Distribution of different carbapenem resistant clones of Acinetobacter baumannii in Tehran Hospitals

Taherikalani Morovat[1], Fatolahzadeh Bahram[2], Emaneini Mohammad[2], Soroush Setareh[1], Feizabadi Mohamad Mehdi[1]

[1]Department of Microbiology, School of Medicine, Ilam University of Medical Sciences, Ilam, Iran;
[2]Department of Microbiology, School of Medicine, Tehran University of Medical Science, Tehran, Iran

SUMMARY

The MICs of imipenem, meropenem, piperacillin-tazobactam, cefotaxime, polymixin B and tigecycline against 80 isolates of *Acintobacter baumanii* from 6 hospitals were determined. A multiplex-PCR was used to detect the genes encoding carbapenemases.

Field Inversion Gel Electrophoresis (FIGE) was then used to investigate the genetic relationships among the carbapenem-resistant isolates. Only 7 isolates were resistant to polymixin B and tigecycline (MIC=16). All isolates were positive for at least 2 carbapenemase genes. At least 10 distinct clones were detected by FIGE. A dominant pattern designated as pulsotype A consisting of 23 isolates was detected from 4 hospitals. The majority of isolates in this pulsotype had a $bla_{\text{OXA-51/23-like}}$ and $bla_{\text{OXA-51/24-like}}$ carbapenemase genes and cultured from the patients at burns and ICU. The pan drug resistant isolates belonged to different FIGE patterns.

Nosocomial infections with different clones of *Acintobacter baumanii* occur at Tehran hospitals. However, inter-hospital transmission with certain pulsotypes is likely.

KEY WORDS: A. baumannii, Carbapenem resistant, FIGE patterns

Received January 20, 2009 *Accepted* April 09, 2009

INTRODUCTION

Strains of *Acinetobacter baumannii* cause pneumonia, endocarditis, meningitis, wound and urinary tract infections. (Bergogne-Berezin *et al.,* 1996; Biendo *et al.,* 1999; Wroblewska *et al.,* 2004). Carbapenems are the treatment of choice in cases of infection with this organism. However, due to production of carbapenem-hydrolyzing- -lactamases such as OXA- carbapenemase, resistance has emerged. Although a short report on the outbreaks of multi and pandrug resistant *A. baumannii* among Iranian burn patients has already been published (Taherikalani *et al.,* 2008), little information was available on the circulation of particular clones at the studied hospitals. The aims of this study were to genetically characterize the carabapenem resistant isolates of *A. baumannii* by FIGE, a prototype of pulsed field gel electrophoresis, and to determine the distribution of genes encoding the Ambler class D carbapenemase, $bla_{\text{OXA-51-like}}$, $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$ and $bla_{\text{OXA-58-like}}$, among different clones.

Corresponding author
Mohammad Mehdi Feizabadi
Department of Microbiology
School of Medicine
Poorsina Street, Tehran, Iran
E-mail: mfeizabadi@sina.tums.ac.ir

MATERIALS AND METHODS

Bacterial isolates

A total of 80 isolates cultured from wound (n=50), trachea (n=10), blood (n=10), CSF (n=4), catheter (n=3) and other samples (n=3) of patients at 6 distinct hospitals (H1-H6) in Tehran during July 2006-December 2007 were investigated. Identification of isolates to species level was based on the phenotypic method of Bouvet and

Grimont (Bouvet *et al.,* 1986). The results were confirmed by amplification of $bla_{\text{OXA-51-like}}$ genes by PCR as all strains of *A. baumannii* intrinsically contain this gene (Turton *et al.,* 2006).

Antibiotic susceptibility testing

The MICs of imipenem, meropenem, piperacillin, piperacillin-tazobactam, cefotaxime, polymixine B were determined by broth microdilution method according to CLSI guidelines (Clinical and Laboratory Standards Institute, 2006). E test was used to determine the MICs of tigecycline after inoculating the isolates in adjusted Muller Hinton agar. The breakpoint for tigecycline was considered as 4.

Escherichia coli ATCC 25922 and ATCC 35218 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality controls in each susceptibility determination.

PCR evaluation of bla_{OXA} genes

A multiplex-PCR targeting $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-58-like}}$ and $bla_{\text{OXA-51-like}}$ genes was used to screen the isolates as described by Woodford *et al.* (Woodford *et al*., 2006). The *Acinetobacter baumannii* reference strains NCTC 13304, NCTC 13302, NCTC 12156 and NCTC 13305 were used as positive controls for the above respectively.

FIGE analysis

To extract the whole chromosomal DNA for FIGE analysis, the pure bacterial cultures were suspended in cell suspension buffer (100 mM Tris: 100 mM EDTA, pH 8.0) and the OD of each sample was adjusted to 1.8 at a wavelength of 600nm using a photometer (Eppendorf, Germany). Cells were then mixed with equal volume of low melting point agarose at 45°C (2% agarose in 1% SDS) and poured into a mould to harden the plugs. The embedded cells in the agarose plugs were incubated with lysis buffer (50 mM Tris: 50 mM EDTA, pH 8.0 + 1% Sarcosyl) in the presence of proteinase K (2mg/ml) for 4 h in a water bath at 55°C. The plugs were washed 6 times by pre-heated distilled water (55°C) and TE buffer (10 mM Tris: 1 mM EDTA, pH 8.0) after the lysis. The plugs containing pure genomic DNA were incubated for 3h with 50U *Apa*I endonuclease. The digested DNAs were loaded into the wells of agarose gel (1%) in 0.5 TBE buffer. Electrophoresis was performed in a FIGE apparatus system (Faculty of Engineering, University of Tehran) for 18 h at 10 C, with an electric field of 5 V/cm^3 in 0.5 TBE buffer. The pulse time increased from 1 to 25 s. A DNA of lambda phage ladder was used as molecular weight marker. After the electrophoresis, the gels were stained with ethidium bromide (1 µg/ml) and visualized by UV illumination.

DNA banding pattern analysis

The DNA patterns were analyzed with Zhen-Negar software (Faculty of Mathematic and Computing Sciences, Sharif University of Technology, Iran) and the strains were clustered by the unweighted pair group method with arithmetic averages strategy (Wofford *et al.* 1996). The DNA banding patterns were interpreted as instructed by Tenover *et al.* (Tenover *et al*., 1995).

RESULTS

High rates of resistance to imipenem (52.5%), meropenem (52.5%), piperacillin (75%), piperacillin-tazobactam (68.7%) and cefotaxime (92.5%) were observed (Table 1). Polymixin B and tigecycline showed good antimicrobial activities (91.2%), though 7 isolates (8.8%) were resistant to all tested antimicrobial agents including polymixin B and tigecycline.

The gene $bla_{\text{OXA-51-like}}$ was identified in all *A. baumannii* strains. However, the positivity for this gene was not necessarily related to increased resistance to carbapenems in itself. When this gene is present, the reduced susceptibility to carbapenems is detectable only when the gene is overexpressed following the insertion of ISAba1 or similar ISs (Turton *et al.,* 2006). $bla_{\text{OXA-23-like}}$ (25%), $bla_{\text{OXA-58-like}}$ (21.2%) and $bla_{\text{OXA-24-like}}$ (15%) genes were detected in this study. Co-existence of two (41.2%), three (6.2%) and four (2.5%) $bla_{\text{OXA-carbapenemase}}$ genes was observed among the isolates. With the exception of 6, all carbapenem resistant isolates had more than one $bla_{\text{OXA-carbapenemase}}$ gene (Table 2).

The 80 isolates were classified into 10 pulsotype with 3 subvariants. Pulsotype A was dominant (n=23), fallowed by pulsotype B (n= 6), pulsotype C (n=6), pulsotype D (n=6) and pulsotype E (n=4). FIGE subvariants including A1 and A2, B1, C1, D1 and D2, E1 and E2, F1 and F2, L1 and M1

TABLE 1 - *In vitro activity of various antimicrobial agents against A. baumannii isolates.*

Antimicrobials	*MIC (μg/ml)*			*%*		
	Range	*MIC50*	*MIC90*	*S*	*I*	*R*
Cefotaxime	1-≥2046	512	1024	7.5	-	92.5
Imipenem	≤0.12-≥256	2	64	42.5	5	52.5
Meropenem	≤0.12-≥256	2	64	42.5	5	52.5
Piperacillin	1-≥2046	512	512	21.2	3.7	75
Piperacillin-tazobactam	1-≥2046	256	512	28.7	2.5	68.7
Polymixin B	≤0.5-64	≤0.5	2	91.2	-	8.8
Tigecycline	≤0.5-64	≤0.5	2	91.2	-	8.8

Abbreviations: S: sensitive; I: intermediate; R: resistance

were also identified among our isolates. A picture showing the banding patterns obtained by FIGE and a dendrogram demonstrating the proportion of dissimilarity between different patterns and their allocated pulsotypes are depicted in figures 1 and 2. Pulsotype A was the predominant pattern particularly among isolates cultured from burn patients. Seven isolates (8.7 %,) with a high level of resistance to carbapenems (MIC=256) contained more than two $bla_{\text{OXA-carbapenemse}}$ genes. These isolates have a different pulsotypes (Table 2). Correlations between pulsotype, $bla_{\text{OXA-carbapenemase}}$ alleles and resistant to carbapenem and other antimicrobials are shown in Table 2.

Isolates within pulsotype A to M contain either $bla_{\text{OXA-51-type}}$ alone or in combination with other genes.

DISCUSSION

A. baumannii is an emerging nosocomial pathogen that is in part due to the capacity of acquiring resistance to multiple antimicrobial agents. Because OXA-producing *A. baumannii* confers resistance to most -lactams, a limited number of antimicrobial agents maintain reliable activity against OXA-producing *A. baumannii*, including polymyxin B and tigecycline (Dalla-Costa *et al.,* 2003). This result is in agreement with those obtained in the European countries (Henwood *et al.,* 2002; Coelhoa *et al.,* 2004) in which tigecycline demonstrated its activity against strains showing a multiple resistance phenotype. Similar findings have also reported from Turkey, a neighboring country to Iran, where tigecycline has been found the most effective antibiotic against MDR strains of Acinetobacter including those producing metal beta lactamase (Eser *et al.,* 2008).

Increasing resistant to antimicrobials, including the carbapenems, has prompted the use of polymyxin B and colistin as a therapeutic agent, and within the last several years it has been used with increasing frequency to treat patients infected with multidrug-resistant gram negative bacteria, including *A. baumannii*. However, problems such as emerging resistance to this antibiotics and its nephrotoxicity should be considered in prescription.

Although all the isolates were resistant to most of the antibiotics tested, some variation was found amongst the group, particularly in their susceptibilities to carbapenems (Table 2). Such variation was seen even amongst isolates with indistinguishable FIGE profiles (Table 2 and Figure 1). The variation in antibiogram results presumably reflects different antibiotic selection pressures in some of the hospitals and/or plasmid acquisition, and is consistent with the theory that isolates may have appeared in some hospitals through selection from a common ancestor,

TABLE 2 - *Correlation between FIGE pulsotype, bla$_{OXA\text{-}carbapenemase}$ alleles and resistant to carbapenem and other antimicrobials among A. baumannii strains.*

Hospital no.	*blaOXA-like genes*	*MIC (μg/ml)*						*FIGE Pulsotype*
		IMP†	*MER*	*PIP*	*PIP/TZ*	*POL*	*TGC*	
H1	blaOXA-51/58	64	16	128	64	2	2	F1
H1	blaOXA-51/23/58	≤256	≤256	128	128	16	16	F
H1	blaOXA-51/24	128	64	64	32	2	2	F
H1	blaOXA-51	8	8	32	32	1	1	A
H1	blaOXA-51	8	8	128	128	2	1	B
H2	blaOXA-51/58	128	64	1024	2040	0.5	0.5	C1
H2	blaOXA-51/23/24/58	≤256	≤256	1024	1024	16	16	B
H2	blaOXA-51	8	8	256	128	1	1	E
H2	blaOXA-51/23	64	32	256	128	1	1	B
H3	blaOXA-51	8	8	256	128	2	1	B
H3	blaOXA-51/23	64	64	64	32	0.5	0.25	K
H3	blaOXA-51/23/58	≤256	≤256	256	256	16	16	K
H3	blaOXA-51/23	64	64	1024	1024	1	1	K
H4	blaOXA-51/58	32	32	1024	1024	2	1	A
H4	blaOXA-51/24	32	32	256	128	0.5	0.5	A
H4	blaOXA-51/58	64	64	128	128	0.5	0.5	A
H4	blaOXA-51/23/58	≤256	≤256	2040	1024	16	16	A
H4	blaOXA-51/58	32	32	256	128	1	2	A
H4	blaOXA-51/23	64	64	128	128	1	1	A
H4	blaOXA-51/24	32	32	128	64	0.5	1	A
H4	blaOXA-51	8	8	256	128	1	2	A
H4	blaOXA-51/ 23	64	32	128	128	1	1	A
H4	blaOXA-51/ 24	64	32	256	256	1	2	A
H4	blaOXA-51/ 58	64	32	1024	1024	0.5	0.25	A
H4	blaOXA-51/ 23	64	32	1024	1024	0.25	1	A
H4	blaOXA-51/ 23	64	32	1024	512	0.5	2	A
H4	blaOXA-51/ 24	64	64	512	512	1	1	A
H4	blaOXA-51/ 23	32	16	512	256	1	1	A
H4	blaOXA-51	8	8	64	64	0.25	0.25	A
H5	blaOXA-51/23	64	64	256	512	1	1	A2
H5	blaOXA-51/58	32	32	512	512	0.5	0.5	G
H5	blaOXA-51/24	32	16	1024	1024	1	1	G
H5	blaOXA-51/23	64	32	1024	512	1	1	G
H5	blaOXA-51/23/58	≤256	≤256	512	256	64	64	E1
H5	blaOXA-51/23	64	64	128	128	1	1	L
H5	blaOXA-51/58	64	32	256	256	1	1	D
H5	blaOXA-51/23/24/58	≤256	≤256	256	128	16	16	D
H5	blaOXA-51/23	64	32	256	128	0.25	0.25	C
H5	blaOXA-51/24	64	64	512	256	1	1	D
H5	blaOXA-51/58	32	16	512	256	0.5	0.5	D1
H5	blaOXA-51/24	128	64	1024	514	1	1	D2
H5	blaOXA-51/23	32	32	512	256	0.5	0.5	M1
H5	blaOXA-51/24	32	16	512	256	0.25	0.25	A
H5	blaOXA-51/23/58	≤256	≤256	1024	512	16	16	A1
H6	blaOXA-51/24	32	16	512	512	0.5	0.5	A
H6	blaOXA-51/58	64	32	1024	512	1	1	A

†IMP: Imipenem; MER: Meropenem; PIP: Piperacillin; PIP/TZ: Piperacillin-tazobactam; POL: Polymyxin B and TGC: Tigecycline.

rather than spread from another hospital (Turton *et al.,* 2004).

Clonal outbreaks of infections with *A. baumannii* containing different OXA-type carbapenemases have been reported from Brazil, French Polynesia, Spain, Southern Europe, the Balkans, Turkey, Korea, and Argentina (Bou *et al.,* 2000; Dalla-Costa *et al.,* 2003; Da Silva *et al*., 1996; Heritier *et al.,* 2005; Jeon *et al.,* 2005; Lopez-Otsoa *et al.,* 2002; Marque *et al.,* 2005; Naas *et al.,* 2005). A single clone or only a few disseminating clones were identified in most situations in the world, and sometimes the coexistence of epidemic cases with unrelated sporadic cases caused by different strains have been demonstrated (Mulin *et al*., 1995; Lyytikäinen *et al.,* 1995; Sader *et al.,* 1996; Marcos *et al.,* 1994; Ratto *et al.,* 1995; Ling *et al.,* 1996; Garcìa-Arata *et al.,* 1997).

This investigation disclosed more than 10 pulsotype in the studied hospitals. The reasons for existence of relatively large number of *A baumannii* clones in the burns units and ICUs may be due to the continuous introduction of new mul-

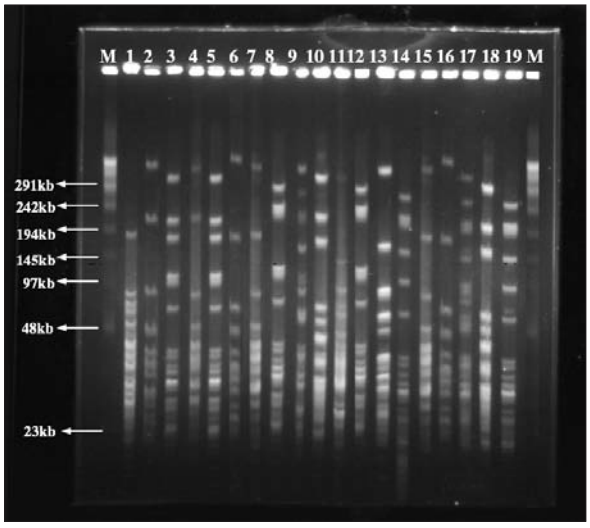

FIGURE 1 - *FIGE profiles of A. baumannii strains isolated from different patients in ICU. Different FIGE types and subtypes identified are indicated. Lateral lanes contain multimers of phage lambda DNA (48 kb) molecular mass markers. Sizes of lambda DNA molecular mass markers are indicated on the left of the panel. (1) Standard strains NCTC 12156, (2, 4 and 14) Pulse types F and F1, (3 and 5) Pulse type H, (6 and 7, 15 and 16) Pulse types D1 and D2; (8 and 12) Pulse type A, (9) Pulse type L, (10 and 18) Pulse type B, (11) Standard strain NCTC 13302, (14 and 19) Pulse type M, (17) Pulse type C.*

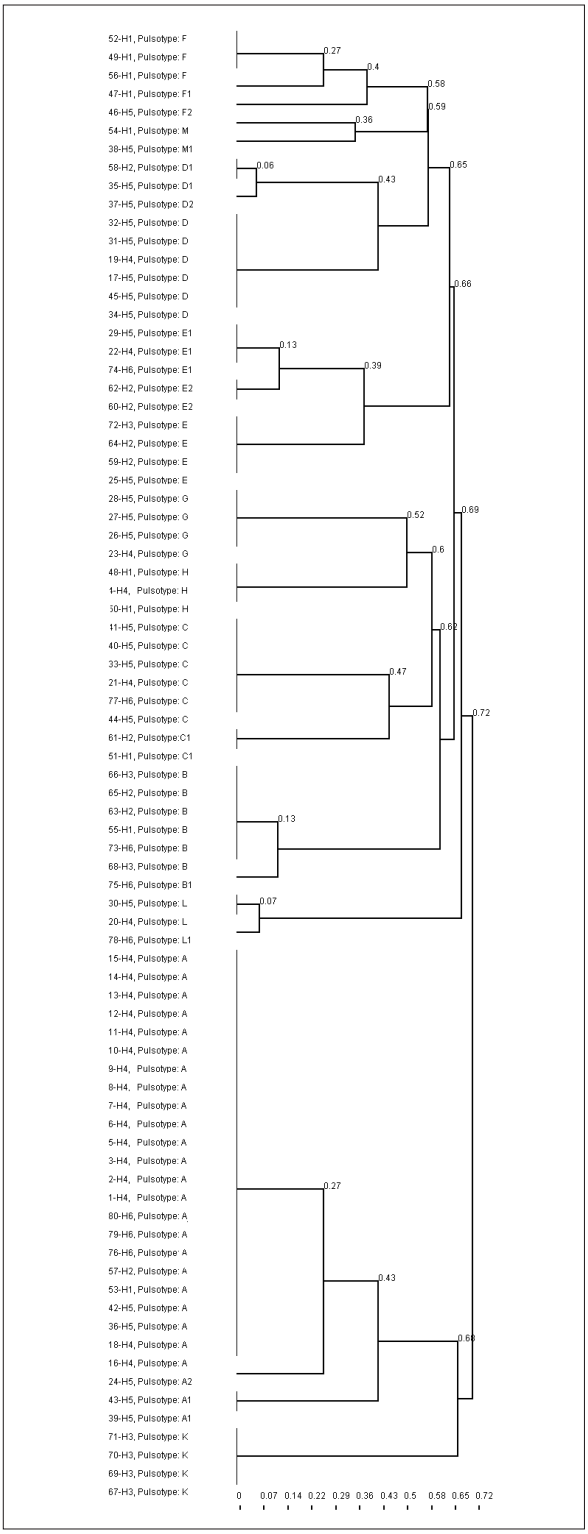

FIGURE 2 - Dendrogram of FIGE macro restriction patterns generated with the RFLPrint computer software. The scale indicates percentage similarity.

tiresistant strains into a setting or occurrence of extensive exchange of resistant genes among multiresistant and partially susceptible *A. baumannii* strains (Flaherty *et al.,* 1996). Despite this heterogeneity, we found certain pulsotypes (for example, pulsotype A) at different hospitals, suggesting that interhospital transmission of certain clones had occurred in Tehran.

FIGE clones A-M variously contain $bla_{\text{OXA-51-type}}$ genes alone or associated with acquired $bla_{\text{OXA-23}}$, $bla_{\text{OXA-24}}$ or $bla_{\text{OXA-58}}$ enzymes. These findings illustrate not only the plasticity of the *A. baumannii* genome but also the ability of individual strains of genomic species 2 to acquire distinct resistance determinants (Woodford *et al.,* 2006). In conclusion, *A. baumannii* strains with different $bla_{\text{OXA-carbapenemase}}$ genes isolated from patients at Tehran belong to different clones. The antimicrobial selective pressure is probably caused by the emergence and spread of OXA-producing epidemic clones of *A. baumannii* among Iranian patients. The incidence and spread of clones of *A. baumannii* suggest the need for a surveillance program to prevent colonization and dissemination. This program would require monitoring infections acquired in burns and intensive care units, and antimicrobial use, as well as molecular typing of bacterial isolates and characterization of antibiotic resistance.

ACKNOWLEDGEMENT

This project was supported by Tehran University of Medical Sciences. The authors thank P. Azimi, H. Shahbazi and M. Ghaffari for their excellent help in data analysis of FIGE patterns.

REFERENCES

Bargogne-Berezin E., Towner K.J. (1996). *Acinetobacter* spp as nosocomial pathogens: Microbiological, clinical and epidemiological features. *Clin. Microbiol. Rev.* **9**, 148-165.

Biendo M., Laurans G., Lefebvre J.F., Daoudi F., Eb F. (1999). Epidemiological study of an *Acinetobacter baumannii* outbreak by using a combination of antibiotyping and ribotyping. *J. Clin. Microbiol.* **37**, 2170-2175.

Bouvet P.J.M., Grimont P.A.D. (1986). Taxonomy of the genus Acinetobacter with the recognition of Acinetobacter baumannii sp. nov., Acinetobacter haemolyticus sp. nov., Acinetobacter johnsonii sp. nov., and Acinetobacter junii sp. nov. and emended descriptions of Acinetobacter calcoaceticus and Acinetobacter lwoffii. *Int. J. Syst. Bacteriol.* **36**, 228-240.

Bou G., Cervero G., Dominguez M.A., Quereda C., Martinez-Beltran J. (2000). Characterization of a nosocomial outbreak caused by a multiresistant *Acinetobacter baumannii* strain with a carbapenem-hydrolyzing enzyme: high-level carbapenem resistance in *A. baumannii* is not due solely to the presence of beta-lactamases. *J. Clin. Microbiol.* **38**, 3299-3305.

Clinical and Laboratory Standards Institute. (2006). Performance standards for antimicrobial susceptibility testing. Approved standard. CLSI document M100-S16. Clinical and Laboratory Standards Institute, Wayne, Pa.

Coelhoa J., Woodforda N., Turton J.F, Livermorea D.M. (2004). Multiresistant acinetobacter in the UK: how big a threat? *J. Hosp. Infect.* **58**, 167-169.

Dalla-Costa L.M., Coelho J.M., Souza H.A., Castro M.E., Stier C.J., Bragagnolo K.L., Rea-Neto A., Penteado-Filho S.R., Livermore D.M., Woodford N. (2003). Outbreak of carbapenem-resistant Acinetobacter baumannii producing the OXA-23 enzyme in Curitiba, Brazil. *J. Clin. Microbiol.* **41**, 3403-3406.

Da Silva G.J., Quinteira S., Bertolo E., Sousa J.C., Gallego L., Duarte A., Peixe L., Portugese Resistance Study Group. (2004). Long-term dissemination of an OXA-40 carbapenemase-producing *Acinetobacter baumannii* clone in the Iberian Peninsula. *J. Antimicrob. Chemother.* **54**, 255-258.

Eser O.K., Ergin A., Tunckanat F., Hasscelik G. (2008). In vitro activity of tigecycline as a therapeutic option against multidrug-resistant Acinetobacter spp. *New Microbiol.* **31**, 535-542.

Flaherty J.P., Weinstein R.A. (1996). Nosocomial infections caused by antibiotic- resistant organisms in the intensive-care unit. *Infect. Control. Hosp. Epidemiol.* **17**, 236-248.

Garcia-Arata M.I., Gerner-Smidt P., Lopez-Brea M. (1997). Epidemiological study of Acinetobacter species isolated from an intensive care unit. *A.P.M.I.S.* **105**, 131-138.

Henwood C.J., Gatward T., Warner M., James D., Sockdale M.W., Spence R.P., Towner K.J., Livermore D.M., Woodford N. (2002). Antibiotic resistance among clinical isolates of Acinetobacter in the UK, and in vitro evaluation of tigecycline (GAR-936). *J. Antimicrob. Chemother.* **49**, 479-487.

Heritier C., Dubouix A., Poirel L., Marty N., Nordmann P. (2005). A nosocomial outbreak of *Acinetobacter baumannii* isolates expressing the carbapenem- hydrolysing oxacillinase OXA-58. *J. Antimicrob. Chemother.* **55**,115-118.

Jeon B.C., Jeong S.H., Bae I.K., Kwon S.B., Lee K., Young D., Lee J.H., Song J.S, Lee S.H. (2005).

Investigation of a nosocomial outbreak of imipenem-resistant *Acinetobacter baumannii* producing the OXA-23 betalactamase in Korea. *J. Clin. Microbiol.* **43**, 2241-2245.

LOPEZ-OTSOA F., GALLEGO L., TOWNER K.J., TYSALL L., WOODFORD N., LIVERMORE D.M. (2002) Endemic carbapenem resistance associated with OXA-40 carbapenemase among *Acinetobacter baumannii* isolates from a hospital in northern Spain. *J. Clin. Microbiol.* **40**, 4741- 4743.

LYYTIKAINEN O., KOLJLG S., HARMA M., VUOPIO-VARKILA J. (1995). Outbreak caused by two multi-resistant Acinetobacter baumannii clones in a burns unit: emergence of resistance to imipenem. *J. Hosp. Infect.* **31**, 41-54.

LING J.M., WISE R., WOO T.H., CHENG A.F. (1996). Investigation of the epidemiology of hospital isolates of Acinetobacter anitratus by two molecular methods. *J. Hosp. Infect.* **32**, 29-38.

MARQUE S., POIREL L., HERITIER C., BRISSE S., BLASCO M.D., FILIP R., COMAN G., NAAS T., NORDMANN P. (2005). Regional occurrence of plasmidmediated carbapenem-hydrolyzing oxacillinase OXA-58 in *Acinetobacter* spp. in Europe. *J. Clin. Microbiol.* **43**, 4885-4888.

MULIN B., TALON D., VIEL J.F., VINC C., LEPRAT R., THOUVEREZ M., MICHEL-BRIAND Y. (1995) Risk factors for nosocomial colonization with multiresistant Acinetobacter baumannii. *Eur. J. Clin. Microbiol. Infect. Dis.* **14**, 569-576.

MARCOS M.A., ABDALLA S., PEDRAZA F., ANDREU A., FERNANDEZ F., GOMEZ-LUS R., JIMENEZ DE ANTA M.T., VILA J. (1994) Epidemiological markers of Acinetobacter baumannii clinical isolates from Spinal Cord Injury Unit. *J. Hosp. Infect.* **28**, 39-48.

NAAS T., LEVY M., HIRSCHAUER C., MARCHANDIN H., NORDMANN P. (2005). Outbreak of carbapenem-resistant *Acinetobacter baumannii* producing the carbapenemase OXA-23 in a tertiary care hospital of Papeete, French Polynesia. *J. Clin. Microbiol.* **43**, 4826-4829.

RATTO P., SORDELLI D.O., ABELEIRA E., TORRERO M., CATALANO M. (1995) Molecular typing of Acinetobacter baumannii-Acinetobacter calcoaceticus complex isolates from endemic and epidemic nosocomial infections. *Epidemiol. Infect.* **114**, 123-132.

SADER H.S., MEMDES C.F., PIGNATARIA A.C., PFALLER M.A. (1996). Use of macrorescriction analysis to demonstrate interhospital spread of multiresistant Acinetobacter baumannii in Sao Paulo, Brazil. *Clin. Infect. Dis.* **23**, 631-634.

TAHERIKALANI M., ETEMADI G., GELIANI K.N., FATOLLAHZADEH B., SOROUSH S., FEIZABADI M.M. (2008). Emergence of multi and pan-drug resistance *Acinetobacter* b*aumannii* carrying $bla_{\text{OXA-type}}$-carbapenemase genes among burn patients in Tehran, Iran. *Saudi. Med. J.* **29**, 623-624.

TURTON J.F., KAUFMANN M.E, WARNER M., COELHO J., DIJKSHOORN L., VAN DER REIJDEN T., PITT T.L. (2004). A prevalent, multiresistant clone of Acinetobacter baumannii in Southeast England. *J. Hosp. Infect.* **58**, 170-179.

TURTON J.F., WOODFORD N., GLOVER J., YARDE S., KAUFMANN M.E., PITT T.L. (2006). Identification of *Acinetobacter baumannii* by detection of the *bla*OXA-51-like carbapenemase gene intrinsic to this species. *J. Clin. Microbiol.* **44**, 2974-2976.

TURTON J.F., WARD M.E., WOOD N., KAUFMANN M.E.E., PIKE R., LIVERMORE D.M., PITT T.L. (2006). The role of ISAba1 in expression of OXA carbapenemase genes in Acinetobacter baumannii. *FEMS Microbiol. Lett.* **258**, 72-77.

TENOVER F.C., ARBEIT R.D., GOERING R.V., MICKELSEN P.A., MURRAY B.E., PERSING D.H., SWAMINATHAN B. (1995). Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial isolate typing. *J. Clin. Microbiol.* **33**, 2233-2239.

WROBLEWSKS M.M., DIJKSHOORN L., MARCHEL H., VAN DEN BARSEAAR M., SWOBODA-KOPEC E., VAN DEN BROEK P.J., LUCZAK M. (2004). Outbreak of nosocomial meningitis caused by *Acinetobacter baumannii* in neurosurgical patients. *J. Hosp. Infect.* **57**, 300-307.

WOODFORD N., ELLINGTON M.J., COELHO J.M., TURTON J.F., WARD M.E., BROWN S., AMYES S.G., LIVERMORE D.M. (2006) Multiplex PCR for genes encoding prevalent OXA carbapenemases in *Acinetobacter* spp. *Int. J. Antimicrob. Agents.* **27**, 351-353.

WOFFORD D.L.S, OLSEN G.J., WADDELL P.J., HILLIS D.M. (1996). Molecular Systematics. Massachusetts, Sinauer Associates, Inc, Sunderland, USA.