β-lactamase. Results of multiplex PCR showed the $bla_{\text{OXA-23-like}}$and $bla_{\text{OXA-24-like}}$genes were detected in 85% and 8.7%, respectively (Fig. 1). Six isolates were carbapenem resistant but these isolates were negative for $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-58-like}}$ and studied metallo-β-lactamase genes. No $bla_{\text{OXA-58-like}}$, bla_{IMP}, bla_{VIM}, bla_{SPM} and bla_{NDM} were detected. $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$and $bla_{\text{OXA-58-like}}$$bla_{\text{IMP}}$, bla_{VIM}, bla_{SPM} and bla_{NDM} were not detected in carbapenem susceptible *A. baumannii* (CSAB) isolates.

REP-PCR. To determine the genetic relationship, all isolates were analyzed by REP-PCR. According to the REP-PCR results, carbapenem resistant isolates were grouped into five clones. The first prevalent genotype was named genotype A and was observed in 75 (36.4%) of 206 isolates (*P*- value < 0.001), thereafter 59 isolates (28.6%) and 57 isolates (27.7%)

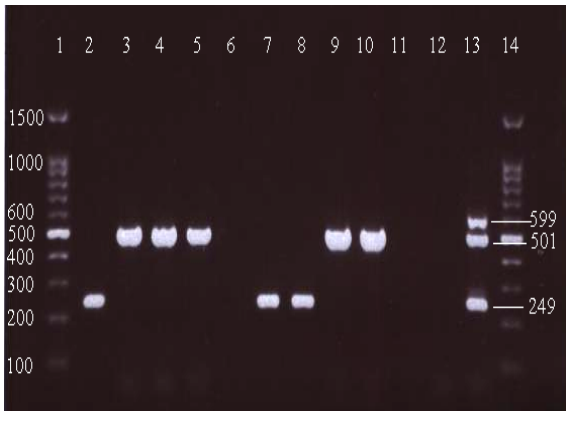

Fig. 1. Electrophoresis results of multiplex PCR. Lanes 1 and 14: 100bp DNA ladder. Lanes 2, 7, 8: isolates with $bla_{\text{OXA-24-like}}$ in 249 bp. Lanes 3, 4, 5, 9 & 10: isolates with $bla_{\text{OXA-23-like}}$ in 501 bp. Lanes 6 & 11 isolates that are negative for $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$ and $bla_{\text{OXA-58-like}}$. Lane 12 negative control (distilled water) and lane 13 Positive control (*A. baumannii* NCTC 13304, NCTC 13302, NCTC 13305 used as positive control for $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$ and $bla_{\text{OXA-58-like}}$ respectively.

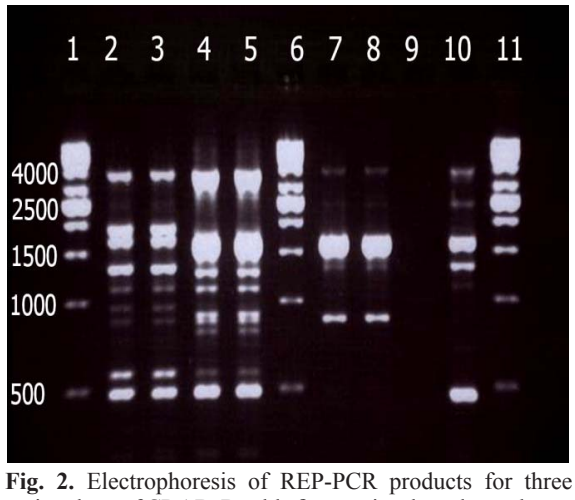

Fig. 2. Electrophoresis of REP-PCR products for three major clone of CRAB. Double fingerprints have been shown for each isolate. Lanes 1, 6 , 11, 1 kb DNA ladder. Lanes 2, 3 (clone A), lanes 4, 5 (clone B), lanes 7, 8 (clone C), Lane 9, negative control and lane 10, *A. baumannii*NCTC 12156 (ATCC 19606).

belonged to genotypes B and C, respectively (Fig. 2). The rates of other genotypes were as follows: D: 5 (2.4%), E: 2 (1%). All clones were distributed throughout the study period. Albeit $bla_{\text{OXA-23-like}}$ was found in all clones, $bla_{\text{OXA-24-like}}$ was seen only in clones A, B, C and D, but clones E was negative for this gene. Eight isolates (3.9%) were carbapenem susceptible and showed different REP pattern from each other. Frequency of each clone in different ICUs was presented in Table 3.

DISCUSSION

A. baumannii is the third causative agent of infection at ICUs patients with mortality rates of 26-68% (20). Increasing rate of carbapenems resistant *A. baumannii* is an important concern (7). Production of Class D oxacillinase which is distributed worldwide is the main mechanism of resistance to carbapenems in this organism. The major carbapenemase genes involved in carbapenem resistance are $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-58-like}}$and $bla_{\text{OXA-143-like}}$. The $bla_{\text{OXA-51-like}}$genes are located in chromosome of all *A. baumannii* strains and are intrinsic to this species (1, 21, 22). In sporadic case or hospital outbreaks of imipenem-resistant, OXA producing *A. baumannii* has been described worldwide (1). Distribution of OXA genes is variable. The rate of $bla_{\text{OXA-23-like}}$has been reported from 31% to 94% in different parts of the world (8, 10, 23-25). Our result for $bla_{\text{Oxa-23-like}}$ (85%) is in the reported ranges. We found 8.7% of isolates were positive for $bla_{\text{Oxa-24-like.}}$ However, the prevalence rate of this gene has been shown previously from 0 to 85.43% (6, 10, 16-

Table 3. Frequency of each clone of carbapenem resistant and carbapenems susceptible isolates in different ICUs.

	ICU								
Clone	Internal	Surgery	A	B	NICU[a]	General 1	General 2	CVICUb	Total
A	36	7	5	9	6	1	9	2	75
B	31	10	1	3	4	6	3	1	59
C	11	8	9	14	8	4	3	0	57
D	2	2	0	0	0	0	1	0	5
E	1	1	0	0	0	0	0	0	2
Sensitive	4	1	1	1	1	0	0	0	8
Total	85	29	16	27	19	11	16	3	206

[a]Neurosurgical ICU
[b]Cardiovascular ICU

24). Despite some authors reported $bla_{\text{OXA-58- like}}$ from 2% to 84.92% in *A. Baumannii* (8, 23,25, 26) but we did not find any $bla_{\text{OXA-58-like}}$ gene in accordance with previous studies (6, 10, 24). Interestingly, we identified 6 isolates that were resistant to imipenem and meropenem that possessed only the intrinsic $bla_{\text{OXA-51-like}}$, but other investigated genes were negative. Resistance to carbapenems in these isolates may be in related to other mechanisms including decreased permeability, alteration of penicillin-binding proteins, AmpC stable derepression and overexpression of efflux pump (27). In *A. baumannii*, metallo-β-lactamase genes such as: bla_{IMP}, bla_{VIM}, bla_{SPM} and bla_{NDM}, have been reported sporadically (28-32) but we did not find any of these genes in our isolates. Similar to our results, negative finding for bla_{IMP}, $bla_{\text{VIM,}}$ bla_{SPM} and bla_{NDM} has been reported by some authors (6, 8, 24, 33). REP-PCR revealed three main genotypes are present in our hospitals and 92.7% of isolates belonged to three genotypes A, B and C. Nosocomial outbreaks of carbapenem resistant *A. baumannii* were reported worldwide and in most cases one or two epidemic isolates were involved (34) and certain clones are disseminated in the hospitals (8, 24, 35). Since, the majority of our isolates belonged to three main genotypes, it is possible that cross-transmission with similar organisms has been occurred between patients. Therefore, it seems that these clones are circulating in our hospitals. Yan *et al*. reported that multidrug resistant *A. baumannii* from same genotype can be transferred between hospitals and even cities (8). Albeit $bla_{\text{Oxa-23-like}}$ was found among all resistant clones, interestingly only genotypes A, B, C and D were positive for $bla_{\text{Oxa-24-like}}$ and this gene was negative in genotypes E. Of note, each sensitive isolates had unique REP-PCR patterns that were different from carbapenem resistant isolates and it is assumed that probable source of this isolates were outside of the studied hospitals. However, it has been reported that carbapenem susceptible isolates may have highly similar or different REP pattern from the carbapenem resistant isolates (35). Our finding revealed that in addition to carbapenems, the rates of resistance to other antimicrobial agents were high. Dizbay *et al*. 2008 in Turkey studied on susceptibility pattern of 66 isolates of *A. baumannii* collected from endotracheal aspirate and found resistance rate to imipenem, and meropenem was 80.3% and 71.2%, respectively. Different rate of resistance to other tested antibiotics were reported by Dizbay et al. (9). The rates of resistance to all antimicrobial agents in our study are higher than reported by Dizbay *et al*.

In conclusion, REP-PCR revealed that three main genotypes involved in the two studied hospitals. In order to prevent the spread of resistant isolates between different wards and patients, we suggest it is necessary to monitor and improve infection-control procedures. Although the research was carefully performed and was reached its aims, but there was one limitation. In our study, difference between colonization and infection was not clear and it suggested that in future studies this subject be surveyed.

ACKNOWLEDGEMENTS

This study was granted an approved research plan (NO.90126) and was financially supported by deputy vice-chancellor for research affairs of Ahvaz Jundishapur University of Medical Sciences, and Infectious & Tropical Disease Research Center, and we thank all of them. We acknowledge especially Research Consultation Center (RCC) for technical support.

REFERENCES

1. Kempf M, Rolain JM. Emergence of resistance to carbapenems in *Acinetobacter baumannii* in Europe: clinical impact and therapeutic options. *Int J Antimicrob Agents* 2012; 39:105-114.
2. Peymani A, Higgins PG, Nahaei MR, Farajnia S, Seifert H. Characterisation and clonal dissemination of OXA-23-producing *Acinetobacter baumannii* in Tabriz, northwest Iran. *Int J Antimicrob Agents* 2012; 39: 526-528.
3. Garnacho-Montero J, Ortiz-Leyba C, Fernandez-Hinojosa E, Aldabo-Pallas T, Cayuela A, Marquez-Vacaro JA, et al. *Acinetobacter baumannii* ventilator-associated pneumonia: epidemiological and clinical findings. *Intensive Care Med* 2005; 31: 649-655.
4. Joseph NM, Sistla S, Dutta TK, Badhe AS, Parija SC. Ventilator-associated pneumonia: role of colonizers and value of routine endotracheal aspirate cultures. *Int J Infect Dis* 2010; 14: 723-729.
5. Shete VB, Ghadage DP, Muley VA, Bhore AV. Multi-drug resistant Acinetobacter ventilator-associated pneumonia. *Lung India* 2010; 27 : 217-220.
6. Manageiro V, Jones-Dias D, Ferreira E, Louro D, Canica M. Genetic diversity and clonal evolution of carbapenem-resistant *Acinetobacter baumannii* isolates from Portugal and the dissemination of ST118. *Int J Antimicrob Agents* 2012; 40: 398-403.
7. Lee HY, Chen CL, Wang SB, Su LH, Chen SH, Liu SY, et al. Imipenem heteroresistance induced by imipenem

in multidrug-resistant *Acinetobacter baumannii*: mechanism and clinical implications. *Int J Antimicrob Agents* 2011; 37: 302-308.
8. Yan ZQ, Shen DX, Cao JR, Chen R, Wei X, Liu LP, et al. Susceptibility patterns and molecular epidemiology of multidrug-resistant *Acinetobacter baumannii* strains from three military hospitals in China. *Int J Antimicrob Agents* 2010; 35: 269-73.
9. Dizbay M, Altuncekic A, Sezer BE, Ozdemir K, Arman D. Colistin and tigecycline susceptibility among multidrug-resistant *Acinetobacter baumannii* isolated from ventilator-associated pneumonia. *Int J Antimicrob Agents* 2008; 32: 29-32.
10. Irfan S, Turton JF, Mehraj J, Siddiqui SZ, Haider S, Zafar A, et al. Molecular and epidemiological characterisation of clinical isolates of carbapenem-resistant *Acinetobacter baumannii* from public and private sector intensive care units in Karachi, Pakistan. *J Hosp Infect* 2011; 78: 143-148.
11. Winn W, Allen S, Janda W, Koneman E, Procop G, Schreckenberger P Woods G(2006). Koneman's Color Atlas and Textbook of Diagnostic microbiology 6 ed. Lippincott Williams & Wilkins, Philadelphia.
12. Clinical and Laboratory Standards Institute, 2011. Performance standards for antimicrobial susceptibility testing: 21th informational supplement. CLSI document M100-S21. CLSI, Wayne, Pa.
13. Wyeth Pharmaceuticals. Tygacil (tigecycline) for injection [Package insert]. 2005. Wyeth Pharmaceuticals Inc., Philadelphia, PA.
14. Bou G, Cervero G, Dominguez MA, Quereda C, Martinez-Beltran J. PCR-based DNA fingerprinting (REP-PCR, AP-PCR) and pulsed-field gel electrophoresis characterization of a nosocomial outbreak caused by imipenem- and meropenem-resistant *Acinetobacter baumannii*. *Clin Microbiol Infect* 2000; 6: 635-643.
15. Woodford N, Ellington MJ, Coelho JM, Turton JF, Ward ME, Brown S, et al. Multiplex PCR for genes encoding prevalent OXA carbapenemases in Acinetobacter spp. *Int J Antimicrob Agents* 2006; 27: 351-3.
16. Poirel L, Walsh TR, Cuvillier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. *Diagn Microbiol Infect Dis* 2011; 70: 119-123.
17. Zong Z, Lu X, Valenzuela JK, Partridge SR, Iredell J. An outbreak of carbapenem-resistant *Acinetobacter baumannii* producing OXA-23 carbapenemase in western China. *Int J Antimicrob Agents* 2008; 31: 50-4.
18. Martin-Lozano D, Cisneros JM, Becerril B, Cuberos L, Prados T, Ortiz-Leyba C, et al. Comparison of a repetitive extragenic palindromic sequence-based PCR method and clinical and microbiological methods for determining strain sources in cases of nosocomial *Acinetobacter baumannii* bacteremia. *J Clin Microbiol* 2002; 40: 4571-4575.
19. Snelling AM, Gerner-Smidt P, Hawkey PM, Heritage J, Parnell P, Porter C, et al. Validation of use of whole-cell repetitive extragenic palindromic sequence-based PCR (REP-PCR) for typing strains belonging to the *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex and application of the method to the investigation of a hospital outbreak. *J Clin Microbiol* 1996; 34: 1193-1202.
20. Nomanpour B, Ghodousi A, Babaei A, Abtahi H, Tabrizi M, Feizabadi M. Rapid, cost-effective, sensitive and quantitative detection of *Acinetobacter baumannii* from pneumonia patients. *IranJ microbiol* 2011; 3: 162-9.
21. D'Arezzo S, Principe L, Capone A, Petrosillo N, Petrucca A, Visca P. Changing carbapenemase gene pattern in an epidemic multidrug-resistant *Acinetobacter baumannii* lineage causing multiple outbreaks in central Italy. *J Antimicrob Chemother* 2011; 66: 54-61.
22. Feizabadi MM, Fathollahzadeh B, Taherikalani M, Rasoolinejad M, Sadeghifard N, Aligholi M, et al. Antimicrobial susceptibility patterns and distribution of blaOXA genes among Acinetobacter spp. Isolated from patients at Tehran hospitals. *Jpn J Infect Dis* 2008; 61: 274-278.
23. Touati M, Diene SM, Racherache A, Dekhil M, Djahoudi A, Rolain JM. Emergence of blaOXA-23 and blaOXA-58 carbapenemase-encoding genes in multidrug-resistant *Acinetobacter baumannii* isolates from University Hospital of Annaba, Algeria. *Int J Antimicrob Agents* 2012; 40: 89-91.
24. Carvalho KR, Carvalho-Assef AP, Peirano G, Santos LC, Pereira MJ, Asensi MD. Dissemination of multidrug-resistant *Acinetobacter baumannii* genotypes carrying bla(OXA-23) collected from hospitals in Rio de Janeiro, Brazil. Int. *Int J Antimicrob Agents* 2009; 34: 25-8.
25. Ergin A, Hascelik G, Eser OK. Molecular characterization of oxacillinases and genotyping of invasive *Acinetobacter baumannii* isolates using repetitive extragenic palindromic sequence-based polymerase chain reaction in Ankara between 2004 and 2010. *Scand J Infect Dis* 2013; 45: 26-31.
26. Ben RJ, Yang MC, Hsueh JC, Shiang JC, Chien ST. Molecular characterisation of multiple drug-resistant *Acinetobacter baumannii* isolates in southern Taiwan**.** *Int J Antimicrob Agents* 2011; 38: 403-408.
27. Kulah C, Mooij MJ, Comert F, Aktas E, Celebi G, Ozlu N,et al. Characterisation of carbapenem-resistant *Acinetobacter baumannii* outbreak strains producing OXA-58 in Turkey. *Int J Antimicrob Agents* 2010; 36: 114-118.
28. Hrabak J, Stolbova M, Studentova V, Fridrichova M, Chudackova E, Zemlickova H. NDM-1 producing *Acinetobacter baumannii* isolated from a patient repatriated to the Czech Republic from Egypt, July 2011.*Euro Surveill* 2012; 17: 1-3.
29. Peymani A, Nahaei MR, Farajnia S, Hasani A, Mirsalehian A, Sohrabi N, et al. High prevalence of metallo-beta-lactamase-producing *Acinetobacter baumannii* in a teaching hospital in Tabriz, Iran. Jpn J Infect Dis 2011; 64: 69-71.
30. Karthikeyan K, Thirunarayan MA, Krishnan P. Coexistence of blaOXA-23 with blaNDM-1 and armA in clinical isolates of *Acinetobacter baumannii* from India. *J Antimicrob Chemother* 2010; 65: 2253-2254.
31. Mohamed NM, Raafat D. Phenotypic and Genotypic

Detection of Metallo-beta-lactainases in Imipenem-resistant *Acinetobacter baumannii* Isolated from a Tertiary Hospital in Alexandria, Egypt. *Res J Microbiol* 2011; 6: 750-760.
32. Shahcheraghi F, Abbasalipour M, Feizabadi M, Ebrahimipour G, Akbari N. Isolation and genetic characterization of metallo-β-lactamase and carbapenamase producing strains of *Acinetobacter baumannii* from patients at Tehran Hospitals. *Iran J Microbiol* 2011; 3: 68-74.
33. Netsvyetayeva I, Sikora M, Golas M, Swoboda-Kopec E, de Walthoffen SW, Dembicka O, et al. *Acinetobacter baumannii* multidrug-resistant strain occurrence in liver recipients with reference to other high-risk groups. *Transplant Proc* 2011; 43: 3116-3120.
34. Zarrilli R, Giannouli M, Tomasone F, Triassi M, Tsakris A. Carbapenem resistance in *Acinetobacter baumannii*: the molecular epidemic features of an emerging problem in health care facilities. *J Infect Dev Countries* 2009; 3: 335-341.
35. Huang L, Sun L, Xu G, Xia T (2008). Differential susceptibility to carbapenems due to the AdeABC efflux pump among nosocomial outbreak isolates of *Acinetobacter baumannii* in a Chinese hospital. *Diagn Microbiol Infect Dis* 2008; 62: 326-332.