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Full Length Research Paper

Phenotypic and genotypic characterization of clinical multidrug resistant *Acinetobacter baumannii* from Algerian intensive care units

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The aim of this study was to evaluate molecular epidemiology and mechanisms of β-lactams resistance in multidrug resistant *Acinetobacter baumannii* strains causing pneumoniae in two intensive care units (ICUs) of two Algerian University hospitals. Between January 2010 and May 2011, 23 strains were collected. Antibiotics susceptibility testing was performed by the disk diffusion method and agar dilution technique. *bla*$_{OXA-23}$, *bla*$_{OXA-24}$, *bla*$_{OXA-58}$, *bla*$_{OXA-51}$, *bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{CTX-M}$, and *bla*$_{PER}$ β-lactamase genes were searched by polymerase chain reaction and sequencing. Clonally, relationships between strains were performed by pulsed field gel electrophoresis (PFGE) after macro-restriction by ApaI enzyme. Nineteen (19) strains were resistant to all β-lactams, two were susceptible only to carbapenems. They also exhibited resistance to the majority of the other antibiotics tested, except to colistin. *bla*$_{OXA-51}$ gene was found in all isolates, *bla*$_{OXA-23}$ in 14, and *bla*$_{TEM-1}$ in three. PFGE analysis showed different patterns in one ICU; however, a clonal diffusion was identified in the second ICU. These results underline the necessity of a surveillance program that would include monitoring of ICU-acquired infections, antibiotic usage and molecular typing of multidrug resistant *A. baumannii* isolates.

Key words: *Acinetobacter baumannii*, multidrug resistance, β-lactamases, epidemiology.

INTRODUCTION

Acinetobacter baumannii is one of the most opportunistic pathogens responsible for serious infections in intensive care units (ICUs) (Schuetz et al., 2012; Dijkshoorn et al., 2007). Pneumonia is the most reported infection, particularly in mechanically ventilated patients (Corbella et al., 2000). *A. baumannii* is responsible for 7.8 to 23% of mortality by acquired pneumonia in the hospitals and 10 to 43% in ICUs (Kempf et al., 2012; Falagas et al., 2006). *A. baumannii* exhibits a remarkable ability to rapidly develop antibiotic resistance that led to multidrug resistance (MDR) within a few decades (Kusradze et al., 2011). To date, some strains of *A. baumannii* have become almost resistant to all currently available antibacterial agents (Wang et al., 2007; Dijkshoorn et al., 1996; Magiorakos et al., 2012) mostly through the acquisition of mobile genetic elements carrying clusters of genes encoding resistance to several antibiotic families at once, making it more complex both in regards of the prevention and treatment (Ben et al., 2011).

The main mechanism of resistance to β-lactams in *A. baumannii* is enzymatic degradation by β-lactamases (Zarrilli et al., 2009). AmpC cephalosporinase is chromosomally encoded by *A. baumannii*, other extended-spectrum β-lactamases (ESBLs) like TEM-92, TEM-116, SHV-12, CTX-M-2, CTX-M-43,VEB-1, PER-1, PER-2 and a narrow-spectrum β-lactamases like TEM-1 and TEM-2 have been described (Peleg et al., 2008).

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Table 1. Primer pairs used for the amplification of β-lactamases gene.

bla gene	Primer	Sequence 5' to 3'	Gene size (bp)	Reference
$bla_{oxa-51-like}$	OXA-51like-F	TAA TGC TTT GAT CGG CCT TG	353	Feizabadi et al. (2008)
	OXA-51like-R	TGG ATT GCA CTT CAT CTT GG		
$bla_{oxa-23-like}$	OXA-23like-F	GAT CGG ATT GGA GAA CCA GA	501	Feizabadi et al. (2008)
	OXA-23like-R	ATT TCT GAC CGC AT TTC CAT		
$bla_{oxa-24-like}$	OXA-24like-F	GGT TAG TTG GCC CCC TTA AA	246	Feizabadi et al. (2008)
	OXA-24like-R	AGT TGA GCG AAA AGG GGA TT		
$bla_{oxa-58-like}$	OXA-58like-F	AAG TAT TGG GGC TTG TGC TG	599	Feizabadi et al. (2008)
	OXA-58like-R	CCC CTC TGC GCT CTA CAT AC		
bla_{TEM}	OT3	ATG AGT ATT CAA CAT TTC CG	850	Ktari et al. (2006)
	OT4	CCA ATG CTT AAT CAG TGA GG		
bla_{SHV}	OS5	TTA TCT CCC TGT TAG CCA CC	800	Ruppé et al. (2009)
	OS6	GAT TTG CTG ATT TCG CTC GG		
bla_{CTX-M}	MA-1	SCS ATG TGC AGY ACC AGT AA	550	Poirel et al. (2000, 2008a, b , 2006)
	MA-2	CCG CRA TAT GRT TGG TGG TG		
bla_{PER}	PER UP	ATG AAT GTC ATT ATA AAA GC	927	Nasehi et al. (2010)
	PER LOW	AAT TTG GGC TTA GGG CAG AA		

Oxacillinases found in *A. baumannii* can be sub-devised into five distinct groups: intrinsic OXA-51-like and acquired, OXA-23-like, OXA-24-like, OXA-58-like and OXA-143; (Ansaldi et al., 2011; Higgings et al., 2009). Metallo-β-lactamases (MBLs) have been identified in *A. baumannii* such as IMP, VIM, SIM (Bou et al., 2000) and recently NDM-1 ,NDM-2 (Pfeifer et al., 2011; Boulanger et al., 2012) and KPC (Robledo et al., 2011; Azimi et al., 2012).

This work constitutes the first molecular research in Algeria on the resistance of *A. baumannii* to β-lactams. The aims of this study was to evaluate molecular epidemiology and mechanisms of β-lactams resistance in MDR *A. baumannii* strains isolated in the microbiological laboratory of Doctor Dorban University hospital of Annaba, Algeria.

MATERIALS AND METHODS

Bacterial strains

Twenty three (23) non redundant multidrug resistant *A. baumannii* isolated from protected distal bronchial sampling of 23 patients hospitalized in two intensive care units (5 in IBN ROCHED Hospital and 18 in IBN SINA Hospital) were collected between January 2010 and May 2011. The isolates were obtained from patients belonging to different age groups: 18 adults (20 to 92 years) and five children (2 to 14 months); seven females and 16 males. Strain identification was performed by standard techniques and the analytical profile index procedure (API 20NE system; bioMérieux, Marcy l'Etoile, France) and confirmed by polymerase chain reaction (PCR) amplification of the endogen $bla_{OXA-51-like}$ (Donald et al., 2000).

Antibiotic susceptibility testing

The disk diffusion method on Mueller–Hinton agar was employed to evaluate susceptibility to the following antimicrobial agents: ticarcillin, ticarcillin-clavulanic acid, piperacillin, piperacillin-tazobactam, ceftazidime, cefepime, imipenem, meropenem, aztreonam, cefsulodin, gentamicin, tobramycin, netilmicin, amikacin, kanamycin, nalidixic acid, ciprofloxacin, tigecycline and colistin. MIC values of ticarcillin, ticarcillin-clavulanic acid, ceftazidime, aztreonam, imipenem, meropenem and cefepime were determined by agar dilution technique. MBLs production was detected using the imipenem-EDTA disk synergy test (Pitout et al ., 2007) .

Current quality control testing was performed using the following organisms: *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25923. The interpretation of the results was referred to the guidelines defined by the Clinical Laboratory Standards Institute (CLSI , 2011).

PCR amplification and sequencing

Genomic DNA of the isolates was extracted to amplify seven genes encoding acquired β-lactamases ($bla_{oxa-23-like}$, $bla_{oxa-24-like}$, $bla_{oxa-58-like}$, bla_{TEM}, bla_{SHV}, bla_{CTX-M} and bla_{PER}). Simplex PCR assays were run using primers listed in Table 1, as previously described (Peleg et al., 2008).

PCR products from representative strains were purified using a purification Kit (Qiagen). DNA sequencing was performed by the dideoxy chain terminator method with Big Dye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) and analyzed using an ABI Prism 3100 genetic analyzer (Applied Biosystems). Similarity searches and alignments of both the nucleotide sequences were performed with the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST) (Caravalho et al., 2009; Hammami et al., 2011).

Pulsed-field gel electrophoresis (PFGE)

Isolates were typed by PFGE following digestion of intact genomic DNA with ApaI (BioRAD, Marnes la Coquette, France). DNA fragments were separated on 1% (w/v) agarose gels in 0.5% TBE buffer using a CHEF DRIII apparatus (Bio-Rad, Hercules, CA) with 6 V/cm, pulsed from 5 to 20 s, for 22 h at 14°C. Gels were stained with ethidium bromide and photographed under ultraviolet light. The ApaI restriction profiles were compared by visual inspection according to the criteria by Van Belkum et al. (2007).

Table 2. Antimicrobial susceptibility and beta-lactams MICs values of the 23 *A. baumannii* isolates.

Strain number	Antibiotic resistance profile	MIC value (µg/l)						
		TIC	TCC	CAZ	FEP	IMP	MER	AZT
1	TC- TCC-PIP- TZP-CAZ-ATM-FEP-GM	2048	512	512	64	2	1	256
2	TC-TCC-PIP-TZP-CAZ--IMP-MER-ATM- FEP -K-NA-CIP	>2048	>1024	512	64	128	32	512
3	TC-TCC-PIP-TZP-CAZ--IMP-MER-ATM- FEP -AN-K-NA-CIP	>2048	>1024	>2048	256	256	64	>512
4	TC-TCC-PIP-TZP-CAZ--IMP-MER-ATM- FEP GM- K	2048	>1024	256	256	128	32	512
5	TC-TCC-PIP-TZP-CAZ--IMP-MER-ATM- FEP -NA-CIP	>2048	>1024	512	>512	128	32	512
6	TC-TCC-PIP-TZP-CAZ-ATM-FEP-GM-TM-K-NA-CIP	1024	128	512	128	2	4	512
7	TC-TCC-PIP-TZP-CAZ--IMP-MER-ATM- FEP -GM	512	512	512	>512	64	16	512
8	TC-TCC-PIP-TZP-CAZ--IMP-MER-ATM- FEP -GM- AN-K -NA-CIP	>2048	>1024	>2048	256	>2048	64	16
9	TC-TCC-PIP-TZP-CAZ--IMP-MER-ATM- FEP -GM-NA-CIP	1024	1024	256	256	128	32	>512
10	TC-TCC-PIP-TZP-CAZ--IMP-MER-ATM- FEP -GM-K-NA-CIP	>2048	>1024	>2048	>512	64	16	>512
11	TC-TCC-PIP-TZP-CAZ--IMP-MER-ATM- FEP –GM-AN-K-NA-CIP	>2048	>1024	>2048	128	128	32	512
12	TC-TCC-PIP-TZP-CAZ--IMP-MER-ATM- FEP -GM-TM-K- NA-CIP	2048	>1024	>2048	>512	128	32	512
13	TC-TCC-PIP-TZP-CAZ--IMP-MER-ATM- FEP -GM-TM-K-NA-CIP	1024	1024	512	128	8	4	512
14	TC-TCC-PIP-TZP-CAZ--IMP-MER-ATM- FEP -GM-AN-K-NA-CIP	>2048	>1024	>2048	256	256	32	512
15	TC-TCC-PIP-TZP-CAZ--IMP-MER-ATM- FEP -GM-AN-K -NA-CIP	>2048	>1024	>2048	>512	256	64	512
16	TC-TCC-PIP-TZP-CAZ--IMP-MER-ATM- FEP -GM-K-NA-CIP	2048	1024	>2048	>512	256	32	512
17	TC-TCC-PIP-TZP-CAZ--IMP-MER-ATM- FEP -AN-K--NA-CIP	>2048	>1024	>2048	>512	256	64	512
18	TC-TCC-PIP-TZP-CAZ--IMP-MER-ATM- FEP -NA-CIP	>2048	>1024	256	128	256	32	512
19	TC-TCC-PIP-TZP-CAZ--IMP-MER-ATM- FEP -NA-CIP	2048	1024	256	256	128	32	512
20	TC-TCC-PIP-TZP-CAZ--IMP-MER-ATM- FEP -AN-K-NA-CIP	>2048	>1024	>2048	64	128	64	512
21	TC-TCC-PIP-TZP-CAZ--IMP-MER-ATM- FEP -TM-AN-K-NA-CIP	2048	>1024	256	64	128	32	512
22	TC-TCC-PIP-TZP-CAZ--IMP-MER-ATM- FEP -GM-AN-K-NA-CIP	>2048	>1024	>2048	256	256	64	>512
23	TC-TCC-PIP-TZP-CAZ--IMP-MER-ATM- FEP -GM-TM-AN-K-NA-CIP	512	512	>2048	512	128	32	16

TIC, ticarcillin; TCC, ticarcillin-clavulanic acid; PIP, piperacillin; TZP, piperacillin-tazobactam; CAZ, ceftazidime; FEP, cefepime; IMP, imipenem; MER, meropenem; ATM, aztreonam; GM, gentamicin; TM, tobramycin; AN, amikacin; K, kanamycin; NA, nalidixic acid; CIP, ciprofloxacin.

RESULTS

Resistance phenotype for *A. baumanni*'s strains

Antibiogram data determined by the disk diffusion method and interpreted according to the guidelines defined by the Clinical Laboratory Standbards Institute (CLSI) revealed that all strains of *A. baumannii* were resistant to ticarcillin, ticarcillin-clavulanic acid, piperacillin, piperacillin-tazobactam, ceftazidime, cefepime and aztreonam. Only two strains were susceptible to carbapenems, no strain was resistant to colistin, netilmicin and tigecyclin (Table 2).The resistance to β-lactams of 23 *A. baumannii* strains was confirmed by MICs values (Table 2).

PCR amplification of β-lactamase genes and sequencing

All strains were positive for bla_{OXA-51} gene, confirming the strain identification. PCR and sequencing confirmed the presence of bla_{OXA-23} and bla_{TEM-1} in 14 (Figure 1) and three strains (Figure 2), respectively. However, $bla_{OXA-24-like}$, $bla_{OXA-58-like}$, bla_{PER}, bla_{SHV} and bla_{CTX-M} were not detected in any of our isolates. Two strains contained both bla_{OXA-23} and bla_{TEM-1} (Table 3).

Pulsed-field gel electrophoresis (PFGE)

Among the 23 strains, only 19 were typed by PFGE. Seven different profiles were detected. Strains isolated at Ibn Rached Hospital showed three different profiles patterns (C, E and G). However, in Ibn Sina Hospital, four genotypes were identified (A, B, F and D); genotype A: A_1 (n= 5) and A_2 (n=5) was found as the most common cluster type (10/16) with 60% harboring the gene bla_{OXA-23}, followed by B cluster: B_1 (n=2) and B2 (n=2) (Table 3).

DISCUSSION

Acinetobacter are widely distributed in nature; they are

Table 3. MDR *A. baumannii* strains: Demographic data and molecular characterization.

Hospital	Strain number	Age	Sex	Date of isolation	*bla* genes			PFGE
					OXA-51	TEM-1	OXA-23	pattern
Ibn Roched Hospital	1	5M	M	21/01/2010	+	+		ND
	2	14M	M	13/04/2010	+			ND
	3	4 M	F	29/06/2010	+		+	E
	4	2 M	F	18/11/2010	+			G
	5	6M	F	05/01/2011	+		+	C
Ibn Sina Hospital	6	38Y	F	17/01/2010	+			A_1
	7	89Y	M	19/01/2010	+		+	A_1
	8	24Y	M	03/02/2010	+		+	D
	9	48Y	M	04/02/2010	+		+	ND
	10	20Y	F	15/02/2010	+			B_2
	11	58Y	M	17/02/2010	+		+	A_1
	12	56Y	M	08/03/2010	+	+	+	B_1
	13	52Y	M	09/03/2010	+			B_1
	14	74Y	M	22/04/2010	+		+	ND
	15	35Y	M	25/05/2010	+		+	A_2
	16	71Y	M	13/06/2010	+	+	+	A_2
	17	34Y	M	17/06/2010	+		+	A_1
	18	29Y	F	06/09/2010	+		+	A_2
	19	63Y	M	24/10/2010	+			A_2
	20	92Y	M	07/11/2010	+			A_1
	21	21Y	M	07/11/2010	+		+	F
	22	73Y	M	19/11/2010	+		+	A_2
	23	32Y	F	01/05/2011	+			B_2

M, Man; F, Female; ND , not determined; M, months; Y, years.

endogenous organisms in various types of soil and water (Peleg et al., 2008). *A. baumannii* have been associated with healthcare-associated infections, especially in debilitated patients. The main sites of infection are the respiratory tract, urinary tract, bloodstream, wounds, and burns. Patients with burns and those in intensive care units and/or using mechanical ventilation are at greater risk (Kempf et al., 2012; Kusradze et al., 2011). An increase in the prevalence of resistant strains has been seen worldwide and treatment of multidrug-resistant strains can be difficult (Drissi et al., 2010; Magiorakos et al., 2012). The carbapenems have been the drug of choice against this pathogen, but the number of isolates resistant to these antimicrobial agents has considerably increased (Ben et al., 2011; Poirel et al., 2008a).

The present study aimed to define the genetic basis of 23 MDR *A. baumannii* strains isolated in 2 ICUs of two Algerian hospitals. They caused pneumonia in debilitated patients using mechanical ventilation.

All strains were resistant to the most antimicrobials tested mainly to β-lactamin. In this study, we report a high prevalence of imipenem-resistant (91.30%). There is only few data available on carbapenem-resistance in *A. baumannii* from North Africa, especially in Algeria, that the percentage of imipenem-resistant *A. baumannii* strains differed among countries and ranged from 5.2% in Algeria to 28.8% in Tunisia (Amazian et al., 2006). No strain was resistant to colistin. Despite its renal toxicity, colistin has become useful antibiotic for treating infections caused by carbapenem resistant pathogens (Lolans et al., 2006; Nordmann et al.,2002), but dissemination of *A. baumannii* resistant to colistin is worrying. In another side, many studies provide the activity of tigecycline against multidrug *A. baumannii* clinical isolates (Kempf et al., 2012; Betriu et al., 2002). All our strains were susceptible to this novel antibiotic.

Majority of the isolates exhibited resistance to other antibiotics such as, aminoglycosides and quinolones; carbapenem resistance in *A. baumannii* is associated with a variety of combined mechanisms. The major mechanism is the production of carbapenem hydrolyzing β-lactamases. These specific groups of β-lactamases are categorized into class B metallo β-lactamases (MBLs) and most frequently class D (oxacillinases) (Kempf et al., 2012) Other mechanisms include efflux pump, loss or low level expression of 29 kDa (CarO), 33-39 kDa, and 43 kDa (homologous to *P. aeruginosa* imipenem resistance protein, OprD) proteins, and altered affinity in penicillin binding proteins (PBPs) (Ben et al., 2011; Peleg et al ., 2008).

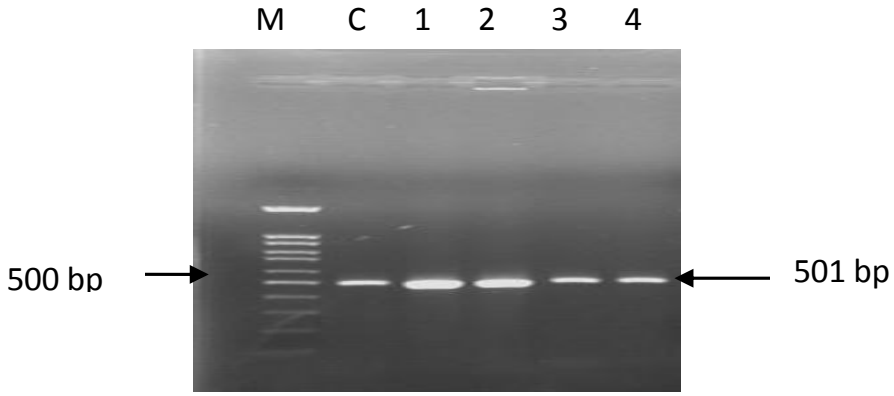

Figure 1. PCR detection of *bla*$_{OXA-23-\ like}$ gene. M, 100 pb DNA ladder (Promega); C, positive control; 1-4, representative strains tested.

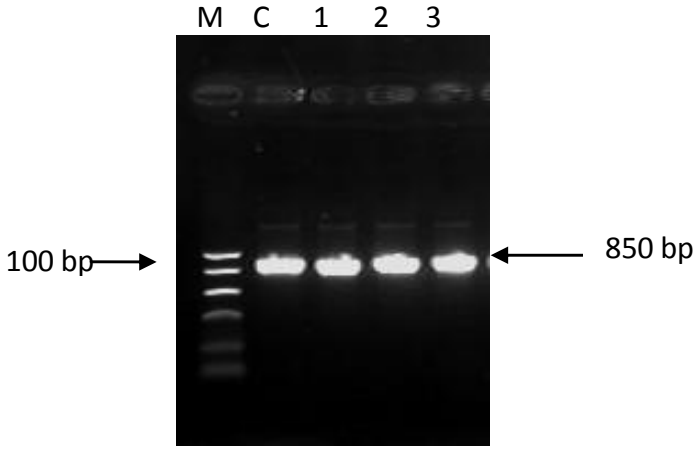

Figure 2. PCR detection of *bla*$_{TEM.}$ M: ϕx174 (Promega); C: positive control habouring *bla*$_{TEM}$;1-3, representative strains tested.

The *bla*$_{OXA-51}$ gene, considered as a natural component of the species chromosome has been used to identify *A. baumannii* (Carvalho et al., 2009). This gene may be associated with resistance to carbapenems when ISAbaI-type insertion sequences, which carry strong promoters, are found upstream to *bla*$_{OXA}$ gene, resulting in increased expression and concomitant resistance to carbapenems (He´ritier et al., 2006). In seven strains, where only *bla*$_{OXA-51}$ was detected, resistance can be explained by non enzymatic mechanisms (Peleg et al., 2008) or insertion of ISAbaI sequences (Kusradze et al 2011; Heritier et al., 2006).

Some studies showed that the presence of ISAbaI upstream to *bla*$_{AmpC}$ was correlated with cefepime resistance (Lin et al., 2011). All ours trains were also resistant to cefepime, but this insertion sequence was not searched in ours strains.

The first identified OXA-type enzyme with carbapenem-hydrolyzing activity was from a clinical *A. baumannii* strain isolated in 1985 from Edinburgh, Scotland. The plasmid-encoding this resistance determinant, initially named ARI-1, was found to be transferable, and the gene was later sequenced and named *bla*$_{OXA-23}$ (Donal et al., 2000; Nordman et al., 2000). Of note, the origin of *bla*$_{OXA-23}$ was recently identified as the chromosome of *Acinetobacter radioresistens*, a commensal species of the human skin (poirel et al., 2008a, b). This enzyme type now contributes to carbapenem resistance in *A. baumannii* globally, causing nosocomial outbreaks or sporadic infections (Ben et al., 2011; Kempf et al., 2012). To the best of our knowledge, the first *A. baumannii* clinical strain harboring *bla*$_{OXA-23}$ identified in Algeria was in 2004 (Mugnier et al., 2010).

Among the five oxacillinase groups found in *A. baumannii*, apart from the intrinsic OXA-51 enzyme, only OXA-23 was detected in ours strains (14 strains), the OXA-23 group was identified worldwide (Mugnier et al 2010). However, *bla*$_{OXA-24-like}$ and *bla*$_{OXA\ -58-like}$ were not

detected in any of ours strains. OXA-24-like enzymes were found in Spain, Belgium, Portugal, the Czech Republic, Georgia, France and the USA; and OXA-58-like enzymes were identified in France, Spain, Belgium, Italy, Australia, USA (Kempf et al.,2012) , including Algeria (Drissi et al., 2010 , Touati et al., 2012), Tunisia (Poirel et al., 2006, 2008a,b ,2000) and recently in Turkey (Metan et al., 2012). Usually, OXA-type enzymes exhibit a weak hydrolysis of carbapenems and may not always show resistance profile, but when they are associated with insertion elements, they may have an increase in its expression and show resistance to carbapenems (Heritier et al., 2006). Further investigations are necessary to explore the genetic environment of bla_{OXA} in ours strains. MBL production was not detected in any of our strains (negative imipenem-EDTA synergy test).

Other β-lactamases have been reported in *A. baumannii*. These include the TEM-1 type, SHV type, CTX-M type , PER-1 , and VEB-1 β-lactamases (Peleg et al .,2008). Although they are important, it is difficult to assess their impact on resistance in the presence of the AmpC cephalosporinase (Corvec et al ., 2003). Three of ours strains possess bla_{TEM-1}.

However, bla_{PER}, bla_{SHV} and bla_{CTX-M} were not detected in any of our isolates. A recent study has reported that the prevalence of bla_{TEM-1} genes analyzed by multiplex-PCR-based (Woodford et al .,2006) among *A. baumannii* isolates in Kaohsiung Armed Forces General Hospital (Kaohsiung, Taiwan) was 79.40% (Ben et al ., 2011). The same study has showed that in multidrug resistant *A. baumannii*, the presence of bla_{TEM-1} predicts resistance to ceftazidime. Strain 1, harboring only bla_{TEM-1} was resistant to all β-lactams except carbapenems (Tables 2 and 3).

Strains isolated at Ibn Sina Hospital showed the dissemination of the genotype A which was the most common cluster type (10/16) followed by B cluster (4/16). However, different PFGE patterns were found in Ibn Roched Hospital. These data can be related to the dissemination characteristics of this microorganism, its permanence for long periods in hospitals, its main form of dissemination, human contact, and the mobility of patients and staff (Mugnier et al., 2010; Lin et al., 2011).

Conclusion

The identification of bla_{OXA-23} in this study confirms the wide geographical distribution of carbapenemases among *A. baumannii* as well as their parallel appearance in outbreak strains. Additionally, it is important to follow antibiotic restriction policies to avoid excessive use of carbapenem and other broad spectrum antibiotics in our country. Also, it is of great importance to study the local epidemiology of *A. baumannii* isolates to establish the best treatment and use the correct epidemiological control.

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REFERENCES

Amazian K, Fendri C, Missoum MF, Bouzouaia N, Rahal K, Savey A, Saadatian-Elahi M, Fabry J (2006). Multicenter pilot survey of resistant bacteria in the Mediterranean area. Eur. J. Clin. Microbiol. Infect. Dis. 25:340-343.

Ansaldi F, Canepa P, Bassetti M, Zancolli M, Molinari MP, Talamini A, Ginocchio F, Durando P, Mussap M , Orengo G, Viscoli C, Icardi G (2011). Sequential outbreaks of multidrug-resistant *Acinetobacter baumannii* in intensive care units of a tertiary referral hospital in Italy: combined molecular approach for epidemiological investigation. J. Hosp. Infect. 79:134-140.

Azimi L, Alaghehbandan R, Mohammadpoor M , Rastegar Lari A (2012). Identification of KPC-Producing *Pseudomonas aeruginosa* and *Acinetobacter baumannii* in a Burned Infant: A Case Report. J. Med. Bacteriol. 1:46-49.

Ben RJ, Yang MC, Hsueh JC, Shiang JC, Chien ST (2011). Molecular characterisation of multiple drug-resistant *Acinetobacter baumannii* isolates in southern Taiwan. In. J. Antimicrob. Agents. 38:403-408.

Betriu C, Rodrı´guez-Avial I, Sánchez BA, Gómez M, Álvarez J, Picazo JJ (2002). In Vitro Activities of Tigecycline (GAR-936) against Recently Isolated Clinical Bacteria in Spain. Antimicrob. Agents. Chemother. 46:892-895.

Boulanger A, Naas T, Fortineau N, Figueiredo S, Nordmann P (2012). NDM-1-producing- *Acinetobacter baumannii* from Algeria. Antimicrob. Agents. Chemother. 56:2214-2215.

Carvalho KR, D'Alincourt Carvalho-Assef AP, Peirano G, Galvão dos Santos LC, Felix Pereira MJ, Asensi MD (2009). Dissemination of multidrug-resistant *Acinetobacter baumannii* genotypes carrying bla_{OXA-23} collected from hospitals in Rio de Janeiro, Brazil. Int. J. Antimicrob. Agents 34:25-28.

Clinical and Laboratory Standards Institute (2011). Performance standards for antimicrobial susceptibility testing;eighteenth informational supplement M100eMS21. CLSI document. Wayne, PA

Corbella X, Montero A, Pujol M, Domi´nguez MA, Ayats J, Argerich MJ, Garrigosa F, Ariza J (2000). Emergence and Rapid Spread of Carbapenem Resistance during a Large and Sustained Hospital Outbreak of Multiresistant *Acinetobacter baumannii*. J. Clin. Microbiol. 38:4086-4095.

Corvec S, Caroff N, Espaze E, Giraudeau C, Drugeon H, Reynaud A (2003). AmpC cephalosporinase hyperproduction in *Acinetobacter baumannii* clinical strains. J. Antimicrob. Chemother. 52:629-635.

Dijkshoorn L ,Nemec A, Seifert H (2007). An increasing threat in hospitals: multidrug-resistant *Acinetobacter baumannii*. Nat. Rev. Microbiol. 5:939-951.

Dijkshoorn L, Aucken H, Gerner-Smidt P,Janssen P, Kaufmann ME, Garaizar J, Ursing J, Pitt TL (1996). Comparison of outbreak and non outbreak *Acinetobacter baumannii* strains by genotypic and phenotypic methods. J. Clin. Microbiol. 34:1519-1525.

Donald HM, Scaife W, Amyes SGB, Young HK (2000). Sequence Analysis of ARI-1, a Novel OXA β-Lactamase, Responsible for Imipenem Resistance in *Acinetobacter baumannii* 6B92. Antimicrob. Agents Chemother. 44:196-199.

Drissil M, Poirel L, Mugnier PD, Ahmed ZB, Nordmann P (2010). Carbapenemase-producing *Acinetobacter baumannii*, Algeria. Eur. J. Clin. Microbiol. Infect. Dis. 29:1457-1458.

Falagas ME, Kopterides P, Siempos II (2006). Attributable mortality of *Acinetobacter baumannii* infection among critically ill patients. Clin. Infect. Dis. 43:389-390.

Feizabadi MM, Fathollahzadeh B, Taherikalani M (2008). Antimicrobial susceptibility patterns and distribution of *bla*$_{OXA}$ genes among *Acinetobacter* spp. isolated from patients at Tehran Hospitals. Jpn. J. Infect. Dis. 61:274-278.

Hammami S, Ghozzi R, Saïdani M, Ben Redjeb S (2011). Carbapenem-resistant *Acinetobacter baumannii* producing the carbapenemase OXA-23 in Tunisia. Tunis. Med. 89:638-643.

Heritier C, Poirel L, Nordman P (2006). Cephalosporinase over-expression resulting from insertion of ISAba1 in *Acinetobacter baumannii*. Clin. Microbiol. Infect. 12:23-13.

Higgings PG, Poirel L, Lehmann M, Nordmann P, Seifert H (2009). OXA-143, a novel carbapenem-hydrolyzing class D -lactamase in *Acinetobacter baumannii*. Antimicrob. Agents. Chemother. 53:5035-5038.

Kempf M, Rolain JM (2012). Emergence of resistance to carbapenems in *Acinetobacter baumannii* in Europe: clinical impact and therapeutic options. Int. J. Antimicrob. Agent 10:105-114 .

Ktari S, Arlet G, Mnif B, Gautier V, Mahjoubi F, Ben Jmeaa M, Bouaziz M, Hammami A (2006). Emergence of Multidrug-Resistant *Klebsiella pneumoniae* Isolates Producing VIM-4 Metallo-β-Lactamase, CTX-M-15 Extended-Spectrum β-Lactamase, and CMY-4 AmpC β-Lactamase in a Tunisian University Hospital. Antimicrob. Agents. Chemother. 50:4198-4201.

Kusradze I, Diene SM, Goderdzishvili M, Rolai JM (2011). Molecular detection of OXA carbapenemase genes in multidrug-resistant *Acinetobacter baumannii* isolates from Iraq and Georgia. In. J. Antimicrob. Agent. 38:164-168.

Lin MF, Kuo HY, Yeh HW, Yang CM, Sung CH, Tu CC, Huang ML, Liou LM (2011). Emergence and dissemination of *bla*$_{OXA-23}$-carrying imipenem-resistant *Acinetobacter* sp in a regional hospital in Taiwan. J. Microbiol. Immunol. Infect. 44:39-44.

Lolans K, Rice TW, Munoz-Price LS, Quinn JP (2006). Multicity Outbreak of Carbapenem-Resistant *Acinetobacter baumannii* Isolates Producing the Carbapenemase OXA-40. Antimicrob. Agents. Chemother. 50:2941-2945.

Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB,Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin. Microbiol. Infect. 18:268-281.

Metan G , Sariguzel F, Sumerkan B, Reijden TV, Dijkshoorn L (2012). Clonal diversity and high prevalence of OXA-58 among *Acinetobacter baumannii* 3 isolates from blood cultures in a tertiary care centre in Turkey. Infect. Genet. Evol. 14:92-97.

Mugnier PD, Poirel L, Naas T (2010). Worldwide dissemination of the *bla*$_{OXA-23}$ carbapenemase gene of *Acinetobacter baumannii*. Emerg. Infect. Dis. 16:35-40.

Nasehi L, Shahcheraghi F, Nikbin VS, Nematzadeh S (2010). PER, CTX-M, TEM and SHV Beta-lactamases in Clinical Isolates of *Klebsiella pneumoniae* Isolated from Tehran, Iran. Iran J. Basic Med. Sci. 13:111-118.

Nordmann P, Poirel L (2002). Emerging carbapenemases in Gram-negative aerobes. Clin. Microbiol. Infect. 8:321-331.

Peleg AY, Seifert H, Paterson DL (2008). *Acinetobacter baumannii*: Emergence of a Successful Pathogen. Clin. Microbiol. Rev. 21:538-582.

Pfeifer Y, Wilharm G, Zander E, Wichelhaus TA, Göttig S, Hunfeld KP, , Seifert H, Witte W, Higgins PG (2011). Molecular characterization of *bla*$_{NDM-1}$ in an *Acinetobacter baumannii* strain isolated in Germany. J. Antimicrob. Chemother. 66:1998-2001.

Pitout JD, Chow BL, Gregson DB, Laupland KB, Elsayed S, Church DL (2007). Molecular epidemiology of metallo-β-lactamase-producing *Pseudomonas aeruginosa* in the Calgary Health Region: emergence of VIM-2-producing isolates. J. Clin. Microbiol. 45:294-298.

Poirel L, Figueiredo S, Cattoir V, Carattoli A, Nordmann P (2008a). Acinetobacter radioresistens as a silent source of carbapenem resistance for *Acinetobacter* spp. Antimicrob. Agents. Chemother. 52:1252-1256.

Poirel L, Nordman P (2006). Carbapenem resistance in *Acinetobacter baumannii*: mechanisms and epidemiology. Clin. Microbiol. Infect. 12:826-883.

Poirel L, Mansour W, Bouallegue O, Nordmann P (2008b). Carbapenem-Resistant *Acinetobacter baumannii* Isolates from Tunisia Producing the OXA-58-Like Carbapenem-Hydrolyzing Oxacillinase OXA-97. Antimicrob. Agents Chemother. 52:1613-1617.

Poirel L, Thomas I, Naas T, Karim A, Nordmann P (2000). Biochemical Sequence Analyses of GES-1, a Novel Class A Extended-Spectrum b-Lactamase, and the Class 1 Integron In52 from *Klebsiella pneumonia*. Antimicrob. Agents Chemother. 44:622-632.

Robledo IE, Aquino EE, Va´zquez GJ (2011). Detection of the KPC Gene in *Escherichia coli, Klebsiella pneumoniae,Pseudomonas aeruginosa*, and *Acinetobacter baumannii* during a PCR-Based Nosocomial Surveillance Study in Puerto Rico. Antimicrob. Agents Chemother. 55:2968-2970.

Ruppé E, Hem S, Lath S, Gautier V, Ariey F, Sarthou JL, Monchy D, Arlet G (2009). CTX-M β-Lactamases in *Escherichia coli* from community-acquired Urinary Tract Infections, Cambodia. Emerg. Infect. Dis. 15:1080-6059.

Schuetz AN, Huard RC, Eshoo MW, Massire C, Della-Latta P, Wu F, Jenkins SG (2012). Identification of a novel *Acinetobacter baumannii* clone in a US hospital outbreak by multilocus polymerase chain reaction/ electrospray-ionization mass spectrometry. Diag. Microbiol. Infect. Dis. 72:14-19.

Touati M , Diene S, Racherache A, Dekhil M, Djahoudi A, Rolain J-M (2012). Emergence of *bla*$_{OXA-23}$ and *bla*$_{OXA-58}$ carbapenemase-encoding genes in multidrug-resistant *Acinetobacter baumannii* isolates from University Hospital of Annaba, Algeria. Int. J. Antimicrob. Agents 40:89-91

Van Belkum A, Tassios PT, Dijkshoorn L, Haeggman S, Cookson B , Fry N.K , Fussing V ,Green J ,Feil E , Gerner-Smidt P ,Brisse S , Struelens M (2007). Guidelines for the validation and application of typing methods for use in bacterial epidemiology. Clin. Microbiol. Infect. 13:1-46.

Wang H, Guo P, Sun H, Wang H, Yang Q, Chen M, Xu Y, Zhu Y(2007). Molecular epidemiology of clinical isolates of carbapenem-resistant *Acinetobacter* spp. from Chinese Hospitals. Antimicrob. Agents Chemother. 51:4022-4028.

Woodford N, Ellington MJ, Coelho JM, Turton JF , Ward ME, Brown S, Amyes SGB, Livermore DM (2006). Multiplex PCR for genes encoding prevalent OXA carbapenemases in *Acinetobacter* spp. Int. J. Antimicrob. Agents 27:351-353.

Zarrilli R, Giannouli M, Tomasone F, Triassi M, Tsakris A (2009). Carbapenem resistance in *Acinetobacter baumannii*: the molecular epidemic features of an emerging problem in health care facilities. J. Infect. Dev. Ctries. 5:335-34.