

## A Plasmid-Borne *bla*<sub>OXA-58</sub> Gene Confers Imipenem Resistance to *Acinetobacter baumannii* Isolates from a Lebanese Hospital<sup>∇</sup>

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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*<sub>OXA-58</sub> 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*<sub>OXA-58</sub> gene was flanked by IS18 and ISAb3 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  $\beta$ -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- $\beta$ -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*<sub>OXA-23</sub>, *bla*<sub>OXA-24</sub>, and *bla*<sub>OXA-58</sub>-like genes (13). Plasmid-borne *bla*<sub>OXA-23</sub> and *bla*<sub>OXA-58</sub> genes have been shown to contribute significantly to carbapenem resistance in *A. baumannii* (3, 10, 13, 18, 21, 22). In particular, the *bla*<sub>OXA-58</sub> 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 ISAb1, ISAb2, ISAb3, and IS18 regulate *bla*<sub>OXA-58</sub> gene expression (22), and ISAb3 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 (bioMérieux, Marcy-l'Étoile, 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*<sub>OXA-58</sub> (30) and one sporadic *A. baumannii* isolate of PFGE type 2 negative for *bla*<sub>OXA-58</sub> (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*<sub>IMP</sub>, *bla*<sub>VIM</sub>, *bla*<sub>SIM</sub>, *bla*<sub>OXA-23</sub>-like, *bla*<sub>OXA-24</sub>-like, *bla*<sub>OXA-51</sub>-like, and *bla*<sub>OXA-58</sub>) was performed as previously described (30). PCR characterization of the *bla*<sub>OXA-58</sub>-surrounding IS was performed as described previously (22). The colinearity between IS elements and the *bla*<sub>OXA-69</sub> gene was analyzed using primers for IS elements described previously by Poirel and Nordmann (22) and for the *bla*<sub>OXA-51</sub>-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*<sub>OXA-58</sub> 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

TABLE 1. Antibiotic susceptibility profile of *A. baumannii* outbreak isolates<sup>a</sup>

Antibiotic	MIC <sub>50</sub> (mg/liter)	MIC <sub>90</sub> (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

<sup>a</sup> *A. baumannii* isolates of PFGE type A were analyzed by a microdilution method for MIC determination according to CSLI guidelines.

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.

**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*<sub>OXA-58</sub> 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*<sub>IMP</sub>-type, *bla*<sub>VIM</sub>-type, or *bla*<sub>SIM</sub>-type MBLs or *bla*<sub>OXA-23</sub> or *bla*<sub>OXA-24</sub> 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*<sub>OXA-69</sub> 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  $2 \times 10^{-5}$  to  $1.5 \times 10^6$ . 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*

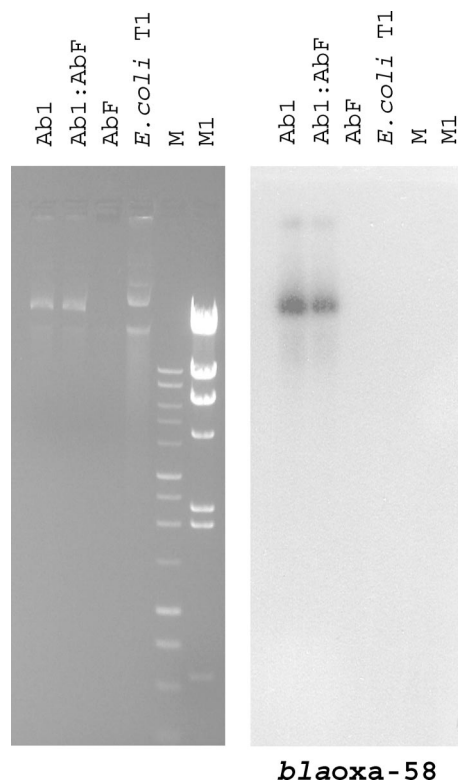


FIG. 1. Plasmid profiles and identification of the plasmid carrying the *bla*<sub>OXA-58</sub> gene in *A. baumannii* isolates. Agarose (0.8%) gel electrophoresis in  $1 \times$  Tris-acetate-EDTA buffer of plasmid preparations from *A. baumannii* isolates Ab 1, Ab 1:Ab F, and Ab F and from a *K. pneumoniae*-*E.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

*coli* transconjugant (2) and that hybridized with a PCR-generated probe for *bla*<sub>OXA-58</sub>; in contrast, no plasmid DNA was isolated from imipenem-susceptible *A. baumannii* isolate Ab F (Fig. 1).

**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-

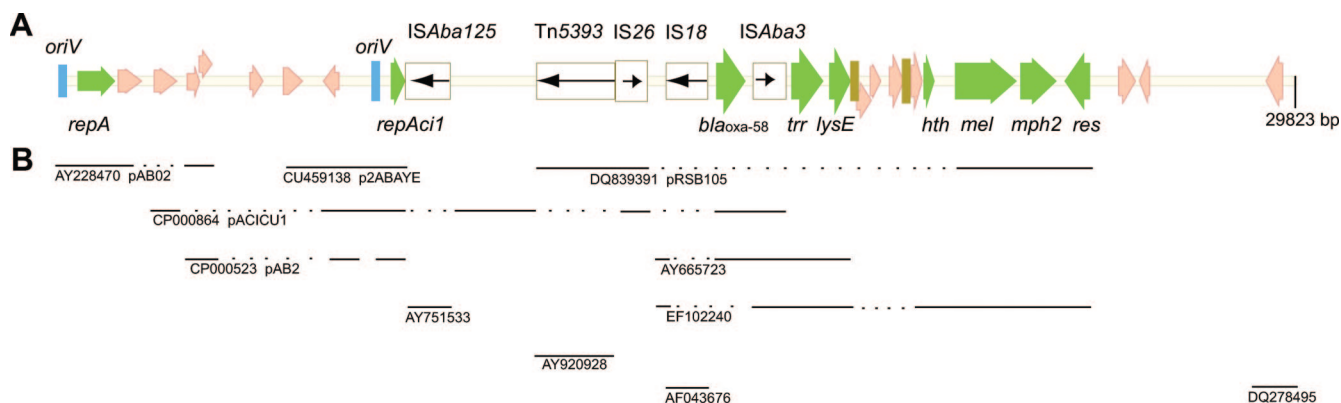


FIG. 2. Schematic map of plasmid pABIR. (A) Linear map of pABIR with relevant features. ORFs are represented by arrow-shaped boxes. IS elements are represented by empty rectangle boxes filled with black arrows indicating the transposase gene and the direction of the transcription. Repeat regions are 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 *ISAb125* 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*<sub>OXA-58</sub> 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*<sub>OXA-58</sub> gene that was flanked by *IS18* and *ISAbA3* elements at the 5' and 3' ends, respectively, as previously described for *A. baumannii* isolate CH29 (22). Two additional ISs, *Tn5393* and *IS26*, flanked *IS18* in pABIR. *IS26* 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*<sub>OXA-58</sub> gene. In accordance with our data, a single copy of the *bla*<sub>OXA-58</sub> 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*<sub>OXA-58</sub> 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 *ISAbA3* 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*<sub>OXA-97</sub> 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*<sub>OXA-58</sub> gene and of IS flanking regions of several plasmids have been characterized (3, 22, 26), only two complete plasmids carrying the *bla*<sub>OXA-58</sub> 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*<sub>OXA-58</sub> 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<sup>a</sup>

Feature	Position	Gene product (no. of amino acids)	Properties and/or putative function	Homology (GenBank/EMBL accession no. match)
<i>oriV</i>	1–200		Origin of DNA replication	pAB02 (AY228470)
Repeat region	217–312		Imperfect 4-repeat iterons; control of DNA replication	
<i>repA</i>	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)
<i>oriV</i>	7450–7650		Origin of DNA replication	
Repeat region	7651–7760		5-repeat iterons; control of DNA replication	
<i>repAci1</i>	7815–8150	112	Plasmid replication protein;	
	9238–9855	205	truncated by <i>ISAb125</i>	
<i>ISAb125</i>	8160–9230	322	<i>ISAb125/ISAb125</i> transposase	<i>ISAb125</i> (AY751533)
<i>Tn5393</i>	11279–13148	611	<i>Tn5393</i> transposase disrupted by <i>IS26</i> insertion	<i>Salmonella enterica</i> serovar Paratyphi A (AM412236); uncultured bacterium pRSB105 (DQ839391)
<i>IS26</i>	13329–13916	195	<i>IS26</i> transposase	pACICU1 (CP000864), uncultured bacterium pRSB105 (DQ839391)
<i>IS18</i>	14349–15422	320	<i>IS18/IS18</i> transposase	<i>Acinetobacter</i> sp. strain BM2716 (AF043676)
<i>bla</i> <sub>OXA-58</sub>	15553–16395	280	Carbapenem-hydrolyzing oxacillinase	AY665723, pACICU1 (CP000864), EF102240
<i>ISAb3</i>	16433–17222	145	IS element <i>ISAb3/ISAb3</i> transposase	AY665723, pACICU1 (CP000864), EF102240
<i>trr</i>	17366–18193	275	Aracl binding protein; arabinose operon control protein; transcriptional regulator	AY665723, EF102240
<i>lysE</i>	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	<i>Geobacter metallireducens</i> 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
<i>mel</i>	21242–22717	491	Macrolide efflux protein	EF102240, uncultured bacterium pRSB105 (DQ839391)
<i>mph2</i>	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	<i>Acinetobacter venetianus</i> pAV1 (DQ278485)

<sup>a</sup> 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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## REFERENCES

- Altschul, S. F., T. L. Madden, A. A. Schaffer, J. Zhang, Z. Zhang, W. Miller, and D. J. Lipman. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* **25**:3389–3402.
- Bagattini, M., V. Crivaro, A. Di Popolo, F. Gentile, A. Scarcella, M. Triassi, P. Villari, and R. Zarrilli. 2006. Molecular epidemiology of extended-spectrum  $\beta$ -lactamase-producing *Klebsiella pneumoniae* in a neonatal intensive care unit. *J. Antimicrob. Chemother.* **57**:979–982.
- Bertini, A., L. Poirel, S. Bernabeu, D. Fortini, L. Villa, P. Nordmann, and A. Carattoli. 2007. Multicopy *bla*<sub>OXA-58</sub> gene as a source of high-level resistance to carbapenems in *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* **51**:2324–2328.
- British Society for Antimicrobial Chemotherapy. 2006. British Society for Antimicrobial Chemotherapy methods for antimicrobial susceptibility testing, version 6, January 2006. British Society for Antimicrobial Chemotherapy, Birmingham, United Kingdom.

5. Busiello, I., R. Acquaviva, A. Di Popolo, T. G. Blanchard, V. Ricci, M. Romano, and R. Zarrilli. 2004. *Helicobacter pylori*  $\gamma$ -glutamyltranspeptidase upregulates COX-2 and EGF-related peptides expression in human gastric cells. *Cell. Microbiol.* **6**:255–267.
6. Chang, H. C., Y. F. Wei, L. Dijkshoorn, M. Vaneechoutte, C. T. Tang, and T. C. Chang. 2005. Species-level identification of isolates of the *Acinetobacter calcoaceticus*-*Acinetobacter baumannii* complex by sequence analysis of the 16S-23S rRNA gene spacer region. *J. Clin. Microbiol.* **43**:1632–1639.
7. Chen, T. L., R. C. Wu, M. F. Shaio, C. P. Fung, and W. L. Cho. 2008. Acquisition of a plasmid-borne *bla*<sub>OXA-58</sub> gene with an upstream IS1008 insertion conferring a high level of carbapenem resistance to *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* **52**:2573–2580.
8. Clinical and Laboratory Standards Institute. 2008. Performance standards for antimicrobial susceptibility testing; eighteenth informational supplement. Approved standard M100-S18. Clinical and Laboratory Standards Institute, Wayne, PA.
9. Cobucci-Ponzano, B., F. Conte, D. Benelli, P. Londei, A. Flagello, M. Monti, P. Pucci, M. Rossi, and M. Moracci. 2006. The gene of an archaic  $\alpha$ -L-fucosidase is expressed by translational frameshifting. *Nucleic Acids Res.* **34**:4258–4268.
10. Coelho, J. M., J. F. Turton, M. E. Kaufmann, J. Glover, N. Woodford, M. Warner, M.-F. Palepou, R. Pike, T. L. Pitt, B. C. Patel, and D. M. Livermore. 2006. Occurrence of carbapenem-resistant *Acinetobacter baumannii* clones at multiple hospitals in London and Southeast England. *J. Clin. Microbiol.* **44**:3623–3627.
11. Dijkshoorn, L., A. Nemeč, and H. Seifert. 2007. An increasing threat in hospitals: multidrug-resistant *Acinetobacter baumannii*. *Nat. Rev. Microbiol.* **5**:939–951.
12. Dorsey, C. W., A. P. Tomaras, and L. A. Actis. 2006. Sequence and organization of pMAC, an *Acinetobacter baumannii* plasmid harboring genes involved in organic peroxide resistance. *Plasmid* **56**:112–123.
13. Heritier, C., L. Poirel, T. Lambert, and P. Nordmann. 2005. Contribution of acquired carbapenem-hydrolyzing oxacillinases to carbapenem resistance in *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* **49**:3198–3202.
14. Hernandez-Alles, S., S. Alberti, D. Alvarez, A. Domenech-Sanchez, L. Martinez-Martinez, J. Gil, J. M. Tomas, and V. J. Benedi. 1999. Porin expression in clinical isolates of *Klebsiella pneumoniae*. *Microbiology* **145**:673–679.
15. Iacono, M., L. Villa, D. Fortini, R. Bordoni, F. Imperi, R. J. P. Bonnal, T. Sicheritz-Ponten, G. De Bellis, P. Visca, A. Cassone, and A. Carattoli. 2008. Whole genome pyrosequencing of an epidemic multidrug resistant *Acinetobacter baumannii* of the European clone II. *Antimicrob. Agents Chemother.* **52**:2616–2625.
16. Lee, K., D. Yong, J. H. Yum, Y. S. Lim, A. Bolmström, A. Qwärnström, Å. Karlsson, and Y. Chong. 2005. Evaluation of Etest MBL for detection of *bla*<sub>IMP-1</sub> and *bla*<sub>VIM-2</sub> allele-positive clinical isolates of *Pseudomonas* spp. and *Acinetobacter* spp. *J. Clin. Microbiol.* **43**:942–944.
17. Mussi, M. A., A. S. Limansky, and A. M. Viale. 2005. 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. *Antimicrob. Agents Chemother.* **49**:1432–1440.
18. Perez, F., A. M. Hujer, K. M. Hujer, B. K. Decker, P. N. Rather, and R. A. Bonomo. 2007. Global challenge of multidrug-resistant *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* **51**:3471–3484.
19. Poirel, L., E. Lebessi, C. Heritier, A. Patsoura, M. Foustoukou, and P. Nordmann. 2006. Nosocomial spread of OXA-58-positive carbapenem-resistant *Acinetobacter baumannii* isolates in a pediatric hospital in Greece. *Clin. Microbiol. Infect.* **12**:1138–1141.
20. Poirel, L., W. Mansour, O. Bouallegue, and P. Nordmann. 2008. Carbapenem-resistant *Acinetobacter baumannii* isolates producing the OXA-58-like carbapenem-hydrolyzing oxacillinase OXA-97 from Tunisia. *Antimicrob. Agents Chemother.* **52**:1613–1617.
21. Poirel, L., S. Marqué, C. Heritier, C. Segonds, G. Chabanon, and P. Nordmann. 2005. OXA-58, a novel class D  $\beta$ -lactamase involved in resistance to carbapenems in *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* **49**:202–208.
22. Poirel, L., and P. Nordmann. 2006. Genetic structure at the origin of acquisition and expression of the carbapenem-hydrolyzing oxacillinase gene *bla*<sub>OXA-58</sub> in *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* **50**:1442–1448.
23. Pournaras, S., A. Markogiannakis, A. Ikonomidis, L. Kondyli, K. Bethimouti, A. N. Maniatis, N. J. Legakis, and A. Tsakris. 2006. Outbreak of multiple clones of imipenem-resistant *Acinetobacter baumannii* isolates expressing OXA-58 carbapenemase in an intensive care unit. *J. Antimicrob. Chemother.* **57**:557–561.
24. Riccio, M. L., N. Franceschini, L. Boschi, B. Caravelli, G. Cornaglia, R. Fontana, and G. M. Rossolini. 2000. Characterization of the metallo- $\beta$ -lactamase determinant of *Acinetobacter baumannii* AC-54/97 reveals the existence of *bla*<sub>IMP</sub> allelic variants carried by gene cassettes of different phylogeny. *Antimicrob. Agents Chemother.* **44**:1229–1235.
25. Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
26. Schulter, A., R. Szczepanowski, N. Kurz, S. Schneider, I. Krahn, and A. Puhler. 2007. Erythromycin resistance-conferring plasmid pRSB105, isolated from a sewage treatment plant, harbors a new macrolide resistance determinant, an integron-containing Tn402-like element, and a large region of unknown function. *Appl. Environ. Microbiol.* **73**:1952–1960.
27. Turton, J. F., S. N. Gabriel, C. Valderrey, M. E. Kauffmann, and T. L. Pitt. 2007. Use of sequence-based typing and multiplex PCR to identify clonal lineages of outbreak strains of *Acinetobacter baumannii*. *Clin. Microbiol. Infect.* **13**:807–815.
28. Turton, J. F., M. E. Ward, N. Woodford, M. E. Kaufmann, R. Pike, D. M. Livermore, and T. L. Pitt. 2006. The role of IS*Aba1* in expression of OXA carbapenemase genes in *Acinetobacter baumannii*. *FEMS Microbiol. Lett.* **258**:72–77.
29. Vallenet, D., P. Nordmann, V. Barbe, L. Poirel, S. Mangenot, E. Bataille, C. Dossat, S. Gas, A. Kreimeyer, P. Lenoble, S. Oztaş, J. Poulain, B. Segurens, C. Robert, C. Arbegel, J.-M. Claverie, D. Raoult, C. Médigue, J. Weissenbach, and S. Cruveiller. 2008. Comparative analysis of acinetobacters: three genomes for three lifestyles. *PLoS ONE* **3**:e1805.
30. Zarrilli, R., R. Casillo, A. Di Popolo, M.-F. Tripodi, M. Bagattini, S. Cucurullo, V. Crivaro, E. Ragone, A. Mattei, N. Galdieri, M. Triassi, and R. Utili. 2007. Molecular epidemiology of a clonal outbreak of multidrug-resistant *Acinetobacter baumannii* in a university hospital in Italy. *Clin. Microbiol. Infect.* **13**:481–489.
31. Zarrilli, R., M. Crispino, M. Bagattini, E. Barretta, A. Di Popolo, M. Triassi, and P. Villari. 2004. Molecular epidemiology of sequential outbreaks of *Acinetobacter baumannii* in an intensive care unit shows the emergence of carbapenem resistance. *J. Clin. Microbiol.* **42**:946–953.