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High prevalence of multidrug-resistance in *Acinetobacter baumannii* and dissemination of carbapenemase-encoding genes $bla_{OXA-23-like}$, $bla_{OXA-24-like}$ and bla_{NDM-1} in Algiers hospitals

Khadidja Khorsi[1], Yamina Messai[1], Moufida Hamidi[2], Houria Ammari[3], Rabah Bakour[1*]

[1]*Laboratory of Cellular and Molecular Biology, Faculty of Biological Sciences, University of Sciences and Technology Houari Boumediene, P.B. 32 El-Alia, Bab-Ezzouar, 16111, Algiers, Algeria*

[2]*Hospital Salim Zemirli, Route de Baraki, BP N°71, El Harrach, 16000, Algiers, Algeria*

[3]*University Hospital Isaad Hassani, Rue Ibrahim Hadjeras, Beni Messous, 16206, Algiers, Algeria*

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ABSTRACT

Objective: To assess and characterize antibiotic resistance in *Acinetobacter baumannii* strains recovered from 5 health-care facilities in Algiers.

Methods: Antibiotic susceptibility testing was performed by agar diffusion and agar dilution methods, resistance genes were identified by PCR and sequencing, and molecular typing of isolates was carried out by enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR).

Results: Among 125 tested isolates, 117 (93.6%) were multidrug-resistant, of which 94 (75.2%) were imipenem resistant. The bla_{ADC} and $bla_{OXA-51-like}$ genes were detected in all isolates, in association with IS*Aba1* sequence in 84% and 8% (imipenem resistant) of isolates, respectively. The $bla_{OXA-23-like}$ and $bla_{OXA-24-like}$ carbapenemase genes were detected in 67.02% and 20.21% of imipenem-resistant isolates, respectively. The $bla_{OXA-23-like}$ gene is linked to IS*Aba1* or IS*Aba4* elements. The metallo-β-lactamase NDM-1 gene was found in 10 (10.6%) imipenem-resistant strains from three hospitals, it is linked to IS*Aba125* element in nine strains. Extended spectrum β-lactamases production was not detected. Imipenem and cefotaxime resistance phenotypes could not be transferred to *Escherichia coli* by conjugation. Outer membrane protein CarO gene was not detected in four imipenem-resistant isolates. The *aac(6′)-Ib*, *sul1*, *sul2*, *tetA* and *tetB* genes were present in 5.31%, 36.17%, 77.65%, 1.06% and 65.92% of strains, respectively. Class 1 integrons were detected in 23.4% strains. ERIC-PCR typing showed a genetic diversity among $bla_{OXA-23-like}$ and $bla_{OXA-24-like}$ positive strains, while clonality was observed among bla_{NDM-1} positives.

Conclusions: This study highlighted the high prevalence of imipenem resistance in *Acinetobacter baumannii* in Algiers hospitals mediated mainly by $bla_{OXA-23-like}$, $bla_{OXA-24-like}$, and bla_{NDM-1} genes.

1. Introduction

Acinetobacter baumannii (*A. baumannii*) is a gram-negative cocobacilli characterized by its ubiquity and its ability to persist in hospital environment and easily acquire antibiotic resistance. It is an opportunistic pathogen responsible for nosocomial infections mainly by cross-transmission, with a propensity for outbreak. *A. baumannii* is naturally resistant to penicillin G, amoxicillin and first and second cephalosporin generations by producing a cephalosporinase of ADC type (*Acinetobacter*-Derived Cephalosporinases) and secondarily through an oxacillinase represented by $bla_{OXA-51-like}$ cluster [1,2]. Overexpression of ADCs through upstream inserting of insertion sequences [2,3] and/or production of extended-spectrum beta-lactamases (ESBLs) are mechanisms of acquired resistance to broad spectrum cephalosporins [1,4]. The resistance to carbapenems is most often related to the acquisition of

*Corresponding author: Rabah Bakour, Laboratory of Cellular and Molecular Biology, Faculty of Biological Sciences, University of Sciences and Technology Houari Boumediene, P.B. 32 El-Alia, Bab-Ezzouar, 16111, Algiers, Algeria.
Tel: +213 21 24 79 13;
fax: +213 21 24 72 17
E-mails: rbakour@yahoo.fr, bakourrabah@gmail.com

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carbapenemases: (i) oxacillinases carbapenem-hydrolyzing class D β-lactamases (CHDLs) distributed into the following main clusters: intrinsic OXA-51-like and acquired OXA-23-like, OXA-24-like/40, OXA-58-like and OXA-143-like; (ii) metallo-β-lactamases (MBLs) of IMP, VIM, SIM, GIM and NDM types; (iii) carbapenemases of Ambler class A as KPC and some variants of GES [1,4,5]. Insertion sequences present at the 5′ end and/or 3′ end of bla_{OXA} genes regulate their expression and mobility, particularly by the formation of transposon structures [5]. MBLs of IMP, VIM and SIM types are often described as gene cassettes of class 1 integrons which are the most prevalent in *A. baumannii* [4], and NDM was found associated with an upstream IS*Aba125* element and can be located within a transposon named Tn125 [6]. Moreover, non-enzymatic mechanisms were described such as modification of membrane permeability through loss of outer membrane proteins, such as CarO [2,7]. Despite worldwide reports on the increase of morbidity and mortality associated with *A. baumannii*, to our best knowledge, few studies were devoted to this germ in Algeria, particularly in Algiers region which has the highest concentration of population and hospitals. More data are needed in order to objectively appreciate the state and epidemiology of antibiotic resistance. The objective of this study was to assess and characterize the antibiotic resistance in clinical *A. baumannii* isolates recovered from 5 health-care facilities in Algiers.

2. Materials and methods

2.1. Bacterial isolates

The study included 125 non repetitive strains of *A. baumannii* collected from five health care institutions in Algiers, Zemirli hospital (n = 91), Beni-Messous hospital (n = 12), Burn Central Clinic (n = 9), Birtraria hospital (n = 7) and Anti-cancer Center Pierre and Marie Curie of Mustapha hospital (n = 6). The strains were isolated during the period 2008–2014 from various clinical specimens: bronchial fluid (n = 43), pus (n = 37), blood (n = 11), urine (n = 10), catheters (n = 6), cerebrospinal fluid (n = 3), bile (n = 1) and unspecified (n = 14). These specimens were from various care units: intensive care (n = 54), neurosurgery (n = 20), orthopedic (n = 10), burn (n = 9), hematology (n = 4), general surgery (n = 2), marrow transplantation (n = 2), internal medicine (n = 1), emergency (n = 1), rehabilitation (n = 1), outpatients (3) and unspecified (n = 18). The strains were identified by standard microbiological techniques, API 20NE identification systems (BioMerieux, France) and by PCR detection of the intrinsic carbapenemase gene $bla_{OXA\text{-}51\text{-}like}$ [8].

2.2. Antibiotic susceptibility testing

Antibiotic susceptibility was determined by the disk diffusion method on Mueller–Hinton agar, according to the recommendations of the Antibiogram Committee of the French Society for Microbiology [9]. The following antibiotic disks (Bio-Rad) were used (μg or International Unit ''IU''/disk): amoxicillin/clavulanic acid (20/10 μg), ticarcillin (TIC) (75 μg), piperacillin (PIP) (75 μg), piperacillin/tazobactam (PTZ) (75/10 μg), cefotaxime (CTX) (30 μg), ceftazidime (CAZ) (30 μg), ceftriaxone (CRO) (30 μg), cefepime (CFP) (30 μg), aztreonam (AZT) (30 μg), imipenem (IMP) (10 μg), ciprofloxacin (CIP) (5 μg), ofloxacine (OFL) (5 μg), pefloxacin (PEF) (5 μg), gentamicin (GM) (15 μg), amikacin (AMK) (30 μg), tetracyclines (TE) (30 μg), trimethoprim/sulfamethoxazole (SXT) (1.25/23.75 μg) and colistin (C) (50 μg). *Escherichia coli* (*E. coli*) ATCC 25922 was used as a control.

The MICs of cefotaxime (CTX), ceftazidime (CAZ), imipenem (IMP), ciprofloxacin (CIP), gentamicin (GM) and trimethoprim/sulfamethoxazole (SXT) were determined by agar-dilution method according to the guidelines of CA-SFM [9].

2.3. Phenotypic detection of production of metallo-β-lactamases (MBLs) and extended-spectrum-β-lactamases (ESBLs)

MBLs production was screened by the imipenem-EDTA double-disk synergy (IEDDS) test using disks of imipenem (10 μg) and EDTA (1.5 mg) spaced at a distance of 15 mm (edge to edge) on Mueller–Hinton agar [10].

ESBLs production was screened by the Double-Disc Synergy Test (DDST) [11] using disks of cefotaxime, ceftazidime and cefepime placed around a disk of amoxicillin/clavulanic acid at a distance of 20 mm from disk center to center on Mueller–Hinton agar supplemented with cloxacillin (500 μg/mL).

2.4. Detection of antibiotic resistance genes, insertion sequences and integrons

Simplex and multiplex PCR were used to screen for the following resistance genes as previously described: OXA carbapenemases: $bla_{OXA\text{-}51\text{-}like}$, $bla_{OXA\text{-}23\text{-}like}$, $bla_{OXA\text{-}24\text{-}like}$, $bla_{OXA\text{-}58\text{-}like}$ [12] and $bla_{OXA\text{-}143}$ [13]; metallo-β-lactamases: bla_{IMP}, bla_{VIM}, bla_{GIM} and $bla_{NDM\text{-}1}$ [14,15]; cephalosporinase: bla_{ADC} [16]; class A beta-lactamases: bla_{TEM}, bla_{SHV} [17], $bla_{CTX\text{-}M}$ [18], bla_{PER}, bla_{VEB}, bla_{GES} [19]; plasmid-mediated quinolone resistance determinants: *qnrA*, *qnrB* and *qnrS* [20]; aminoglycoside-modifying enzyme: *aac(6′)-Ib* [20]; tetracycline efflux pumps: *tetA* and *tetB* [21]; dihydropteroate synthases: *sul1*, *sul2* and *sul3* [22,23]; dihydrofolate reductases: *dfrA1*, *dfrA5*, *dfrA7*, *dfrA8* and *dfrA12* [24].

Insertion sequences IS*Aba1*, IS*Aba4* and IS*Aba125* were searched by PCR as previously described [3,25,26]. The genetic association between IS*Aba1*, IS*Aba4* and IS*Aba125* sequences and bla_{ADC}, $bla_{OXA\text{-}51\text{-}like}$, $bla_{OXA\text{-}23\text{-}like}$, $bla_{OXA\text{-}24\text{-}like}$ and $bla_{NDM\text{-}1}$ genes was investigated by PCR using combinations of forward or reverse primers of IS*Aba* sequences and *bla* genes.

Screening for class 1 and class 2 integrons was done by multiplex PCR targeting *int1*, *sul1* and *qacΔE* genes and by simplex PCR targeting *int2* gene [22,27]. *carO* gene was searched by PCR as previously described [7]. All primers used were presented in Table 1.

Table 1
Primers used in this study.

Target	Primer	Sequence 5′→3′	AT (°C)	ES (bp)	Ref
bla$_{\text{OXA-23-like}}$	OXA-23-F	GATCGGATTGGAGAACCAGA	52	501	[12]
	OXA-23-R	ATTTCTGACCGCATTTCCAT			
bla$_{\text{OXA-24-like}}$	OXA-24-F	GGTTAGTTGGCCCCCTTAAA	52	246	[12]
	OXA-24-R	AGTTGAGCGAAAAGGGGATT			
bla$_{\text{OXA-58-like}}$	OXA-58-F	AAGTATTGGGGCTTGTGCTG	52	599	[12]
	OXA-58-R	CCCCTCTGCGCTCTACATAC			
bla$_{\text{OXA-51-like}}$	OXA-51-F	TAATGCTTTGATCGGCCTTG	52	353	[12]
	OXA-51-R	TGGATTGCACTTCATCTTGG			
bla$_{\text{OXA-143-like}}$	OXA-143-F	TGGCACTTTCAGCAGTTCCT	52	149	[13]
	OXA-143-R	TAATCTTGAGGGGGCCAACC			
bla$_{\text{VIM}}$	VIMgen-F2	GTTTGGTCGCATATCGCAAC	53	382	[14]
	VIMgen-R2	AATGCGCAGCACCAGGATAG			
bla$_{\text{IMP}}$	IMPgen-F1	GAATAGAATGGTTAACTCTC	53	188	[14]
	IMPgen-R1	CCAAACCACTAGGTTATC			
bla$_{\text{GIM}}$	GIM-F1	TCAATTAGCTCTTGGGCTGAC	53	72	[14]
	GIM-R1	CGGAACGACCATTTGAATGG			
bla$_{\text{NDM-1}}$	NDM-Fm	GGTTTGGCGATCTGGTTTTC	52	621	[15]
	NDM-Rm	CGGAATGGCTCATCACGATC			
IS*Aba-1*	ISAba1a	ATGCAGCGCTTCTTTGCAGG	55	393	[3]
	ISAba1b	AATGATTGGTGACAATGAAG			
IS*Aba-4*	ISAba4A	ATTTGAACCCATCTATTGGC	40	611	[25]
	ISAba4B	ACTCTCATATTTTTTCTTGG			
IS*Aba-125*	125F	GGGTAATGCTCGTATCGT	48	148	[26]
	125R	TAGACGTAGACGTGGTCA			
bla$_{\text{TEM}}$	TEM up	ATGATGATTCAACATTTCCG	52	858	[17]
	TEM low	CCAATGCTTAATCAGTGAGG			
bla$_{\text{SHV}}$	SHV up	TTATCTCCCTGTTAGCCACC	50	795	[17]
	SHV low	GATTTGCTGATTTCGCTCGG			
bla$_{\text{CTX-M}}$	CTX-MA	CGCTTTGCGATGTGCAG	55	550	[18]
	CTX-MB	ACCGCGATATCGTTGGT			
bla$_{\text{PER}}$	PER-1F	ATGAATGTCATTATAAAAGC	55	925	[19]
	PER-1B	AATTTGGGCTTAGGGCAGAA			
bla$_{\text{VEB}}$	VEB-1F	CGACTTCCATTTCCCGATGC	55	643	[19]
	VEB-1B	GGACTCTGCAACAAATACGC			
bla$_{\text{GES}}$	GES-1F	ATGCGCTTCATTCACGCAC	55	860	[19]
	GES-1B	CTATTTGTCCGTGCTCAGG			
bla$_{\text{ADC}}$	ADC1	CCGCGACAGCAGGTGGATA	51	420	[16]
	ADC2	TCGGCTGATTTTCTTGGTT			
qnr A	qnrAm F	AGAGGATTTCTCACGCCAGG	54	580	[20]
	qnrAm R	TGCCAGGCACAGATCTTGAC			
qnr B	qnrBm F	GGMATHGAAATTCGCCACTG	54	246	[20]
	qnrBm R	TTTGCYGYYCGCCAGTCGAA			
qnr S	qnrSm F	GCAAGTTCATTGAACAGGGT	54	428	[20]
	qnrSm R	TCTAAACCGTCGAGTTCGGCG			
aac(6′)Ib	aac(6)-F	TTGCGATGCTCTATGAGTGGCTA	55	482	[20]
	aac(6)-R	CTCGAATGCCTGGCGTGTTT			
tet A	TetA-F	GTAATTCTGAGCACTGTCGC	62	954	[21]
	TetA-R	CTGCCTGGACAACATTGCTT			
tet B	TetB-F	CTCAGTATTCCAAGCCTTTG	57	414	[21]
	TetB-R	ACTCCCCTGAGCTTGAGGGG			
intI 1	intI 1-F	CCTTCGAATGCTGTAACCGC	*	248	[22]
	intI 1-R	ACGCCCTTGAGCGGAAGTATC			
*qac*EΔ1	qacEΔ1-F	GAGGGCTTTACTAAGCTTGC	*	200	[22]
	qacEΔ1-R	ATACCTACAAAGCCCCACGC			
*sul*1	Sul1-F	TCAGACGTCGTGGATGTCG	*	346	[22]
	Sul1-R	CGAAGAACCGCACAATCTCG			
*sul*2	Sul2 up	GCGCTCAAGGCAGATGGCATT	69	293	[23]
	Sul2 low	GCGTTTGATACCGGCACCCGT			
sul 3	Sul3 up	GGAAGAAATCAAAAGACTCAA	53	363	[23]
	Sul3 low	CCTAAAAAGAAGCCCATACC			
intI 2	IntI2-F	GTAGCAAACGAGTGACGAAATG	60	789	[27]
	IntI2-R	CACGGATATGCGACAAAAAGGT			
dfr A1	dfr A1-F	GTGAAACTATCACTAATGG	46	471	[24]
	dfr A1-R	ACCCTTTTGCCAGATTTG			
dfr A5	dfr A5-F	GCBAAAGGDGARCAGCT	52	394	[24]
	dfr A5-R	TTTMCCAYATTTGATAGC			
dfr A7	dfr A7-F	AAAATTTCATTGATTTCTGCA	52	471	[24]
	dfr A7-R	TTAGCCTTTTTTCCAAATCT			
dfr A8	dfr A8-F	TTGGGAAGGACAACGCACTT	46	382	[24]

Table 1 (*continued*)

Target	Primer	Sequence 5′→3′	AT (°C)	ES (bp)	Ref
	dfr A8-R	ACCATTTCGGCCAGATCAAC			
dfr A12	dfr A12-F	GGTGAGCARAAGATYTTTCGC	46	309	[24]
	dfr A12-R	TGGGAAGAAGGCGTCACCCTC			
*car*O	CarO-F	AAAGTATTACGTGTTTTAGTG	55	750	[7]
	CarO-R	TTACCAGTAGAAGTTTACACC			
ERIC2	ERIC2	AAGTAAGTGACTGGGGTGACGC	30	/	[28]

AT: Annealing temp; ES: Expected size; *: 65 °C, decrease by 1 °C by cycle for 10 cycles and 55 °C for 24 cycles.

PCR products of positive reactions for $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-23-like}}$, $bla_{\text{NDM-1}}$, IS*Aba125*/$bla_{\text{NDM-1}}$ and *aac(6′)-Ib* were sequenced and analyzed with the BLAST and FASTA programs of the National Center for Biotechnology Information (www.ncbi.nlm.nhi.gov).

2.5. Molecular genotyping of isolates

The clonal relationship between imipenem-resistant isolates was investigated by enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR) using primer ERIC2 [28] at annealing temperature of 30 °C. Fingerprints were visually compared and strains showing dissimilarity by one band or more were considered as different [29].

2.6. Conjugation experiments

Mating experiments were performed using sodium azide resistant *E. coli* BM21 as a recipient. Exponential cultures of *A. baumannii* isolates as donor (1 vol) and recipient (2 vol) were inoculated as a spot on Brain Heart Infusion Agar (BHIA). After overnight incubation at 37 °C, transconjugants were selected on BHIA supplemented with cefotaxime (4 μg/mL) or imipenem (2 μg/mL) and sodium azide (300 μg/mL).

3. Results

Of the 125 *A. baumannii* strains, 87 showed resistance to imipenem and 7 intermediate resistance. All these 94 imipenem-resistant strains were also resistant to ticarcillin, piperacillin, piperacillin/tazobactam, amoxicillin/clavulanic acid, cefotaxime and ceftriaxone, and 93.60%, 97.86% and 98.93% were resistant to ceftazidime, cefepime and aztreonam, respectively. For non-beta-lactams, resistance rates were 90.42%, 90.42% and 91.48% for ciprofloxacin, ofloxacin and pefloxacin, 77.65% and 80.85% for amikacin and gentamicin, and 86.16% and 97.87% for tetracycline and trimethoprim/sulfamethoxazole. All strains were susceptible to colistin (Table 2). Agar dilution MICs of imipenem, cefotaxime, ceftazidime, ciprofloxacin, gentamicin and trimethoprim/sulfamethoxazole were, respectively, from 8

Table 2
Antimicrobial resistance rates of *A. baumannii* isolates from Algiers hospitals (n = 125).

Antibiotic (disc load)	Breakpoint[a] (zone in mm)		Phenotypes[b] of imipenem-resistant isolates (n = 94) (%)			Phenotypes[b] of imipenem-susceptible isolates (n = 31) (%)			Phenotypes[b] of total isolates (n = 125) (%)		
	≥S	<R	R	I	S	R	I	S	R	I	S
Ticarcillin (75 μg)	22	18	100.00	0.00	0.00	64.51	6.45	29.03	91.20	1.60	7.20
Piperacillin (75 μg)	22	18	100.00	0.00	0.00	61.29	12.09	25.80	90.40	3.20	6.40
Piperacillin/Tazobactam (75/10 μg)	19	14	96.80	3.19	0.00	3.22	35.48	61.29	73.60	11.20	15.20
Amoxicilline/Clavulanate (20/10 μg)	23	16	94.68	5.31	0.00	35.48	45.16	29.03	80.00	15.20	4.80
Cefotaxime (30 μg)	26	23	100.00	0.00	0.00	83.87	6.45	9.67	96.00	1.60	2.40
Ceftriaxone(30 μg)	26	23	100.00	0.00	0.00	87.09	3.22	9.67	96.00	1.60	2.40
Ceftazidime (30 μg)	21	19	91.48	2.12	6.38	61.29	9.67	29.03	84.80	3.20	12.00
Cefepime (30 μg)	21	19	88.29	9.57	2.12	29.04	12.09	58.06	72.80	10.40	16.80
Aztreonam (30 μg)	23	21	95.74	3.19	1.06	77.41	6.45	16.12	91.20	3.20	5.60
Imipenem (10 μg)	24	17	92.55	7.44	0.00	0.00	0.00	100.00	69.60	5.60	24.80
Pefloxacin (5 μg)	22	16	84.04	7.44	8.51	41.94	19.35	38.70	72.80	10.40	16.80
Ofloxacin (5 μg)	22	22	90.42	0.00	9.57	64.51	0.00	35.45	85.60	0.00	14.40
Ciprofloxacin (5 μg)	22	22	90.42	0.00	9.57	54.83	0.00	45.16	82.40	0.00	17.60
Gentamicin (15 μg)	16	16	80.85	0.00	19.14	67.74	0.00	32.25	76.80	0.00	23.20
Amikacin (30 μg)	17	15	67.02	10.63	22.34	51.61	6.45	41.93	63.20	9.60	27.20
Tetracycline (30 μg)	19	17	78.72	7.44	13.82	58.06	3.22	38.70	72.80	5.60	21.60
Trimethoprim/Sulfamethoxazole (1,25/23,75 μg)	16	13	94.68	3.19	2.12	67.74	9.67	32.27	88.00	2.40	9.60
Colistin (50 μg)	15	15	0.00	0.00	100.00	0.00	0.00	100.00	0.00	0.00	100.00

[a] Breakpoints and susceptibility were interpreted according to CA-SFM (2013).
[b] S: susceptible; I: intermediate; R: resistant.

Table 3

Antimicrobial resistance patterns and β-lactamase genes of imipenem-resistant *A. baumannii* isolates (n = 94).

Hospital	Care units	Specimens	Dates	Cephalosporinase and carbapenemase genes	IMP MICs μg/mL	Antibiotic resistance patterns (resistant + intermediate) (n)	No. of isolates
ZM	Orthopedic, intensive care	bronchial fluid, pus	2010–2013	bla_{ADC}, $bla_{oxa\text{-}51\text{-}like}$	16–32	TIC-PIP-PPT-AMC-CTX-CRO-CAZ-ATM-FEP-IMP-PEF-CIP-OFX-AN-SXT-TE (1) TIC-PIP-PPT-AMC-CTX-CRO-CAZ-ATM-FEP-IMP-PEF-CIP-OFX-GM-AN-SXT-TE (1)	2
ZM BM	Intensive care neurosurgery orthopedic outpatient	bronchial fluid, catheter, blood, pus, urine	2013–2014	bla_{ADC}, $bla_{oxa\text{-}51\text{-}like}$, $bla_{oxa\text{-}24\text{-}like}$	8–128	TIC-PIP-PPT-AMC-CTX-CRO-CAZ-ATM-FEP-IMP-PEF-CIP-OFX-GM-AN-TE (1) TIC-PIP-PPT-AMC-CTX-CRO-CAZ-ATM-IMP-PEF-CIP-OFX-GM-AN-SXT-TE (2) TIC-PIP-PPT-AMC-CTX-CRO-CAZ-ATM-FEP-IMP-PEF-CIP-OFX-GM-SXT-TE (2) TIC-PIP-PPT-AMC-CTX-CRO-CAZ-ATM-FEP-IMP-PEF-CIP-OFX-GM-AN-SXT-TE (14)	19
ZM BM BCC BR PMCC	Neurosurgery, hematology intensive care, orthopedic, burn, general surgery rehabilitation, emergency not specified	bronchial fluid, pus, blood, cerebrospinal fluid, not specified	2009–2014	bla_{ADC}, $bla_{oxa\text{-}51\text{-}like}$, $bla_{oxa\text{-}23\text{-}like}$	8–128	TIC-PIP-PPT-AMC-CTX-CRO-IMP-AN-SXT (1) TIC-PIP-PPT-AMC-CTX-CRO-ATM-IMP-SXT-TE (1) TIC-PIP-PPT-AMC-CTX-CRO-IMP-GM-AN-SXT (1) TIC-PIP-PPT-AMC-CTX-CRO-ATM-FEP-IMP-GM-AN-SXT-TE (2) TIC-PIP-PPT-AMC-CTX-CRO-CAZ-ATM-FEP-IMP-GM-SXT-TE (1) TIC-PIP-PPT-AMC-CTX-CRO-CAZ-ATM-FEP-IMP-GM-AN-SXT (1) TIC-PIP-PPT-AMC-CTX-CRO-CAZ-ATM-FEP-IMP-GM-AN-SXT-TE (1) TIC-PIP-PPT-AMC-CTX-CRO-ATM-FEP-IMP-OFX-GM-AN-SXT-TE (1) TIC-PIP-PPT-AMC-CTX-CRO-ATM-FEP-IMP-PEF-CIP-OFX-AN-SXT-TE (1) TIC-PIP-PPT-AMC-CTX-CRO-CAZ-ATM-FEP-IMP-PEF-CIP-OFX-SXT-TE (5) TIC-PIP-PPT-AMC-CTX-CRO-CAZ-ATM-FEP-IMP-PEF-CIP-OFX-GM-SXT (2) TIC-PIP-PPT-AMC-CTX-CRO-CAZ-ATM-FEP-IMP-PEF-CIP-OFX-GM-AN-TE (1) TIC-PIP-PPT-AMC-CTX-CRO-CAZ-ATM-FEP-IMP-PEF-CIP-OFX-AN-SXT-TE (6) TIC-PIP-PPT-AMC-CTX-CRO-CAZ-ATM-FEP-IMP-PEF-CIP-OFX-GM-SXT-TE (5) TIC-PIP-PPT-AMC-CTX-CRO-CAZ-ATM-FEP-IMP-PEF-CIP-OFX-GM-AN-SXT-TE (34)	63
ZM BM PMCC	Neurosurgery, orthopedic intensive care, marrow-transplantation, internal medicine	bronchial fluid, cerebrospinal fluid, catheter, pus, bile	2012–2014	bla_{ADC}, $bla_{oxa\text{-}51\text{-}like}$, $bla_{NDM\text{-}1}$	32–128	TIC-PIP-PPT-AMC-CTX-CRO-CAZ-ATM-FEP-IMP-PEF-CIP-OFX-GM-SXT (8) TIC-PIP-PPT-AMC-CTX-CRO-CAZ-ATM-FEP-IMP-PEF-CIP-OFX-GM-SXT-TE (1) TIC-PIP-PPT-AMC-CTX-CRO-CAZ-ATM-FEP-IMP-PEF-CIP-OFX-GM-AN-SXT-TE (1)	10

TIC: ticarcillin; PIP: piperacillin; PPT: piperacillin/tazobactam; AMC: amoxicillin/clavulanate; CTX: cefotaxime; CRO: ceftriaxone; CAZ: ceftazidime; ATM: aztreonam; FEP: cefepime; IMP: imipenem; PEF: pefloxacin; CIP: ciprofloxacin; OFX: ofloxacin; GM: gentamicin; AN: amikacin, SXT: trimethoprim/sulfamethoxazole; TE: tetracycline. ZM: Zemirli hospital; BCC: Burn central clinic; BM: Beni-Messous hospital; BR: Birtraria hospital; PMCC: Pierre and Marie Curie Centre; MIC: Minimal inhibitory concentration.

to 128 μg/mL, 8 to 2 048 μg/mL, 2 to 2 048 μg/mL, 0.5 to 128 μg/mL, 1 to >64 μg/mL and 2/38 to >32/608 μg/mL.

The prevalence of antibiotic resistance was relatively lower for some antibiotics in the 31 imipenem-susceptible strains, namely ticarcillin (70.96%), piperacillin (73.38%), piperacillin/tazobactam (38.70%), ceftazidime (70.96%), cefepime (41.13%), ciprofloxacin (54.83%), ofloxacin (64.51%), pefloxacin (61.29%), gentamicin (67.74%) and tetracycline (61.26%) (Table 2).

Sixteen resistance profiles including 9–17 antibiotics were defined among imipenem-resistant strains, and 24 profiles including 3–16 antibiotics among imipenem-susceptible strains. All imipenem-resistant strains and almost all imipenem-susceptible strains (74.2%) were multidrug-resistant (MDR), they were resistant to at least three classes of antibiotics (Table 3).

The ADC type cephalosporinase gene was detected in all isolates and its association with IS*Aba1* in 105 isolates, 87 of the 94 imipenem-resistant and 18 of the 31 imipenem-susceptible. The screening for ADC/IS*Aba125* association in the 20 remaining strains was negative.

Phenotypic detection of ESBL and PCR screening for β-lactamases SHV, CTX-M, PER, VEB and GES genes were negative, while TEM gene was detected in 32 strains.

The screening for OXA-type carbapenemases revealed the presence the $bla_{\text{OXA-51-like}}$ gene in all isolates. IS*Aba1* was found upstream of $bla_{\text{OXA-51-like}}$ gene in only 10 (8%) imipenem-resistant strains, while it was detected in 116 (92.8%) strains, 93 of 94 imipenem-resistant strains and 23 of 31 imipenem-susceptible strains. $bla_{\text{OXA-23-like}}$ gene was detected by PCR and sequencing in 63 imipenem-resistant strains from the 5 hospitals. The presence of IS*Aba1* and IS*Aba4* upstream of $bla_{\text{OXA-23-like}}$ gene in opposite orientation was showed in 37 and 18 imipenem-resistant strains, respectively. The 8 remaining $bla_{\text{OXA-23-like}}$ positive strains did not show association with these insertion sequences. $bla_{\text{OXA-24-like}}$ gene was found in 19 imipenem-resistant strains from Zemirli and Beni Messous hospitals, without association with any insertion sequences. Genes encoding OXA-58-like and OXA-143-like carbapenemases were not detected (Table 3).

Phenotypic test for metallo-β-lactamases production was positive for 10 imipenem-resistant strains (Figure 1), all harbored the NDM-1 gene detected by PCR and sequencing. These strains from various clinical specimens were from Zemirli hospital (5 isolates), Pierre and Marie Curie Center (4 isolates) and Beni Messous hospital (1 isolate) (Table 3). The insertion sequence IS*Aba125* was detected in the 10 strains, and found upstream of $bla_{\text{NDM-1}}$ gene in the same orientation in 9 strains. The NDM-1 strain without association with IS*Aba125* was from Beni Messous hospital. Metallo-β-lactamases IMP, VIM and GIM were not detected.

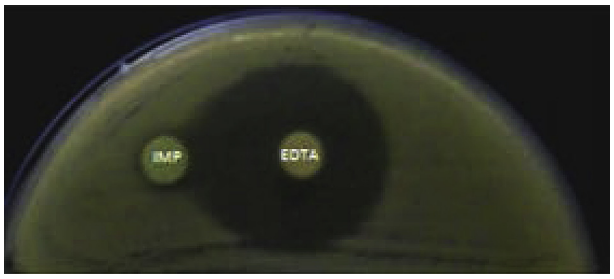

Figure 1. Detection of metallo-β-lactamase production by the imipenem-EDTA synergy test.

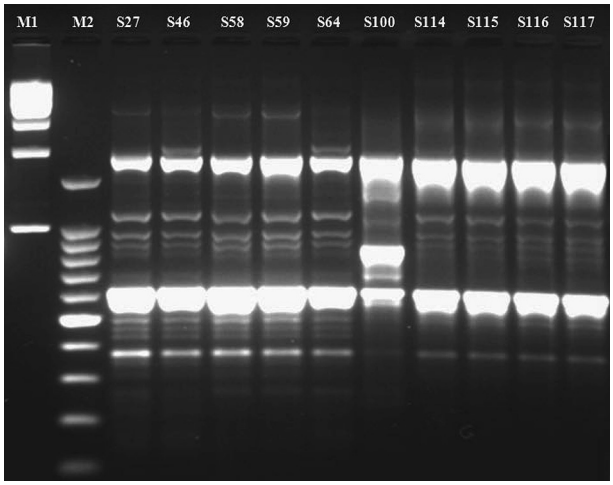

Figure 2. ERIC-PCR fingerprints of NDM-1 positive imipenem-resistant *A. baumannii* isolates (n = 10).
S27, S46, S58, S59, S64: isolates from Zemirli hospital; S100: isolate from Beni-Messous hospital; S114, S115, S116, S117: isolates from Pierre and Marie Curie Centre; M1 and M2: 1 kb and 100 bp DNA markers ladder (Promega).

Conjugation experiments performed on imipenem-resistant strains have not allowed transfer of cefotaxime or imipenem resistance to the recipient strain *E. coli* BM21.

The gene of the outer membrane protein CarO was not detected in four imipenem-resistant strains harboring OXA-51-like (n = 1), OXA-51-like + OXA-23-like (n = 1) and OXA-51-like + IS*Aba1*/OXA-23-like (n = 2).

The two remaining imipenem-resistant strains without OXA or NDM-1 carbapenemases had an IS*Aba1*/OXA-51-like association or a loss of the outer membrane protein CarO gene.

The screening for resistance genes to other classes of antibiotics in the 94 imipenem-resistant strains showed the absence of PMQR [*qnr* and *aac(6′)-Ib-cr*], *dfr* and *sul3* genes, while *aac(6′)-Ib*, *sul1*, *sul2*, *tetA* and *tetB* were detected in 5 (5.31%), 34 (36.17%), 73 (77.65%), 1 (1.06%) and 62 (65.92%) strains, respectively. Class 1 integrons were detected in 22 (23.4%) strains, while class 2 integrons were absent.

Molecular typing of the 94 imipenem-resistant strains performed by ERIC-PCR identified 57 DNA profiles. The 63 OXA-23 strains presented 41 different genetic profiles and the 19 OXA-24 strains showed 10 different DNA profiles. The 10 NDM-1 strains showed 4 DNA profiles, the same genetic profile for the 4 strains from Pierre and Marie Curie Center, two for the 5 strains from Zemirli hospital, and a specific profile for the single strain from Beni-Messous hospital (Figure 2).

4. Discussion

The widespread of carbapenem-resistant *A. baumannii* constitutes a global public health threat. Molecular characterization of mechanisms and epidemiology of multidrug-resistance is a main step to tackle its spreading and develop therapeutic strategies. Seventy five percent of *A. baumannii* strains collected from 5 hospitals in Algiers were resistant to imipenem. This rate is higher than that previously reported in Algeria in a national survey (45.32%) [30], but similar to that more recently reported in Western Algeria (70.8%) [31]. Overall, carbapenem

resistance has rapidly increased worldwide and prevalence of imipenem-resistant strains has reached 100% in some countries [32]. This evolution may be due to a heavy use of carbapenems, notably following the pandemic of ESBLs, particularly CTX-M type, and also to the no compliance with hygiene and isolation procedures in some countries.

All our imipenem-resistant strains were multidrug resistant, as commonly reported worldwide [33,34], this is maybe inherent to the accumulation of mutations selected by various antibiotics before introduction of carbapenems, and to the multiple mechanisms of carbapenem resistance in *A. baumannii* conferring simultaneous resistance to antibiotics of other classes.

Resistance patterns of our strains leave very few therapeutical options. In this study, like in previous reports from Algeria [31,35], all our isolates were susceptible to colistin. Overall, MDR *Acinetobacter* strains remain susceptible to colistin, fosfomycine and tigecycline. *In vitro* and animal studies support the role of combination therapy of colistin with fosfomycine or tigecycline and with other molecules as rifampicine and vancomycine or teicoplamine [4,6,36]. Besides imipenem, it also should be noted the very high prevalence of aztreonam resistance in our strains. This antibiotic, which normally is not or weakly hydrolyzed by OXA- and MBL-carbapenemases types (including NDM-1), constitutes a therapeutic solution in combination with a large broad spectrum serine beta-lactamases inhibitor [6].

The ADC gene was present in all strains in agreement with the ubiquitous nature of this gene responsible for *A. baumannii* natural resistance to penicillins and 3rd generation cephalosporins [16,26]. Resistance to cephalosporins may also be due to the acquisition of ESBL; no synergy between clavulanic acid and antibiotic markers was observed in our strains. The search for beta-lactamases previously identified in *A. baumannii*, namely TEM, SHV, CTX-M, PER, VEB and GES, was positive for only TEM. In agreement with our results, no ESBLs were detected in imipenem-resistant *A. baumannii* in Western Algeria [31] and the narrow spectrum beta-lactamases TEM-1 and TEM-2 were described as prevalent in *Acinetobacter* [1,4].

The screening for OXA-type enzymes with potent carbapenemase activity showed the presence of OXA-51-like in all strains, these enzymes are known as ubiquitous and intrinsic to *A. baumannii* species [8,27].

OXA-23-like and OXA-24-like were detected in 67.02% and 20.21% of imipenem resistant strains, respectively, with a total of 87.23%. OXA-23-like is the most prevalent of carbapenamases with a global distribution and OXA-23-producing *A. baumannii* strains were described as cause of nosocomial outbreaks [5,27,37]. OXA-24-like is also a widely disseminated cluster, however at a lesser prevalence than OXA-23-like [5]. In Algeria, Oxa-23-like and OXA-24-like were previously reported in Eastern and Western Algeria [31,35,38] and just recently in a hospital in Algiers [39].

Because of their low expression, bla_{ADC} and bla_{OXA} genes have weak hydrolytic activity on oxymino-beta-lactams and carbapenems, their expression level becomes clinically significant by the presence of upstream insertion sequences providing them with strong promoters. bla_{ADC} gene present in all strains was found linked to IS*Aba1* in 84% of strains, this association is commonly reported in *A. baumannii*, resulting in high resistance to broad spectrum cephalosporins, particularly ceftazidime [3,16,26], as was observed in our strains. $bla_{\text{OXA-51-like}}$ were found associated with IS*Aba1* in only 10 strains, while this IS was detected in 116 (92.8%) strains and it was described as the most frequently associated with $bla_{\text{OXA-51-like}}$ [40]. However, other IS such as IS*Aba825* can provide effective promoters to $bla_{\text{OXA-51-like}}$ gene, contributing to carbapenem resistance [41]. Almost all detected $bla_{\text{OXA-23-like}}$ genes (87.3%) were found associated with IS*Aba1* or IS*Aba4*, in agreement with previous studies showing the role of these sequences in overexpression of $bla_{\text{OXA-23-like}}$ in *A. baumannii* [25,27,40]. $bla_{\text{OXA-24-like}}$ genes were not found associated with any insertion sequence in our strains, as was already reported [4,42]. In addition to their role as "mobile promoter", insertion sequences are involved in mobilization of resistance genes conferring them a high potential of diffusion.

Ten strains (10.63%) harbored the MBL gene $bla_{\text{NDM-1}}$, they were recovered from different clinical specimens in three hospitals, Zemirli, Pierre and Marie Curie Center and Beni Messous. NDM-1 was previously reported by other authors in Western Algeria [31] and just recently in Eastern Algeria and in a hospital in Algiers [39]. This MBL detected originally in enterobacteria was later described in *Acinetobacter*, with a link with the Indian subcontinent and in some cases with the Balkan region and the Middle East [6]. However, this enzyme has rapidly spread throughout the world reaching at last Central and South America [43,44]. NDM-1 strains were recovered from national patients who do not seem to have had any recent contact with abroad. The same observation was reported about NDM-1 strains in Western Algeria [31]. This would suggest that NDM-1 is possibly endemic in Algeria. Nine of the 10 NDM-1 strains have been found associated with IS*Aba*125, as already described [45]. This sequence can positively affect the expression of NDM-1 and contribute to its mobilization through a composite transposon consisting of two IS*Aba125* (Tn125) [45].

Conjugation assays have not allowed transfer of cefotaxime and imipenem resistance to *E. coli*; this may be related to chromosomal location of carbapenemase genes, instability of *Acinetobacter*-derived plasmids in *E. coli* or non self transferability of plasmids.

CarO outer membrane protein was described as contributing in the influx of carbapenems in *A. baumannii* and a reduced susceptibility to carbapenems can result from insertional inactivation of CarO [7]. In our study, the non detection of *car*O gene in four strains suggests its deletion, the same observation was already reported [46].

Concerning non-beta-lactam resistance genes, plasmid-borne quinolone resistance genes [*qnr* and *aac(6′)-Ib-cr*] were not detected in this study, in agreement with the rarity of PMQR determinants in *A. baumannii* [47,48]. The most frequently resistance mechanisms to fluoroquinolones are mutations in topoisomerases genes and efflux pumps such as AdeABC [2]. *aac(6′)-Ib* gene was found in five gentamicin- and amikacin-resistant strains from Beni-Messous hospital, it is commonly reported in *A. baumannii* [1]. In agreement in our study, the prevalence of trimethoprim/sulfamethoxazole resistance in *A. baumannii* is high in many geographic regions [4]. Although acquired-*dfr* genes were previously reported within integron structures [4,49], none of *dfr* gene clusters was detected in our strains. Trimethoprim resistance in *Acinetobacter* can be related to housekeeping *dfr* genes and to efflux systems [50,51]. Sulfonamides resistance genes *sul1* and *sul2* genes were highly present in our strains, they are commonly described in *Acinetobacter* and are predictive of the

presence of integrons [49,52]. *tetB* gene was highly prevalent among our tetracycline-resistant strains (76.54%) compared to *tetA*, as previously reported in *Acinetobacter* [53], *tetB* also confers resistance to minocycline [2]. The tetracycline resistance of *tet*-negative strains is probably due to other efflux pumps [53]. Integrons provide an additional means of mobility and expression to resistance genes, class 1 integrons were identified in 23.4% of imipenem-resistant isolates which were *sul1* positive. Class 1 integrons are very common in multidrug resistant *A. baumannii* [27,54] and most acquired MBL genes were located within these genetic elements [4,55].

ERIC-PCR analysis revealed globally a genetic diversity among OXA-23-like and OXA-24-like positive strains, multiple clones have coexisted in hospitals and care units indicating a polyclonal dissemination, which may be due to multiple reservoirs as fecal carriers and environmental sources, and to the diffusion of mobile genetic elements. Except the single strain from Beni Messous hospital, a clonal relatedness was observed for strains harboring NDM-1 gene from Pierre and Marie Curie Center and Zemirli hospital. This finding suggests clonal emergence of NDM-1-producing strains, maybe promoted by cross-transmission.

In conclusion, this study reported the high prevalence of multidrug resistant *A. baumannii* in Algiers hospitals, with imipenem resistance mediated by IS*Aba1*- and IS*Aba4*-associated $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$ and IS*Aba125*-associated $bla_{\text{NDM-1}}$. A genetic diversity was observed for OXA-23-like- and OXA-24-like-producing strains, while clonality has characterized the NDM-1 strains. These findings report a critical situation that requires compliance with the rules of hygiene, patient isolation, identification of germ sources, implementation of treatment strategies, and careful monitoring of antibiotic resistance evolution.

Conflict of interest statement

We declare that we have no conflicts of interest.

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References

[1] Bonnin RA, Nordmann P, Poirel L. Screening and deciphering antibiotic resistance in *Acinetobacter baumannii*: a state of the art. *Expert Rev Anti Infect Ther* 2013; **11**(6): 571-583.
[2] Gootz TD, Marra A. *Acinetobacter baumannii*: an emerging multidrug-resistant threat. *Expert Rev Anti Infect Ther* 2008; **6**(3): 309-325.
[3] Héritier C, Poirel L, Nordmann P. Cephalosporinase overexpression resulting from insertion of IS*Aba1* in *Acinetobacter baumannii*. *Clin Microbiol Infect* 2006; **12**(2): 123-130.
[4] Peleg AY, Seifert H, Paterson DL. *Acinetobacter baumannii*: emergence of a successful pathogen. *Clin Microbiol Rev* 2008; **21**(3): 538-582.
[5] Poirel L, Naas T, Nordmann P. Diversity, epidemiology, and genetics of class D beta-lactamases. *Antimicrobial Agents Chemother* 2010; **54**(1): 24-38.
[6] Dortet L, Poirel L, Nordmann P. Worldwide dissemination of the NDM-type carbapenemases in gram-negative bacteria. *Biomed Res Int* 2014; **2014**: 249856.
[7] Mussi MA, Limansky AS, Viale AM. Acquisition of resistance to carbapenems in multidrug-resistant clinical strains of *Acinetobacter baumannii*: natural insertional inactivation of a gene encoding a member of a novel family of beta-barrel outer membrane proteins. *Antimicrobial Agents Chemother* 2005; **49**(4): 1432-1440.
[8] Turton JF, Woodford N, Glover J, Yarde S, Kaufmann ME, Pitt TL. Identification of *Acinetobacter baumannii* by detection of the $bla_{\text{OXA-51-like}}$ carbapenemase gene intrinsic to this species. *J Clin Microbiol* 2006; **44**(8): 2974-2976.
[9] Antibiogramm Committee of French Society for Microbiology (CA-SFM). Communiqué 2013. [Online] Available at: www.sfm-microbiologie.org.
[10] Lee K, Chong Y, Shin HB, Kim YA, Yong D, Yum JH. Modified Hodge test and EDTA-disk synergy tests to screen metallo-β-lactamase-producing strains of *Pseudomonas* and *Acinetobacter* species. *Clin Microbiol Infect* 2001; **7**(2): 88-91.
[11] Jarlier V, Nicolas M, Fournier G, Philippon A. Extended broad-spectrum beta-lactamases conferring transferable resistance to newer beta-lactam agents in Enterobacteriaceae: hospital prevalence and susceptibility patterns. *Rev Infect Dis* 1988; **10**(4): 867-878.
[12] 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**(4): 351-353.
[13] Higgins PG, Lehmann M, Seifert H. Inclusion of OXA- 143 primers in a multiplex polymerase chain reaction (PCR) for genes encoding prevalent OXA carbapenemases in *Acinetobacter* spp. *Int J Antimicrob Agents* 2010; **35**: 305.
[14] Mendes RE, Kiyota KA, Monteiro J, Castanheira M, Andrade SS, Gales AC, et al. Rapid detection and identification of metallo-b-lactamase encoding genes by multiplex real-time PCR assay and melt curve analysis. *J Clin Microbiol* 2007; **45**(2): 544-577.
[15] Nordmann P, Poirel L, Carrër A, Toleman MA, Walsh TR. How to detect NDM-1 producers. *J Clin Microbiol* 2011; **49**(2): 718-721.
[16] Ruiz M, Marti S, Fernandez-Cuenca F, Pascual A, Vila J. Prevalence of IS(*Aba1*) in epidemiologically unrelated *Acinetobacter baumanii* clinical isolates. *FEMS Microbiol Lett* 2007; **274**(1): 63-66.
[17] Verdet C, Benzerar Y, Gautier V, Adam O, Ould-Hocine Z, Arlet G. Emergence of DHA-1-producing *Klebsiella* spp. in the parisian region: genetic organization of the *ampC* and *ampR* genes originating from *Morganella morganii*. *Antimicrobial Agents Chemother* 2006; **50**(2): 607-617.
[18] Messai Y, Iabadene H, Benhassine T, Alouache S, Tazir M, Gautier V, et al. Prevalence and characterization of extended-spectrum beta-lactamases in *Klebsiella pneumoniae* in Algiers hospitals (Algeria). *Pathol Biol* 2008; **56**(5): 319-325.
[19] Kim J, Park YJ, Kim SI, Kang MW, Lee SO, Lee KY. Nosocomial outbreak by *Proteus mirabilis* producing extended-spectrum β-lactamase VEB-1 in a Korean university hospital. *J Antimicrob Chemother* 2004; **54**(6): 1144-1147.
[20] Figueira V, Vaz-Moreira I, Silva M, Manaia CM. Diversity and antibiotic resistance of *Aeromonas* spp. in drinking and waste water treatment plants. *Water Res* 2011; **45**(17): 5599-5611.
[21] Guardabassi L, Dijkshoorn L, Collard JM, Olsen JE, Dalsgaard A. Distribution and *in-vitro* transfer of tetracycline resistance determinants in clinical and aquatic *Acinetobacter* strains. *J Med Microbiol* 2000; **49**(10): 929-936.
[22] Murinda SE, Ebner PD, Nguyen LT, Mathew AG, Oliver SP. Antimicrobial resistance and class 1 integrons in pathogenic *Escherichia coli* from dairy farms. *Foodborne Pathog Dis* 2005; **2**(4): 348-352.
[23] Frank T, Gautier V, Talarmin A, Bercion R, Arlet G. Characterization of sulphonamide resistance genes and class 1 integron gene cassettes in *Enterobacteriaceae*, Central African Republic (CAR). *J Antimicrob Chemother* 2007; **59**(4): 742-745.
[24] Šeputiene V, Povilonis J, Ruzauskas M, Pavilonis A, Suziedeliene E. Prevalence of trimethoprim resistance genes in *Escherichia coli* isolates of human and animal origin in Lithuania. *J Med Microbiol* 2010; **59**(3): 315-322.

[25] Corvec S, Poirel L, Naas T, Drugeon H, Nordmann P. Genetics and expression of the carbapenem-hydrolyzing oxacillinase gene bla_{OXA-23} in *Acinetobacter baumannii*. *Antimicrobial Agents Chemother* 2007; **51**(4): 1530-1533.
[26] Lopes BS, Amyes SG. Role of IS*Aba1* and IS*Aba125* in governing the expression of bla_{ADC} in clinically relevant *Acinetobacter baumannii* strains resistant to cephalosporins. *J Med Microbiol* 2012; **61**(8): 1103-1108.
[27] Valenzuela JK, Thomas L, Partridge SR, Van der Reijden T, Dijkshoorn L, Iredell J. Horizontal gene transfer in a polyclonal outbreak of carbapenem-resistant *Acinetobacter baumannii*. *J Clin Microbiol* 2007; **45**(2): 453-460.
[28] Decré D, Burghoffer B, Gautier V, Petit JC, Arlet G. [Outbreak of multi-resistant *Klebsiella oxytoca* involving strains with extended-spectrum beta-lactamases and strains with extended-spectrum activity of the chromosomal beta-lactamase]. *J Antimicrob Chemother* 2004; **54**(5): 881-888.
[29] Van Belkum A, Tassios PT, Dijkshoorn L, Haeggman S, Cookson B, Fry NK, et al. European Society of Clinical Microbiology and Infectious Diseases (ESCMID) Study Group on Epidemiological Markers (ESGEM). Guidelines for the validation and application of typing methods for use in bacterial epidemiology. *Clin Microbiol Infect* 2007; **13**(Suppl. 3): 1-46.
[30] Rahal K, Belouni R, Tali-Maamar H, Boudouane M, Missoum MFK, Benslimani A, et al. *13ème Rapport d'évaluation (Janvier à Décembre 2011): Surveillance de la résistance des bactéries aux antibiotiques*. Algérie: Ministère de la Santé; 2012.
[31] Mesli E, Berrazeg M, Drissi M, Bekkhoucha SN, Rolain JM. Prevalence of carbapenemase-encoding genes including New Delhi metallo-β-lactamase in *Acinetobacter* species, Algeria. *Int J Infect Dis* 2013; **17**(9): 739-743.
[32] Martins HS, Bomfim MR, França RO, Farias LM, Carvalho MA, Serufo JC, et al. Resistance markers and genetic diversity in *Acinetobacter baumannii* strains recovered from nosocomial bloodstream infections. *Int J Environ Res Public Health* 2014; **11**(2): 1465-1478.
[33] Nordmann P, Poirel L, Walsh TR, Livermore DM. The emerging NDM carbapenemases. *Trend Microbiol* 2011; **19**(12): 588-595.
[34] Mishra SK, Rijal BP, Pokhrel BM. Emerging threat of multidrug resistant bugs – *Acinetobacter calcoaceticus baumannii* complex and methicillin resistant *Staphylococcus aureus*. *BMC Res Notes* 2013; **6**: 98.
[35] Bakour S, Kempf M, Touati A, Ait Ameur A, Haouchine D, Sahli F, et al. Carbapenemase-producing *Acinetobacter baumannii* in two university hospitals in Algeria. *J Med Microbiol* 2012; **61**(9): 1341-1343.
[36] Hornsey M, Wareham DW. *In vivo* efficacy of glycopeptide-colistin combination therapies in a *Galleria mellonella* model of *Acinetobacter baumannii* infection. *Antimicrobial Agents Chemother* 2011; **55**(7): 3534-3537.
[37] Mugnier PD, Poirel L, Naas T, Nordmann P. Worldwide dissemination of the bla_{OXA-23} carbapenemase gene of *Acinetobacter baumannii*. *Emerg Infect Dis* 2010; **16**(1): 35-40.
[38] Touati M, Diene SM, Racherache A, Dekhil M, Djahoudi A, Rolain JM. 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* 2012; **40**(1): 89-91.
[39] Bakour S, Touati A, Bachiri T, Sahli F, Tiouit D, Naim M, et al. First report of 16S rRNA methylase ArmA-producing *Acinetobacter baumannii* and rapid spread of metallo-b-lactamase NDM-1 in Algerian hospitals. *J Infect Chemother* 2014; **20**(11): 696-701.
[40] Turton JF, Ward ME, Woodford N, Kaufmann ME, Pike R, Livermore DM, et al. The role of IS*Aba1* in expression of OXA carbapenemase genes in *Acinetobacter baumannii*. *FEMS Microbiol Lett* 2006; **258**(1): 72-77.
[41] Lopes BS, Al-Hassan L, Amyes SGB. IS*Aba825* controls the expression of the chromosomal bla_{OXA-51}-like and the plasmid borne bla_{OXA-58} gene in clinical isolates of *Acinetobacter baumannii* isolated from the USA. *Clin Microbiol Infect* 2012; **18**(11): 446-451.
[42] Walther-Rasmussen J, Høiby N. [OXA-type carbapenemases]. *J Antimicrob Chemother* 2006; **7**(3): 373-383.
[43] Pasteran F, Albornoz E, Faccone D, Gomez S, Valenzuela C, Morales M, et al. Emergence of NDM-1-producing *Klebsiella pneumoniae* in Guatemala. *J Antimicrob Chemother* 2012; **67**(7): 1795-1797.
[44] Pérez JAE, Olarte-Escobar NM, Castro-Cardozo B, Valderrama-Márquez IA, Garzón Aguilar MI, de la Barrera LM, et al. Outbreak of NDM-1-producing *Klebsiella pneumoniae* in a neonatal unit in Colombia. *Antimicrobial Agents Chemother* 2013; **58**(12): 1957-1960.
[45] Pfeifer Y, Wilharm G, Zander E, Wichelhaus TA, Göttig S, Hunfeld KP, et al. Molecular characterization of bla_{NDM-1} in an *Acinetobacter baumannii* strain isolated in Germany in 2007. *J Antimicrob Chemother* 2011; **66**(9): 1998-2001.
[46] Jiang MJ, Zhao SP, Li JM, Zhang FS. Molecular epidemiological study and detection of multi-drug resistant *Acinetobacter baumannii*-related resistance genes. *Afr J Microbiol Res* 2013; **7**(48): 5496-5502.
[47] Perez F, Hujer AM, Hujer KM, Decker BK, Rather PN, Bonomo RA. Global challenge of multidrug-resistant *Acinetobacter baumannii*. *Antimicrobial Agents Chemother* 2007; **51**(10): 3471-3484.
[48] Strahilevitz J, Jacoby GA, Hooper DC, Robicsek A. Plasmid-mediated quinolone resistance: a multifaceted threat. *Clin Microbiol Rev* 2009; **22**(4): 664-689.
[49] Taitt CR, Leski TA, Stockelman MG, Craft DW, Zurawski DV, Kirkup BC, et al. Antimicrobial resistance determinants in *Acinetobacter baumannii* isolates taken from military treatment facilities. *Antimicrobial Agents Chemother* 2014; **58**(2): 767-781.
[50] Su XZ, Chen J, Mizushima T, Kuroda T, Tsuchiya T. AbeM, an H-coupled *Acinetobacter baumannii* multidrug efflux pump belonging to the MATE family of transporters. *Antimicrobial Agents Chemother* 2005; **49**(10): 4362-4364.
[51] Mak JK, Kim MJ, Pham J, Tapsall J, White PA. Antibiotic resistance determinants in nosocomial strains of multidrug-resistant *Acinetobacter baumannii*. *J Antimicrob Chemother* 2009; **63**(1): 47-54.
[52] Dally S, Lemuth K, Kaase M, Rupp S, Knabbe C, Weile J. DNA microarray for genotyping antibiotic resistance determinants in *Acinetobacter baumannii* clinical isolates. *Antimicrobial Agents Chemother* 2013; **57**(10): 4761-4768.
[53] Coyne S, Courvalin P, Périchon B. Efflux-mediated antibiotic resistance in *Acinetobacter* spp. *Antimicrobial Agents Chemother* 2011; **55**(3): 947-953.
[54] Lin MF, Chang KC, Yang CY, Yang CM, Xiao CC, Kuo HY, et al. Role of integrons in antimicrobial susceptibility patterns of *Acinetobacter baumannii*. *Jpn J Infect Dis* 2010; **63**(6): 440-443.
[55] Zarrilli R, Pournaras S, Giannouli M, Tsakris A. Global evolution of multidrug-resistant *Acinetobacter baumannii* clonal lineages. *Int J Antimicrob Agents* 2013; **41**(1): 11-19.