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A Plasmid-Borne bla_{OXA-58} Gene Confers Imipenem Resistance to *Acinetobacter baumannii* Isolates from a Lebanese Hospital▽

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We investigated the basis of the carbapenem resistance of 17 multidrug-resistant *Acinetobacter baumannii* clinical isolates collected from 2004 to 2005 at the Saint George University Hospital in Beirut, Lebanon. *A. baumannii* isolates were clonally related and were susceptible to colistin and trimethoprim-sulfamethoxazole, susceptible or intermediate to ampicillin-sulbactam and meropenem, and resistant to all other antimicrobials. Conjugation experiments demonstrated that resistance to imipenem could be transferred along with a plasmid containing the carbapenem-hydrolyzing oxacillinase bla_{OXA-58} gene. The plasmid that we called pABIR was 29,823 bp in size and showed a novel mosaic structure composed of two origins of replication, four insertion sequence (IS) elements, and 28 open reading frames. The bla_{OXA-58} gene was flanked by IS*18* and IS*Aba3* elements at the 5′ and 3′ ends, respectively. The production of the carbapenem-hydrolyzing oxacillinase OXA-58 was apparently the only mechanism for carbapenem resistance in *A. baumannii* isolates causing the outbreak at the Lebanese Hospital.

Acinetobacter baumannii is an important opportunistic pathogen responsible for a variety of nosocomial infections, especially in intensive care unit (ICU) patients (11, 18). These organisms are frequently resistant to multiple antimicrobial agents including broad-spectrum β-lactams, carbapenems, aminoglycosides, and fluoroquinolones (11, 18, 31). *A. baumannii* may develop resistance to carbapenems through various mechanisms including decreased permeability because of porin modifications or reduced expression, the overexpression of efflux pumps, and the production of carbapenemases, such as metallo-β-lactamase or carbapenem-hydrolyzing oxacillinases (CHDLs) (3, 17–24).

The emergence of carbapenem resistance in *A. baumannii* has been reported worldwide (11, 18) and has been correlated in Europe with the acquisition of CHDLs (10, 18–23). Three main acquired CHDL gene clusters have been identified in *A. baumannii*, represented by the bla_{OXA-23}-, bla_{OXA-24}-, and bla_{OXA-58}-like genes (13). Plasmid-borne bla_{OXA-23} and bla_{OXA-58} genes have been shown to contribute significantly to carbapenem resistance in *A. baumannii* (3, 10, 13, 18, 21, 22). In particular, the bla_{OXA-58} gene has been identified in carbapenem-resistant *A. baumannii* isolates worldwide (3, 13, 20, 23, 30). Recent studies have shown that the flanking insertion sequence (IS) elements IS*Aba1*, IS*Aba2*, IS*Aba3*, and IS*18* regulate bla_{OXA-58} gene expression (22), and IS*Aba3* possibly regulates its acquisition (19).

An outbreak of multidrug-resistant *Acinetobacter baumannii* was observed between November 2004 and October 2005 in the Saint George University Hospital of Beirut, Beirut, Lebanon. The aim of the present study was to (i) assess the genetic relatedness and the antimicrobial susceptibility of *A. baumannii* isolates in the hospital, (ii) study the horizontal gene transfer of the carbapenem resistance of the *A. baumannii* isolates, and (iii) analyze plasmid DNA sequences involved in the acquisition of carbapenem resistance of the *A. baumannii* isolates.

MATERIALS AND METHODS

Microbiological methods. *A. baumannii* isolates were identified as being *A. baumannii* spp. by using the Vitek 2 automatic system with an ID-GNB card for the identification of gram-negative bacilli according to the manufacturer's instructions (bioMerieux, Marcy-l'Etoile, France). Species identification was confirmed by PCR amplification and sequence analysis of the 16S-23S rRNA intergenic spacer region (6).

Antimicrobial susceptibility testing. MICs were determined by a microdilution method according to Clinical and Laboratory Standards Institute (CLSI) document M7-A6 (8). Breakpoint values were those recommended by the CLSI (8). Breakpoints for colistin were those from the British Society for Antimicrobial Chemotherapy (4). Etest MBL strips (AB Biodisk, Solna, Sweden) were used to evaluate the presence of metallo-beta-lactamase (MBL) activity according to the manufacturer's procedures (16). *Pseudomonas aeruginosa* ATCC 27853 was used as an MBL-negative reference strain, and *A. baumannii* AC-54/97 producing IMP-2 MBL (24) was used as the MBL-positive reference strain. The relative contribution of oxacillinases to carbapenem resistance was assessed by analyzing carbapenem MICs with and without 200 mM of NaCl (23) through a liquid microdilution method. *A. baumannii* isolates of pulsed-field gel electrophoresis (PFGE) type 1 carrying bla_{OXA-58} (30) and one sporadic *A. baumannii* isolate of PFGE type 2 negative for bla_{OXA-58} (30) were used as CHDL-positive and CHDL-negative reference strains, respectively. The contribution of AmpC beta-lactamase was tested by determining carbapenem MICs with and without 200 mg/liter of cloxacillin (23) through a liquid microdilution method.

PFGE analysis and sequencing typing. DNA macrorestriction of *A. baumannii* isolates, PFGE, and dendrogram analysis were performed as previously reported (30). Sequencing typing (ST) was performed as described previously (27).

Mating experiments. Filter mating was performed using *A. baumannii* isolates Ab 1 or Ab 8 of PFGE type A, resistant to imipenem and susceptible to

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trimethoprim-sulfamethoxazole, and Ab F isolate of PFGE type B, susceptible to imipenem while resistant to trimethoprim-sulfamethoxazole, as donor and recipient cells, respectively. Transconjugants were selected on brain heart infusion agar plates containing imipenem (16 mg/liter) plus trimethoprim-sulfamethoxazole (250 mg/liter). The frequency of transfer was calculated as the number of transconjugants divided by the number of surviving recipients.

Plasmid DNA characterization and PCR analysis. Plasmid DNA preparations were performed by using the QIAfilter Plasmid Purification Maxi kit adapted for low-copy-number plasmids (Qiagen Corporation, Milan, Italy) according to the manufacturer's procedure. Southern hybridization of plasmid profiles was performed as described previously by Sambrook et al. (25). PCR analysis for carbapenemase-encoding genes in *Acinetobacter* spp. (bla_{IMP}, bla_{VIM}, bla_{SIM}, bla_{OXA-23}-like, bla_{OXA-24}-like, bla_{OXA-51}-like, and bla_{OXA-58}) was performed as previously described (30). PCR characterization of the bla_{OXA-58}-surrounding IS was performed as described previously (22). The colinearity between IS elements and the bla_{OXA-69} gene was analyzed using primers for IS elements described previously by Poirel and Nordmann (22) and for the bla_{OXA-51}-like gene described previously by Turton et al. (28). PCR amplification of the complete *carO* gene was performed as described previously (17).

Outer membrane protein analysis. Outer membrane protein fractions were prepared by sonication and solubilization in 2% sodium lauroyl sarcosinate and analyzed by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis under reducing conditions as previously described (14). N-terminal sequence analysis by automated Edman degradation and protein analysis by matrix-assisted laser desorption-ionization mass spectrometry or liquid chromatography online tandem mass spectrometry were performed as previously described (5, 9).

Plasmid DNA sequencing and computer analysis of sequencing data. A "walking primer" approach starting with primers derived from the 5′ and 3′ ends of the bla_{OXA-58} gene was adopted to obtain the complete DNA sequence of the plasmids. Direct sequencing of Qiagen-purified plasmid DNA was performed using the ABI Prism BigDye Terminator v3.1 Ready Reaction cycle sequencing kit and the 3730 DNA analyzer (Applied Biosystems, Foster City, CA). DNA sequences were assembled using the program Autoassembler, version 1.4 (Applied Biosystems, Foster City, CA), and annotated using the BLAST program (1) and the sequence annotation tools integrated into the Sequin program, version 7.9 (available at http://www.ncbi.nlm.nih.gov/Sequin/index.html). The graphic view of plasmid DNA sequences was generated using the program VectorNti, version 10 (Invitrogen Corporation, Carlsbad, CA).

Nucleotide sequence accession numbers. The nucleotide sequences of *A. baumannii* plasmid pABIR from *A. baumannii* Ab1:AbF transconjugant 1 and of *carO* genes from *A. baumannii* isolates Ab 1 and Ab F have been deposited in the GenBank nucleotide database under accession numbers EU294228, DQ642020, and DQ642021, respectively. The annotation of plasmid pABIR performed by the National Center for Biotechnology Information is also available in the GenBank genome database under accession number NC_010481.

RESULTS AND DISCUSSION

Molecular epidemiology of *A. baumannii* in the hospital. The molecular epidemiology of a clonal outbreak of carbapenem-resistant *A. baumannii* infection was studied in Saint George University Hospital, Beirut, Lebanon, from November 2004 to October 2005. In the previous 12 months, only sporadic *A. baumannii* strains that were susceptible to carbapenems were isolated in the hospital. From November 2004 to October 2005, *A. baumannii* was isolated from 17 patients: 11 from the medical-surgical ICU and 6 from other wards. Ten patients had ventilator-associated pneumonia, three had wounds or abscesses, one had hospital-acquired pneumonia, one had pleural effusion, one had bacteremia, and one had urinary tract infection. Crude mortality was 35% (6/17).

Molecular typing by PFGE and dendrogram analysis identified one major PFGE pattern in all 17 *A. baumannii* isolates during the outbreak and one additional PFGE pattern in one sporadic isolate from the ICU of the hospital 6 months ahead that differed in the migration of more than six bands and exhibited <70% similarity, which we named A and B, respectively. Of the 17 outbreak *A. baumannii* isolates, 12 showed an identical macrorestriction pattern, which we named pattern A, and 5 showed two- to three-fragment variations in the macrorestriction pattern with a similarity of more than 80% by dendrogram analysis and were classified into three subtypes, A1 to A3. ST analysis assigned the 17 outbreak isolates of PFGE type A to ST group 2 and the sporadic isolate of PFGE type B to ST group 3. During the epidemic, *A. baumannii* isolates of PFGE type A were also obtained from one humidifier and one sink of two rooms of the ICU ward. Data indicate that the *A. baumannii* outbreak in the hospital was caused by the spread of a single epidemic clone.

TABLE 1. Antibiotic susceptibility profile of *A. baumannii* outbreak isolates[a]

Antibiotic	MIC_{50} (mg/liter)	MIC_{90} (mg/liter)	MIC range (mg/liter)
Sulbactam-ampicillin	8	16	8–16
Piperacillin-tazobactam	125	250	125–>250
Ceftazidime	250	250	125–>250
Cefepime	16	32	16–32
Imipenem	16	16	8–16
Meropenem	8	8	4–8
Amikacin	64	125	64–125
Gentamicin	125	250	125–>250
Ciprofloxacin	64	250	32–>250
Trimethoprim-sulfamethoxazole	0.5	0.5	0.5–1
Colistin	2	4	1–4

[a] *A. baumannii* isolates of PFGE type A were analyzed by a microdilution method for MIC determination according to CSLI guidelines.

Antimicrobial susceptibility patterns of *A. baumannii* isolates. All *A. baumannii* isolates of PFGE type A showed an identical multiresistant antibiotype. In particular, they were resistant or intermediate to imipenem, susceptible or intermediate to meropenem and ampicillin-sulbactam, and susceptible to colistin and trimethoprim-sulfamethoxazole (Table 1). In contrast, the sporadic isolate of PFGE type B was susceptible to imipenem and meropenem and resistant to trimethoprim-sulfamethoxazole.

To study the mechanism of carbapenem resistance, the MIC of imipenem and the presence of MBL activity were evaluated for all *A. baumannii* isolates of PFGE type A by using Etest MBL strips. All isolates were resistant or intermediate to imipenem (MICs from 8 to 16 mg/liter) but negative for MBL production (imipenem-EDTA MIC, 8 to 4 mg/liter). To further characterize the carbapenem resistance, we studied the relative contribution of oxacillinases and AmpC beta-lactamase to imipenem resistance by analyzing imipenem MICs in the presence of 200 mM NaCl or 200 mg/liter cloxacillin for *A. baumannii* isolates of PFGE type A through a microdilution method. These experiments showed that imipenem MICs (16 mg/liter) were inhibited by up to eightfold in the presence of NaCl (2 mg/liter) but not in the presence of cloxacillin. No changes in ceftazidime MICs (250 mg/liter) were observed in the presence of NaCl or cloxacillin. The above-described data suggested that oxacillinase activity, but not AmpC activity, contributed to imipenem resistance in epidemic *A. baumannii* isolates.

Molecular analysis of carbapenem resistance in *A. baumannii* isolates. PCR and sequence analysis identified a bla_{OXA-58} gene in DNA from all imipenem-resistant *A. baumannii* iso-

lates of PFGE type A but not from the imipenem-susceptible isolate of PFGE type B. No amplification products were obtained using primers for bla_{IMP}-type, bla_{VIM}-type, or bla_{SIM}-type MBLs or bla_{OXA-23} or bla_{OXA-24} CHDLs. This suggested that OXA-58 was the oxacillinase contributing to carbapenem resistance. Also, PCR experiments failed to identify any IS element upstream of the naturally occurring bla_{OXA-69} gene in imipenem-resistant *A. baumannii* isolates of PFGE type A, thus excluding that IS-mediated overexpression of this oxacillinase may account for the resistance to imipenem (28).

Molecular analysis of the outer membrane protein profile of *A. baumannii* isolates. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis showed an outer membrane profile composed of two major protein bands with apparent molecular masses of 24 and 37 kDa in the imipenem-resistant *A. baumannii* isolates Ab 1 and Ab 8 and 27 and 37 kDa in the imipenem-susceptible *A. baumannii* isolate Ab F. Automated Edman degradation analysis of the 24- and 27-kDa proteins from the imipenem-resistant and imipenem-susceptible isolates showed identical N-terminal sequences that corresponded to those of the outer membrane protein CarO, whose loss has been associated with carbapenem resistance (17). Mass spectrometry analysis of the CarO protein from imipenem-resistant isolate Ab 1 identified the peptides expected from the hydrolysis of the deduced amino acid sequence that was identical to the deduced amino acid sequence reported previously by Mussi et al. (GenBank accession number AY684798) (17) except for a glutamine replacing the lysine at residue 197. This demonstrated that the full-length CarO protein was expressed in imipenem-resistant isolate Ab 1 and ruled out that the apparent lower molecular weight of the CarO protein from imipenem-resistant isolates was caused by a truncation at the C terminus. Therefore, data excluded that carbapenem resistance in epidemic *A. baumannii* isolates was contributed by modifications in the outer membrane protein profile.

Conjugative transfer of imipenem resistance. To further study the mechanism responsible for imipenem resistance, we asked whether imipenem resistance might have been transferred through conjugation. Filter-mating experiments demonstrated that resistance to imipenem was transferred from isolates Ab 1 and Ab 8 of PFGE type A and ST group 2 to imipenem-susceptible isolate Ab F of PFGE type B and ST group 3 at a frequency ranging from 2×10^{-5} to 1.5×10^{6}. All the transconjugants showed the PFGE profile and the ST group of the recipient isolate Ab F (data not shown). The antimicrobial susceptibility profile of the transconjugants was identical to that of the recipient isolate Ab F with the exclusion of imipenem and meropenem, being susceptible to colistin, intermediate to ampicillin-sulbactam and meropenem, and resistant to all other antimicrobials including imipenem. Imipenem MICs for transconjugants were similar (16 mg/liter) to those for donor isolates and were inhibited by up to eightfold in the presence of 200 mM NaCl, thus suggesting that imipenem resistance was contributed by oxacillinase activity in the transconjugants also.

Both imipenem-resistant *A. baumannii* isolate Ab 1 and the Ab 1:Ab F transconjugant were shown to carry a single plasmid molecule that migrated faster than an approximately 80-kb plasmid extracted from a *Klebsiella pneumoniae-Escherichia coli* transconjugant (2) and that hybridized with a PCR-generated probe for bla_{OXA-58}; in contrast, no plasmid DNA was isolated from imipenem-susceptible *A. baumannii* isolate Ab F (Fig. 1).

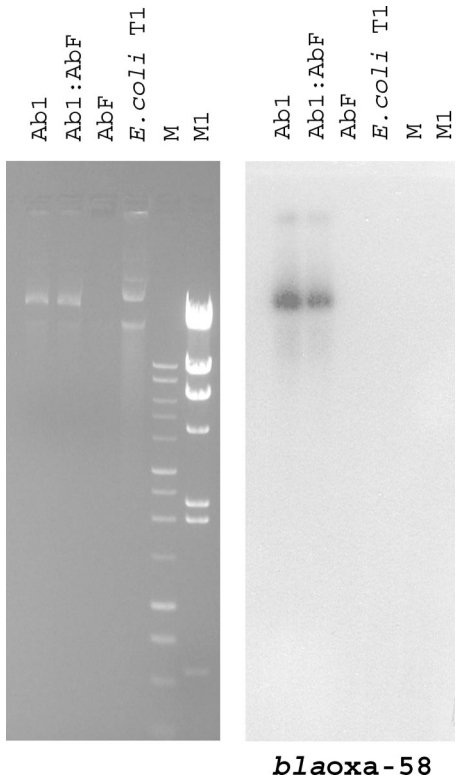

FIG. 1. Plasmid profiles and identification of the plasmid carrying the bla_{OXA-58} gene in *A. baumannii* isolates. Agarose (0.8%) gel electrophoresis in 1× Tris-acetate-EDTA buffer of plasmid preparations from *A. baumannii* isolates Ab 1, Ab 1:Ab F, and Ab F and from a *K. pneumoniae-E. coli* transconjugant (*E. coli* T1) stained with ethidium bromide and visualized under UV light and Southern blot hybridization with the bla_{OXA-58} probe are shown. M is a 1-kb DNA ladder (Promega, Milan, Italy), and M1 is HindIII-digested lambda DNA (Invitrogen, Milan, Italy).

Genetic structure of plasmid pABIR. The direct sequence of plasmid preparations from isolate Ab 1 and one Ab1:AbF transconjugant identified an identical plasmid that was designated pABIR, for *Acinetobacter baumannii* imipenem resistance. Plasmid pABIR was 29,823 bp in size, with a G+C content of 36.8%. The analysis of pABIR identified 28 open reading frames (ORFs). Of these ORFs, 22 were transcribed in a clockwise orientation, while the remaining 6 were transcribed counterclockwise. The main features of pABIR sequences and the regions of similarity with other known sequences are depicted in Fig. 2. Table 2 lists the putative functions, the characteristics, and the closest relatives for the predicted product of each ORF. The origin of replication (*oriV*), a repeat region composed of four imperfect direct iterons, and the *repA* gene, coding for RepAB replicase, were located at the beginning of the pABIR sequences and were homologous with those found in the *A. baumannii* plasmid pAB02 replicon, the partial sequence for which is available (GenBank accession number AY2284790). An additional origin of replication, five direct-

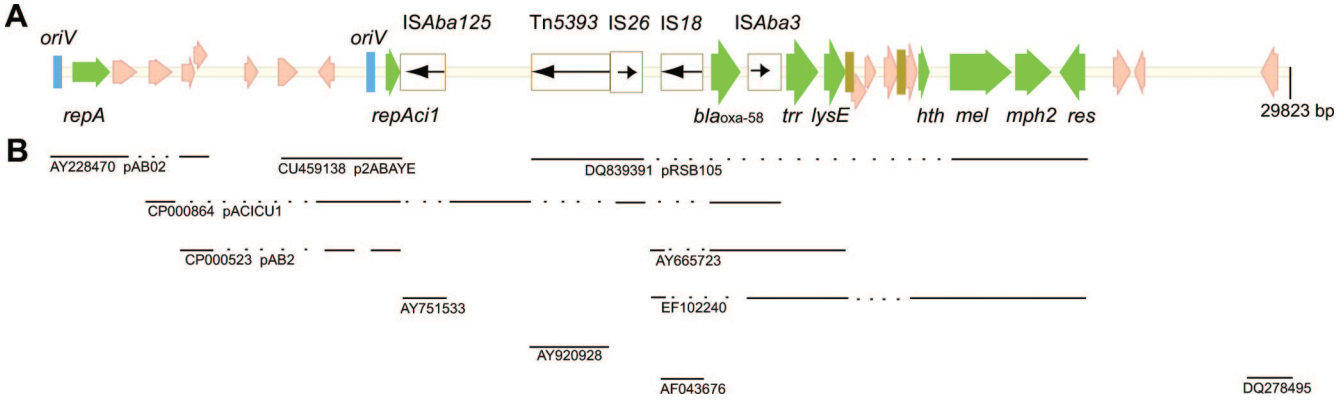

FIG. 2. Schematic map of plasmid pABIR. (A) Linear map of pABIR with relevant features. ORFs are represented by arrow-shaped boxes. IS elements are represented by empty rectangle boxes filled with black arrows indicating the transposase gene and the direction of the transcription. Repeat regions are indicated by vertical bars. Names of various features are reported below or above the map. (B) Regions of identity or of high similarity with other sequences reported in the GenBank/EMBL database are indicated by continuous lines.

repeat iterons, and a gene coding for a DNA replicase, homologous to the *repAci1* gene identified in *A. baumannii* plasmid pACICU1 (15), were also found in plasmid pABIR, where the coding sequences of the *repAci1* gene were truncated by the insertion of an IS*Ab125* element. The additional replicon of pABIR showed high homology with those found in three other *A. baumannii* plasmids, pACICU1, a 28-kb plasmid containing the bla_{OXA-58} gene isolated from *A. baumannii* strain ACICU, the sequence of which has been recently reported (15); pAB2, an 11-kb plasmid from *A. baumannii* ATCC 17978 (CP000523); and p2ABAYE, an approximately 10-kb plasmid isolated from multidrug-resistant *A. baumannii* strain AYE (29) (Fig. 2 and Table 2). The presence of two replicons suggests that pABIR is a mosaic plasmid originating from the fusion of two separate molecules. Similarly, two replicons have also been found in plasmid pACICU1 from *A. baumannii* strain ACICU (15) and in the erythromycin resistance plasmid pRSB105 isolated from activated sludge bacteria, where they have been postulated to represent a cointegrate of two formerly separate replicons (26).

pABIR contained a single copy of the bla_{OXA-58} gene that was flanked by IS*18* and IS*Aba3* elements at the 5′ and 3′ ends, respectively, as previously described for *A. baumannii* isolate CH29 (22). Two additional ISs, Tn*5393* and IS*26*, flanked IS*18* in pABIR. IS*26* was identical to the element found in plasmid pACICU1 from *A. baumannii* strain ACICU (15) but was inserted in the opposite orientation with respect to the bla_{OXA-58} gene. In accordance with our data, a single copy of the bla_{OXA-58} gene in plasmid pOUR from *A. baumannii* strain 183 showing an imipenem MIC of 16 mg/liter was described previously (3), while two copies of the bla_{OXA-58} gene were found in plasmid pACICU1 (15), and three copies of the gene were found in plasmids from *A. baumannii* isolates showing higher MICs (3). Downstream of the IS*Aba3* element, the *trr* and *lysE* genes and two sequences similar to those defined as the Re27-1 and Re27-2 structures in *A. baumannii* pMAD and hypothesized to be involved in homologous recombination processes (22) were identified. A genetic region containing genes involved in macrolide resistance and the *res* gene encoding a resolvase site-specific recombinase was identified and was identical to that found on an *A. baumannii* plasmid carrying the bla_{OXA-97} carbapenemase gene (GenBank accession number EF102240) (20) and on plasmid pRSB105, isolated from a sewage treatment plant (26).

No genes coding for the conjugative apparatus, the secretion system, or any mobilization protein were found in plasmid pABIR, thus suggesting that the plasmid is not self-conjugative and that the genes that mediate the transfer have been provided in *trans*. Although the genetic structure of the bla_{OXA-58} gene and of IS flanking regions of several plasmids have been characterized (3, 22, 26), only two complete plasmids carrying the bla_{OXA-58} gene have been described so far (7, 15). One of these, pTVICU53, is an 11-kb plasmid that contains a predicted origin-of-transfer DNA region and a gene coding for a mobilization protein (7) and should be transconjugated through a helper plasmid, as previously demonstrated for its homologous plasmid pMAC (12). The other, pACICU1, is a 28-kb plasmid that does not contain sequences for conjugation or mobilization but has been postulated to be mobilized in *trans* by a complete *tra* locus, encoding a conjugative apparatus and type IV secretion system, carried by plasmid pACICU2, of 64 kb, which coresides in bacteria with pACICU1 (15). The presence of a single plasmid molecule in donor cells of the isolates described herein led us to exclude that *trans*-mobilization occurred through a conjugation process promoted by another plasmid coresident within the same cell. *trans*-Mobilization mediated by chromosomally located transfer systems can also be hypothesized. In further support of this, the loci responsible for the conjugative transfer have been reported to have a chromosomal location in multidrug-resistant *A. baumannii* strain AYE, which belongs to ST group 2 as *A. baumannii* donor cells of the conjugation experiments described herein (29).

In conclusion, the acquisition of resistance to carbapenems in *A. baumannii* from the Lebanese hospital was caused by the spread of plasmid pABIR, carrying the bla_{OXA-58} gene. The mosaic genetic structure of pABIR might have been generated by multiple recombination events mediated by IS elements.

TABLE 2. Genetic regions and ORFs of plasmid pABIR[a]

Feature	Position	Gene product (no. of amino acids)	Properties and/or putative function	Homology (GenBank/EMBL accession no. match)
oriV	1–200		Origin of DNA replication	pAB02 (AY228470)
Repeat region	217–312		Imperfect 4-repeat iterons; control of DNA replication	
repA	357–1244	295	RepA AB; replicase	pAB02 (AY228470)
ORF	1321–1884	188	Unknown	
ORF	2179–2721	180	Hypothetical protein similar to COG 0790 FOG; TPR repeat, SEL1 subfamily	pMAC (AY541806), pACICU1 (CP000864)
ORF	2964–3260	98	Putative inner membrane protein	pAB02 (AY228470), pAB2 (CP000523)
ORF	3247–3561	104	Putative cytoplasmic protein	pAB02 (AY228470), pAB2 (CP000523)
ORF	4455–4766	103	Unknown	
ORF	5235–5714	153	Unknown	p2ABAYE (CU459138)
ORF	6210–6581	123	Unknown	pACICU1 (CP000864), p2ABAYE (CU459138), pAB2 (CP000523)
oriV	7450–7650		Origin of DNA replication	
Repeat region	7651–7760		5-repeat iterons; control of DNA replication	
repAci1	7815–8150	112	Plasmid replication protein; truncated by IS*Ab125*	
	9238–9855	205		
IS*Ab125*	8160–9230	322	IS*Ab125*/IS*Ab125* transposase	IS*Aba125* (AY751533)
Tn*5393*	11279–13148	611	Tn*5393* transposase disrupted by IS*26* insertion	*Salmonella enterica* serovar Paratyphi A (AM412236); uncultured bacterium pRSB105 (DQ839391)
IS*26*	13329–13916	195	IS*26* transposase	pACICU1 (CP000864), uncultured bacterium pRSB105 (DQ839391)
IS*18*	14349–15422	320	IS*18*/IS*18* transposase	*Acinetobacter* sp. strain BM2716 (AF043676)
bla_{OXA-58}	15553–16395	280	Carbapenem-hydrolyzing oxacillinase	AY665723, pACICU1 (CP000864), EF102240
IS*Aba3*	16433–17222	145	IS element IS*Aba3*/IS*Aba3* transposase	AY665723, pACICU1 (CP000864), EF102240
trr	17366–18193	275	Arac1 binding protein; arabinose operon control protein; transcriptional regulator	AY665723, EF102240
lysE	18243–18848	201	Threonine efflux protein	AY665723, EF102240
Recombination point	18843–18869		Repeat region 1/Re27-1	AY665723, EF102240
ORF	18914–19243	109	Unknown	
ORF	19244–19525	93	Putative transcriptional regulator	*Geobacter metallireducens* GS-15 (CP000148) (73% identity)
ORF	19605–20030	141	Unknown	
Recombination point	20062–20088		Repeat region 2/Re27-2	AY665723, EF102240
ORF	20164–20484	106	Unknown	EF102240
ORF	20477–20749	90	Helix-turn-helix XRE family cds	EF102240
mel	21242–22717	491	Macrolide efflux protein	EF102240, uncultured bacterium pRSB105 (DQ839391)
mph2	22773–23657	294	Macrolide 2′-phosphotransferase	
Resolvase	23847–24449	200	Resolvase	
ORF	25126–25533	135	Unknown	
ORF	25629–25877	82	Unknown	
ORF	28643–29032	129	Unknown	*Acinetobacter venetianus* pAV1 (DQ278485)

[a] Sequences in the GenBank/EMBL database showing identity or high similarity are indicated. Microorganisms from which DNA sequences have been isolated are indicated when different from *A. baumannii*. COG, conserved domain in bacteria; cds, coding sequences.

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