JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Sept. 2016. Vol. **10**(3), p. 1675-1682

Molecular Characterization of Carbapenem Resistant *Acinetobacter baumannii* and Investigation of Genetic Diversity Between Local and International Clones

Salman Shaheer Ahmed[1,2], Gokcen Dinc[2,3], Baldan Rossella[4], Emine Alp[1], Aysegul Ulu-Kilic[1], Willem JG Melchers[5], Joost Hopman[5] and Andreas Voss[5]

[1]Department of Infectious diseases, Faculty of Medicine, Erciyes University, Kayseri, Turkey.
[2]Department of Molecular Microbiology, Genome and Stem Cell Center, Erciyes University, Kayseri, Turkey.
[3]Department of Medical Microbiology, Faculty of Medicine, Erciyes University, Kayseri, Turkey.
[4]Emerging Bacterial Pathogens Unit, San Raffaele Scientific Institute, Milan, Italy.
[5]Department of Medical Microbiology, Radboud University Medical Center, Nijmegen, The Netherlands.

https://doi.org/10.22207/JPAM.10.3.01

(Received: 11 May 2016; accepted: 03 July 2016)

The emergence of Carbapenem-resistant *Acinetobacter baumannii* is a threat to modern medicine and has been recognised globally. Epidemic clones of *A.baumannii* are associated with hospital epidemics throughout the world causing serious ramifications. There are number of molecular typing techniques used for molecular characterisation, however, due to lack of resources molecular epidemiology remains unfledged in developing countries. In this study a total number of 205 carbapenem-resistant *Acinetobacter* species were collected and compared with European Clone I, II and III. PCR for and $bla_{\text{OXA-51-like}}$ was performed for confirmation and multiplex PCR was performed for $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$ and $bla_{\text{OXA-58-like}}$ genes. Isolates harbouring carbapenemases were selected for molecular typing using PFGE, RAPD-PCR and REP-PCR and discriminatory index was performed. The cost of all molecular typing methods was calculated. The $bla_{\text{OXA-51-like}}$ gene was present in 188 (91.7%), and within these 188 isolates; $bla_{\text{OXA-23-like}}$ 36(19%), $bla_{\text{OXA-24-like}}$ 62(33%), 30(16%) carried $bla_{\text{OXA-58-like}}$ genes. PFGE typing revealed 13 clusters and 15 unique types, RAPD-PCR, 11 clusters and 23 unique types and REP-PCR, 8 clusters and no unique types. With 80% similarity cut-off value, 11 isolates were similar to EU clone I, 5 isolates were similar to EU II and 3 isolates were similar to EU III. Cost of molecular methods was compared between PFGE, RAPD-PCR and REP-PCR. PFGE was found to have high discriminatory power and RAPD-PCR was economical.

Keywords: carbapenem; hospital infections; molecular typing.

In recent years, *Acinetobacter baumannii* has emerged as a predominant cause of healthcare-associated infections worldwide. From an epidemiological point of view, its most remarkable features as a nosocomial pathogen are its ability to survive in the hospital environment and to develop resistance to multiple antimicrobial agents. It is responsible for various opportunistic nosocomial infections including skin, wound and urinary tract infections often targeting vulnerable hospitalized patients[1]. In a recent European study *A.baumannii* was among the eight most common causes of nosocomial pneumonia and the second most common pathogen in bloodstream infections acquired in hospitals. Carbapenem resistance is often mediated by OXA-carbapenemases-encoding genes; extrinsically by $bla_{\text{OXA-23-like}}$,

* To whom all correspondence should be addressed.
Tel.: +90 531 381 9526;
E-mail: salmansahmed@erciyes.edu.tr

$bla_{\text{OXA-24-like}}$ and $bla_{\text{OXA-58-like}}$, and intrinsically by $bla_{\text{OXA-51-like}}$ carbapenemases[2]. Outbreaks of this organism in hospitals are frequently caused by strains belonging to the European clones I-III. Resistance to several antibiotics, in particular carbapenems, is the hallmark of the most successful clones[3]. Despite the widely accepted idea that a few genotypic groups are responsible for a large proportion of the burden of *A. baumannii* infections, the genetic distinctness of clones among themselves and from other genotypes remains to be established.

Strain typing of *A. baumannii* has become an indispensable epidemiological tool to identify reservoirs and pattern of transmission. Pulsed-field gel electrophoresis (PFGE), a macrogenomic strain typing approach, is the most commonly employed method in health care facilities. However, PFGE remains an expensive, effort-intensive and technically demanding procedure with a long turnaround time, urging to find alternatives[4]. In resource limited countries, semi-automated PCR-based technologies such as Random amplification of polymorphic DNA (RAPD) PCR and Repetitive element palindromic (REP) PCR, remain therefore the choice of typing. Typing data generated by these methods are generally available within 12 hours[5]. There are many studies in which these typing methods were used to study *A.baumannii* isolates exclusively [6]. However, comparison between these three methods and their cost-effectiveness in resource limited settings has not been extensively examined yet.

The aims of this study are to (i) determine the prevalence of carbapenem resistance genes in a large series of isolates, (ii) obtain epidemiological typing data for carbapenem-resistant *A.baumannii*, (iii) establish the clonal relationship of Turkish isolates with the European clones I-III, (iv) compare different typing methods such as PFGE, RAPD-PCR and REP-PCR, and their cost-effectiveness for resource limited countries.

MATERIALS AND METHODS

Bacterial isolates

A total of 205 clinical isolates of multidrug resistant *Acinetobacter* species isolates were collected from the Department of Infectious diseases, Erciyes University Hospital between 2011 and 2012. To establish clonal relatedness we used European clone I, II, and III (NIPH 527, NIPH 528, NIPH 1669) provided by Dr. Alexander Nemec, Laboratory of Bacterial Genetics, National Institute of Public Health, Prague, Czech Republic. Out of 205 clinical isolates 97 isolates carrying extrinsic OXA genes were used further for molecular profiling. Clinical data collected included patients' age, sex, number of days in hospital prior to isolation, specimen types, wards, and type of infection. Isolates were collected by previously described method[7], isolated in pure culture on Luria-Bertani agar plates, and identified with the Vitek 2 system (bio-Merieux, Marcy-l'Étoile, France). Identification at the species level was reconfirmed with the API 20NE system (bio-Merieux, Marcy-l'Étoile, France).

Bacterial growth and DNA extraction

All *Acinetobacter* isolates were grown in Luria-Bertani broth (BD Laboratories, NJ, USA) at 37°C for 24 h with shaking. Genomic DNA was extracted using PureLink Spin Column-based kit (Invitrogen, California, USA).

PCR for resistance gene detection

PCR assays for the presence of carbapenemase encoding genes ($bla_{\text{OXA-51-like}}$, $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, and $bla_{\text{OXA-58-like}}$) were performed, using AmpliTaq Gold DNA polymerase (Life Technologies, California, USA) and AmpliTaq Gold PCR Master mix (Life Technologies, California, USA) and primers (Table no.1) were designed from (Invitrogen, California, USA), as previously described[8]. The Thermocycler (Sensoquest, Göttingen, Germany) was programmed as 94°C for 5 min, 30 cycles of 94°C for 25 s, 52°C for 40 s and 72°C for 50 s, and a final elongation at 72°C for 6 min. Amplification products were run on 1.5% agarose gel in 0.5TAE buffer at, 60 V for 1.5 h, and bands visualized with GelRed (0.5 mg/L) (Biotium, California, USA) using a ChemiDoc Imaging system (Biorad, Califronia, USA).

Random Amplified Polymorphic DNA (RAPD)-PCR

RAPD-PCR genomic fingerprinting was performed using AmpliTaq Gold DNA polymerase (Life Technologies, California, USA) and AmpliTaq Gold PCR Master mix (Life Technologies, California, USA) and primers were designed from (Invitrogen, California, USA). The primer and PCR conditions were used as previously described[9]. The Primer 52

-GCTTGTGAAC-32 with the following protocol: initial denaturation at 94°C for 3 min, followed by 34 cycles at 92°C for 30 s, 40°C for 60 s and 72°C for 3 min, a final single cycle at 92°C for 30 s, 40°C for 60 s and 72°C for 10 min. Amplification products were run on 1% agarose gels with 1× TAE buffer at 90 V for 1·5 h, and bands visualized with GelRed (0.5 mg/L)(Biotium, California, USA) using a ChemiDoc Imaging system (Biorad, Califronia, USA).

Repetitive Element Palindromic (REP)-PCR

It was performed using primer pair used in previously described study [10] REP1 5'-IIIGCGCCGICATCAGGC-32 and REP2 5'-ACGTCTTATCAGGCCTAC-32 , the primer REP1 has the nucleotide (I) inosine at ambiguous positions in the REP consensus sequence. Inosine contains the purine base hypoxanthine and is able to base-pair with A, C, G and T. An initial denaturation at 94 °C for 10 min, followed by 30 cycles at 94 °C for 60 s, 45 °C for 60 s and 72 °C for 2 minutes, with a single final elongation at 72 °C for 16 minutes u. Amplification products were run on 1% agarose gels with 1× TAE buffer at 90 V for 1·5 h, stained with GelRed (0.5 mg/L)(Biotium, California, USA) and bands visualized using a ChemiDoc Imaging system (Biorad, Califronia, USA).

Pulsed Field Gel Electrophoresis (PFGE)

Genomic DNA embedded in agarose blocks was digested with 30U *SmaI*[11] (New England Biolabs, Ipswich, UK). Agarose gel electrophoresis was performed in 1% PFGE certified agarose gels (Biorad, California, USA) in 0.5 X TBE buffer using a CHEF DR III apparatus (Biorad, California, USA) for 20 hours at 12ºC. The run conditions were 200 V with a pulse angle of 120° and pulse times of three phases as follows: 20s for 8h, 10s for 8h and 5s for 4h. A Lambda PFGE ladder (Biorad, California, USA) was used to provide molecular size markers, and the gels were stained with GelRed (0.5 mg/L) (Biotium, California, USA). DNA bands patterns were visualized using a ChemiDoc Imaging system (Biorad, Califronia, USA). PFGE, RAPD-PCR and REP-PCR clusters were interpreted using Bionumerics software (version 2.5 (Applied Maths, Kortrijk, Belgium) (Department of Microbiology, Ospedale San Raffaele Institute, Milan, Italy); using the Dice correlation coefficient and Unweighted Pair Group Method with Arithmetic Mean (position tolerance and optimization of 1.5%).

Cost comparison of molecular typing methods

The costs in Euros of PFGE, RAPD-PCR and REP-PCR were compared. Material cost (media, reagents, and commercial kits); instrumentation, chemicals and lab ware were also calculated.

RESULTS

PCR for Resistance genes

The $bla_{\text{OXA-51-like}}$ gene was present in 188 (91.7%) isolates out of 205 isolates collected for 2 years from 2011-2012. Only isolates containing $bla_{\text{OXA-51-like}}$ gene were further processed for PCR study for other resistance genes such as $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$ and $bla_{\text{OXA-58-like}}$. In those isolates, 36(19%) isolates carried $bla_{\text{OXA-23-like}}$, 62(33%) carried $bla_{\text{OXA-24-like}}$ and 30(16%) isolates carried $bla_{\text{OXA-58-like}}$ resistant genes.

PFGE, RAPD-PCR and REP-PCR

With 80% similarity cut-off value, a hundred carbapenem isolates out of 188 isolates those carrying extrinsic $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, and $bla_{\text{OXA-58-like}}$ genes only, were included for DNA profiling studies with 3 EU clones as standards. PFGE typing revealed 13 clusters followed by RAPD-PCR: 11 clusters and REP-PCR: 8 clusters. RAPD-PCR showed 23 unique types followed by PFGE: 15 and REP-PCR showed none unique types. Consequently, PFGE showed highest discriminatory index (0.8814) sharply followed by RAPD-PCR (0.8784) and REP-PCR (0.7261) (Table no. 2). Discriminatory index was calculated by using indices of Hunter's discriminatory formula

$$(9)\quad D = 1 - \frac{1}{N(N-1)} \sum_{j=1}^{s} x_j(x_j - 1)$$

Analysis of 100 isolates by PFGE showed 11 isolates (Table no.2) within cluster A were similar to EU I clone. Five isolates belonging to cluster G and 3 isolates belonging to cluster N were similar to EU II and EU III clone respectively. European clones were mainly isolated from Medical ICU including all 11 EU I clone, (2/5) EU clone 2 isolated from coronary vascular ICU and Infectious Diseases Service and (2/3) EU clone 3 from internal medicine ICU and (1/3) EU clone 3 is isolated from general surgery ICU.

Clinical Characteristics of the data

Analysis of 100 selected isolates data

Table 1. Primers Used for Detection of OXA-Like Genes

Resistance gene	Primers were taken from previous study (R5)	Fragment size
$bla_{\text{OXA-51-like}}$	52 -TAATGCTTTGATCGGCCTTG-32 52 -TGGATTGCACTTCATCTTGG-32	353base pairs
$bla_{\text{OXA-23-like}}$	52 -GATCGGATTGGAGAACCAGA-32 52 -ATTTCTGACCGCATTTCCAT-32	501 base pairs
$bla_{\text{OXA-24-like}}$	52 -GGT TAG TTG GCC CCC TTA AA-32 52 -AGT TGA GCG AAA AGG GGA TT-32	246 base pairs
$bla_{\text{OXA-58-like}}$	52 -AAG TAT TGG GGC TTG TGC TG-32 52 -CCC CTC TGC GCT CTA CAT AC-32	599 base pairs

Table 2. Typing analysis of *A.baumannii* using PFGE, RAPD-PCR and REP-PCR methods

Methods	Clusters	Unique types	Discriminatory index
REP-PCR	8	0	0.7261
RAPD-PCR	11	23	0.8784
PFGE	13	15	0.8814

Table 3. Clinical characteristics of the data

Underlying conditions	All patients, n (%)
Malignancy	33(16.1)
Liver failure	7(3.4)
COPD	31(15.1)
Diabetes mellitus	42(20.5)
Chronic heart failure	24(11.7)
Kidney failure	41(20)
Hypertension	41(20)
Corticosteroid therapy	51(24.9)
Invasive Procedures in Diagnosis and Treatment	
Total parenteral nutrition	62(30.2)
Re-intubation	14(6.8)
Transfusion	118(57.8)
Operation	67(32.7)
Enteral feeding	72(35.1)
Peritoneal dialysis	6(2.9)
Central venous catheter	117(57.6)
Urinary catheter	152(74.1)
Mechanical ventilation	127(62)
Tracheotomy	52(25.4)
Drainage catheter	24(11.7)
Chest tube	25(12.2)
External ventricular drainage	1(0.5)
Bronchoscopy	7(3.4)
Colostomy	8(3.9)

Table 4. Overall cost and efficiency conditions of different molecular techniques

		PFGE	RAPD-PCR	REP-PCR
Reagents/ consumables	DNA extraction	€2	€0.5	€0.5
	Gel separation and analysis	€3	€1	€1
	PFGE DNA extraction kit	€350 for 50 samples	No kit is required if DNA extracted professionally.	No kit is required if DNA extracted professionally.
	Restriction enzymes	€150 with expiry date of 1 year	Not applicable	Not applicable
	primers	Not applicable	€30 with life span of 2-3 years if stored in -20 degrees	€30 with life span of 2-3 years if stored in -20 degrees
	Agarose	Specialized agarose €200 only for PFGE	€20 Normal agarose for molecular biology	€20 Normal agarose for molecular biology
	Buffer	Specialized TAE/TBE buffer is preferred cost €200/1lt 50X	Normal laboratory prepared TAE/TBE yield good results	Normal laboratory prepared TAE/TBE yield good results
Software used	Bionumerics	Only Bionumerics software can be suedCost < €10,000	Bionumerics used for comparison, also other free software can be used	Bionumerics used for comparison, also other free software can be used
Overall cost and efficiency conditions of different molecular	Time	1 week	8 hours	8 hours
	Material cost per sample	€2.7	€1	€1
	Hands on time per 20 sample batch	9.5 hours	2.5 hours	2.5 hours
	Equipment cost	€28,000	€6,000	€6,000
	Labour intensity	Very high	normal	High
	Discriminatory power	High	Normal	Low

revealed 28% (28/100) of patients were colonised and remaining 72% (72/100) were detected with primary infection. Based on 100 selected isolated, the Male: Female ratio was 65:35 and the majority of the patients were aged between 45-75 years old. Most patients were admitted to hospital for medical conditions and had one or more predisposing factors to *A.baumannii* infections. As noted in (Table no. 3), the most common underlying condition was corticosteroid therapy (n=51, 24.9%) followed by diabetes mellitus (n=42, 20.5%), malignancy (n=33, 16.1%) and chronic obstructive pulmonary disease (COPD) (n=31, 15.1%). The most common invasive diagnosis procedure was from urinary catheter (n=152, 74.1%) followed by central venous catheter (n=117, 57.6%). Of all cases, (n=44, 23.4%) patients admitted to ICU of which (n=16, 8.5%) patients had most common reason for admission was respiratory disease.

Cost effectiveness of Typing methods

PFGE apparatus is expensive compared to any thermocycler (Table no. 4). PCR-based typing methods are economically thermocyclers can be used for various PCR based reactions including typing and technically suitable for resource limited laboratories in developing countries. Cost of the molecular techniques was calculated in which RAPD-PCR was the most economic method (5 Euros/sample including consumables), however, PFGE (11 Euros/sample including consumables) is gold standard but laborious, time consuming (1 week) and expensive REP-PCR (6 Euros/sample including consumables) was also economic (Table 4).

DISCUSSION

Previous study reported the presence of European clone II in Turkey[2], however, in this study we have showed the presence of European clone I, II and III in Turkey. Moreover, EU clone III was first to be determined in Turkey. Furthermore, the presence of 11 isolates were similar to EU clone I lineage isolates, 5 were similar to EU clone II and 3 were similar to EU clone III speculating wider multidrug resistance threat within local findings. Our results support the presence of $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$ and $bla_{\text{OXA-58-like}}$. Thirty six isolates (19%) out of 188 carried $bla_{\text{OXA-23-likes}}$ genes, which is low, compared to other studies reporting 100%[12] or high prevalence of $bla_{\text{OXA-23-like}}$ some reported more than 80%[13] in Turkey. Presence of $bla_{\text{OXA-24-like}}$ resistance gene was reported in 62 isolates (33%) which is rarely identified in this geographical region and this is the first study to report $bla_{\text{OXA-58-like}}$ genes from Turkey.

Nowadays, there is a growing interest to analyze *A. baumannii* infection/colonization because one of the main problems with *A. baumannii* is the difficulty to distinguish true infection from colonization and to regulate whether or not the affected patients require therapy against this pathogen. Furthermore, colonization is main risk factor for secondary infection and A.baumannii colonization strains are more resistant to antibiotics than A.baumannii infection strains[14]. In the current study, taking into account all units, the first sources of *A. baumannii* isolates were wound/drainage and skin and soft-tissue samples, representing 26.3% of all samples. This could be explained by the ubiquitous dissemination of *A. baumannii* in all units from patients with trauma, burns, devices or invasive medical procedures such as catheters and drains. Another risk factor for dissemination is the ability of *A baumannii* to survive in hospital environments for an average of 20 days at a relative humidity of 31%[15].

In our setting, 64.8% (122/188) of *A. baumannii* isolates were from ICU patients. Thus, ICU was the main unit with high risk patients of hospital-acquired *A. baumannii* infection/ colonization. As described worldwide, *A. baumannii* has become a nightmare for many ICUs with critically ill patients with multiple co-morbidities, concurrent infections and prolonged courses of antibiotics[16]. In ICU patients, *A. baumannii* was mostly isolated from the respiratory tract (20.7%), followed by bloodstream (19.6%) and urine (10.3%). Thus, the respiratory tract was the most common site of isolation of *A. baumannii*. This is not surprising because *A. baumannii* has the propensity to colonize respiratory or ventilation devices in ICU ventilated patients. In these patients, it is often difficult to distinguish upper airway colonization from ventilator-associated pneumonia.

In addition to this, we also investigated the use of various molecular typing methods employed to characterise *A.baumannii* in resource

limited laboratories. Cost depends of numerous factors, the amount of initial capital outlay for the equipment, its depreciation, which will depend on whether it is out-of-date compared with newer versions or totally new platforms. The frequency and care it is used and finally of any modifications to the working place. In addition, cost of servicing, the price, and need for the any replacement parts and consumables should also be considered. In our study, cost of the molecular techniques was calculated in which RAPD-PCR was the most economic method (5 Euros/sample including consumables), however, PFGE (11 Euros/sample including consumables) is gold standard but laborious, time consuming and expensive. PFGE apparatus is expensive compared to any thermocycler, which makes it generally unaffordable in resource limited laboratories (Table no. 4). Furthermore, thermocyclers can be used for various PCR based reactions including typing.

Amenability to computerised analysis and incorporating of the typing results and electronic database are other important criteria for typing. Since these factors are important for longitudinal comparison of large number of isolates. At local hospital data obtained by robust typing methods can be analysed electronically or assessed visually. Visual interpretation of even small number of isolates requires normalisation of data prior to inspection[17]. Nonetheless, since clones are spreading among hospital and various geographical areas electronic databases helps microbiologist and public health institutes to monitor the spread. In PFGE, results are analysed using BioNumerics software and typing results are incorporated for computer analysis, however, which costs more than €10,000, which could be a negative aspect of this technique. With this software we can analyze RAPD-PCR and REP-PCR gel bands together with PFGE.

A good typing method should discriminate between assigned samples to two different strains sampled randomly from population of given species. It can be expressed as a probability using Simpson's index of diversity. Hunter and Gatson's modification of Simpson's index of diversity and fixed confidence intervals are important criteria used to decide strain's identity or diversity. In our study PFGE has high discriminatory power and the results can be compared by international database called PusleNet, however, unlike RAPD-PCR and REP-PCR[18].

Stability is another criterion for evaluation of typing method in which strains markers assessed by typing method should not change rapidly or should remain stable during storage and subculture. Nearly all RAPD markers are dominant, it is not possible to distinguish whether DNA segment form heterozygous has amplified or from homozygous. Furthermore, it is a PCR based reaction and mismatch between the primer and template could hinder the product formation.

RAPD-PCR provides a much higher level of discrimination for less cost of a diverse cohort to hospital- and community-acquired CD clinical strains [19]. It should also be recognized that RAPD-PCR would also be less expensive to perform if the PCR products were electrophoresed using an agarose gel instead of specialized agarose gel for PFGE which is 10 times more expensive. Considering the overall cost of the technique and procedure PCR based methods are more appropriate, however, PFGE has supremacy over RAPD-PCR and REP-PCR in discriminatory power and reproducibility. Although further studies needed to compare these techniques and other advanced molecular methods.

ACKNOWLEDGMENTS

This study was supported by Erciyes University Scientific Research Projects (BAP kodu 4059) and presented as poster in EACID-2014 Belgrade (P-232). This study was approved by local ethical committee, Erciyes University, Kayseri 38039, Turkey.

REFERENCES

1. Alp E, Coruh A, Gunay GK, Yontar Y, Doganay M: Risk factors for nosocomial infection and mortality in burn patients: 10 years of experience at a university hospital. *J Burn Care Res* 2012, **33**(3):379-385.
2. Ahmed SS, Alp E, Ulu-Kilic A, Dinc G, Aktas Z, Ada B, Bagirova F, Baran I, Ersoy Y, Esen S *et al*: Spread of carbapenem-resistant international clones of Acinetobacter baumannii in Turkey and Azerbaijan: a collaborative study. *Eur J Clin Microbiol Infect Dis* 2016.

3. Hu D, Liu B, Dijkshoorn L, Wang L, Reeves PR: Diversity in the major polysaccharide antigen of Acinetobacter baumannii assessed by DNA sequencing, and development of a molecular serotyping scheme. *PLoS One* 2013, **8**(7):e70329.
4. Bae IK, Kim J, Sun JY, Jeong SH, Kim YR, Wang KK, Lee K: Comparison of pulsed-field gel electrophoresis & repetitive sequence-based PCR methods for molecular epidemiological studies of Escherichia coli clinical isolates. *Indian J Med Res* 2014, **140**(5):679-685.
5. Campioni F, Pitondo-Silva A, Bergamini AM, Falcao JP: Comparison of four molecular methods to type Salmonella Enteritidis strains. *APMIS* 2015, **123**(5):422-426.
6. Ahmed SS, Alp E: Genotyping methods for monitoring the epidemic evolution of A. baumannii strains. *J Infect Dev Ctries* 2015, **9**(4):347-354.
7. Jacobs AC, Sayood K, Olmsted SB, Blanchard CE, Hinrichs S, Russell D, Dunman PM: Characterization of the Acinetobacter baumannii growth phase-dependent and serum responsive transcriptomes. *FEMS Immunol Med Microbiol* 2012, **64**(3):403-412.
8. Woodford N, Ellington MJ, Coelho JM, Turton JF, Ward ME, Brown S, Amyes SG, Livermore DM: Multiplex PCR for genes encoding prevalent OXA carbapenemases in Acinetobacter spp. *Int J Antimicrob Agents* 2006, **27**(4):351-353.
9. Carr E, Eason H, Feng S, Hoogenraad A, Croome R, Soddell J, Lindrea K, Seviour R: RAPD-PCR typing of Acinetobacter isolates from activated sludge systems designed to remove phosphorus microbiologically. *J Appl Microbiol* 2001, **90**(3):309-319.
10. Sung JY, Kwon KC, Cho HH, Koo SH: Antimicrobial resistance determinants in imipenem-nonsusceptible Acinetobacter calcoaceticus-baumannii complex isolated in Daejeon, Korea. *Korean J Lab Med* 2011, **31**(4):265-270.
11. Romalde JL, Castro D, Magarinos B, Lopez-Cortes L, Borrego JJ: Comparison of ribotyping, randomly amplified polymorphic DNA, and pulsed-field gel electrophoresis for molecular typing of Vibrio tapetis. *Syst Appl Microbiol* 2002, **25**(4):544-550.
12. Zeka AN, Poirel L, Sipahi OR, Bonnin RA, Arda B, Ozinel M, Ulusoy S, Bor C, Nordmann P: GES-type and OXA-23 carbapenemase-producing Acinetobacter baumannii in Turkey. *J Antimicrob Chemother* 2014, **69**(4):1145-1146.
13. Cicek AC, Saral A, Iraz M, Ceylan A, Duzgun AO, Peleg AY, Sandalli C: OXA- and GES-type beta-lactamases predominate in extensively drug-resistant Acinetobacter baumannii isolates from a Turkish University Hospital. *Clin Microbiol Infect* 2014, **20**(5):410-415.
14. del Mar Tomas M, Cartelle M, Pertega S, Beceiro A, Llinares P, Canle D, Molina F, Villanueva R, Cisneros JM, Bou G: Hospital outbreak caused by a carbapenem-resistant strain of Acinetobacter baumannii: patient prognosis and risk-factors for colonisation and infection. *Clin Microbiol Infect* 2005, **11**(7):540-546.
15. Jawad A, Seifert H, Snelling AM, Heritage J, Hawkey PM: Survival of Acinetobacter baumannii on dry surfaces: comparison of outbreak and sporadic isolates. *J Clin Microbiol* 1998, **36**(7):1938-1941.
16. Joly-Guillou ML: Clinical impact and pathogenicity of Acinetobacter. *Clin Microbiol Infect* 2005, **11**(11):868-873.
17. van Belkum A, Tassios PT, Dijkshoorn L, Haeggman S, Cookson B, Fry NK, Fussing V, Green J, Feil E, Gerner-Smidt P *et al*: Guidelines for the validation and application of typing methods for use in bacterial epidemiology. *Clin Microbiol Infect* 2007, **13** Suppl 3:1-46.
18. Soyer Y, Alcaine SD, Schoonmaker-Bopp DJ, Root TP, Warnick LD, McDonough PL, Dumas NB, Grohn YT, Wiedmann M: Pulsed-field gel electrophoresis diversity of human and bovine clinical Salmonella isolates. *Foodborne Pathog Dis* 2010, **7**(6):707-717.
19. Pasanen T, Kotila SM, Horsma J, Virolainen A, Jalava J, Ibrahem S, Antikainen J, Mero S, Tarkka E, Vaara M *et al*: Comparison of repetitive extragenic palindromic sequence-based PCR with PCR ribotyping and pulsed-field gel electrophoresis in studying the clonality of Clostridium difficile. *Clin Microbiol Infect* 2011, **17**(2):166-175.