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Characterization and PCR-Based Replicon Typing of Resistance Plasmids in *Acinetobacter baumannii*▽

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Acinetobacter baumannii **is an opportunistic pathogen, especially in intensive care units, and multidrug-resistant isolates have increasingly been reported during the last decade. Despite recent progress in knowledge of antibiotic resistance mechanisms in *A. baumannii*, little is known about the genetic factors driving isolates toward multidrug resistance. In the present study, the *A. baumannii* plasmids were investigated through the analysis and classification of plasmid replication systems and the identification of *A. baumannii*-specific mobilization and addiction systems. Twenty-two replicons were identified by *in silico* analysis, and five other replicons were identified and cloned from previously uncharacterized *A. baumannii* resistance plasmids carrying the OXA-58 carbapenem-hydrolyzing oxacillinase. Replicons were classified into homology groups on the basis of their nucleotide homology. A novel PCR-based replicon typing scheme (the *A. baumannii* PCR-based replicon typing [AB-PBRT] method) was devised to categorize the *A. baumannii* plasmids into homogeneous groups on the basis of the nucleotide homology of their respective replicase genes. The AB-PBRT technique was applied to a collection of multidrug-resistant *A. baumannii* clinical isolates carrying the bla_{OXA-58} or bla_{OXA-23} carbapenemase gene. A putative complete conjugative apparatus was identified on one plasmid whose self-conjugative ability was demonstrated *in vitro*. We showed that this conjugative plasmid type was widely diffused in our collection, likely representing the most important vehicle promoting the horizontal transmission of *A. baumannii* resistance plasmids.**

The foundation of plasmid biology was largely built on the genetic analysis of plasmid strategies for broad-host-range replication in Gram-negative bacteria. Mechanisms which guarantee the autonomous replication, addiction systems based on toxin-antitoxin factors, partitioning systems ensuring stable inheritance during cell division, and other virulence and antimicrobial resistance determinants have been described for plasmids circulating in the *Enterobacteriaceae* family and *Pseudomonas* spp. (17). Enterobacterial plasmids have also been classified into homogeneous groups on the basis of their replication controls by conjugation (plasmid incompatibility) and molecular methods (Southern blot hybridization with replicon probes and PCR-based replicon typing) (5, 8, 10, 11). Currently, 27 incompatibility groups are recognized in the *Enterobacteriaceae* by the Plasmid Section of the National Collection of Type Cultures (Colindale, London, United Kingdom). In contrast, limited information is available on the plasmids circulating in *Acinetobacter* spp., even though *Acinetobacter baumannii* is an important pathogen in intensive care units (13, 28). Moreover, despite recent progress in the study of antibiotic resistance mechanisms in *A. baumannii*, little is known about the genetic factors that have driven the recent evolution of *A. baumannii* toward multidrug resistance. *A. baumannii* may develop resistance to carbapenems through plasmid-mediated acquisition of carbapenem-hydrolyzing class D β-lactamases (CHDLs) (29). In particular, the bla_{OXA-58} and bla_{OXA-23} genes encoding the OXA-58 and OXA-23 CHDLs, respectively, have been reported from *A. baumannii* isolates collected from distant parts of the world in association with plasmids. The aim of the present study was to investigate the *A. baumannii* plasmids through the analysis and classification of plasmid replication systems and identification of *A. baumannii*-specific mobilization and addiction systems. Finally, novel tools for detecting *A. baumannii* resistance plasmids are proposed and the plasmids are categorized into homogeneous families on the basis of the nucleotide homologies of their respective replicase genes.

MATERIALS AND METHODS

In silico **analysis of *A. baumannii* plasmids.** An *in silico* comparative analysis of fully and partially sequenced *Acinetobacter* plasmids was performed at GenBank using the BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Fifteen fully sequenced and eight partially sequenced *A. baumannii* plasmids available at GenBank from six completed genomes from previous studies or identified in this study were analyzed (Table 1). Multiple-sequence alignments of the replicon nucleotide sequences have been performed using DNAMAN software (Lynnon BioSoft, Vaudreuil, Quebec, Canada) set for DNA quick alignment with a gap penalty of 7, a K-tuple of 3, 5 top diagonals, and a window size of 5. Multiple-sequence alignments of the coding sequences were performed by using the DNAMAN software set for protein quick alignment with a gap penalty of 3, a K-tuple of 1, five top diagonals, and a window size of 5.

A. baumannii **PCR-based replicon typing (AB-PBRT) method.** A total of 19 PCR amplifications were devised to detect 27 replicase genes, which were grouped into 19 homology groups (GRs) on the basis of their nucleotide sequence similarities (Table 1 and Fig. 1). These groups include five novel replicase genes (*aci3*, *aci4*, *aci5*, *aci7*, and *aci8* [Table 1]), cloned and sequenced as

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TABLE 1. *A. baumannii* replicase genes analyzed in this study

Strain	Plasmid (EMBL accession no.)	Replicase name	Rep superfamily	Source of homology by BLASTp best hit[a]	% amino acid identity (EMBL accession no.)	Iterons[b]	Rep group	Reference
ACICU	pACICU1 (NC_010605)	Aci1	Rep-3 pfam01051	*Klebsiella pneumoniae* pKPN5	71 (YP_001338806)	Pos	GR2	19
		AciX	Rep-3 pfam01051	*Neisseria lactamica*	55 (ZP_05987942)	Pos	GR10	
	pACICU2 (NC_010606)	Aci6	Rep pfam03090	*Pseudoalteromonas* sp.	41 (YP_001887739)	Neg	GR6	
SDF	p1ABSDF (NC_010395)	p1ABSDF0001	Rep-3 pfam01051	*Moraxella bovis*	59 (YP_001966359)	Pos	GR1	34
	p2ABSDF (NC_010396)	p2ABSDF0001	Rep-3 pfam01051	*Moraxella bovis*	40 (YP_003289297)	Pos	GR12	
		p2ABSDF0025	Rep-3 pfam01051	*Moraxella bovis*	50 (YP_003289297)	Pos	GR18	
	p3ABSDF (NC_010398)	p3ABSDF0002	Rep-3 pfam01051	*Klebsiella pneumoniae* pKPN5	57 (YP_001338806)	Neg	GR7	
		p3ABSDF0009	Rep-3 pfam01051	*Klebsiella pneumoniae* pKPN5	45 (YP_001338806)	Pos	GR9	
		p3ABSDF0018	Rep-3 pfam01051	*Moraxella bovis*	41 (YP_003289297)	Pos	GR15	
AYE	p1ABAYE (NC_010401)	p1ABAYE0001	Rep-3 pfam01051	*Enhydrobacter aerosaccus*	33 (ZP_05619518)	Pos	GR11	34
	p2ABAYE (NC_010402)	p2ABAYE0001	Rep-3 pfam01051	*Klebsiella pneumoniae* pKPN5	71 (YP_001338806)	Pos	GR2	
	p3ABAYE (NC_010404)	p3ABAYE0002	Rep-3 pfam01051	*Pasteurella multocida* pJR2_p4	31 (NP_848174)	Neg	GR13	
	p4ABAYE (NC_010403)	p4ABAYE0001	Rep-1 pfam01446	*Pseudomonas putida*	43 (NP_064737)	Neg	GR14	
ATCC 17978	pAB1 (NC_009083)	A1S_3471	Rep-3 pfam01051	*Klebsiella pneumoniae* pKPN5	59 (YP_001338806)	Pos	GR17	32
	pAB2 (NC_009084)	A1S_3472	Rep-3 pfam01051	*Klebsiella pneumoniae* pKPN5	71 (YP_001338806)	Pos	GR2	
Ab0057	pAB0057 (NC_011585)	AB57_3921	Rep-3 pfam01051	*Klebsiella pneumoniae* pKPN5	71 (YP_001338806)	Pos	GR2	1
Ab49	pAB49 (L77992; partial)	repApAB49	Rep-1 pfam01446	*Bacillus cereus*	38 (ZP_04189469)	Neg	GR16	Unpublished
AbABIR	pABIR (EU294228)	RepA_AB	Rep-3 pfam01051	*Moraxella bovis*	40 (YP_003289297)	Pos	GR12	35
VA-566/00	pABVA01 (NC_012813)	Aci2	Rep-3 pfam01051	*Klebsiella pneumoniae* pKPN5	74 (YP_001338806)	Pos	GR2	9
Ab19606	pMAC02 (AY541809)	RepM-Aci9	Rep-3 pfam01051	*Klebsiella pneumoniae* pKPN5	57 (YP_001338806)	Pos	GR8	12
Ab02	pAB02 (AY228470, partial)	repA_AB	Rep-3 pfam01051	*Moraxella bovis*	40 (YP_003289297)	Pos	GR12	Unpublished
Ab135040	p135040 (GQ861437, partial)	rep135040	Rep-3 pfam01051	*Klebsiella pneumoniae* pKPN5	58 (YP_001338806)	Pos	GR19	18
Ab736	p736 (GU978996; partial)	Aci7	Rep-3 pfam01051	*Klebsiella pneumoniae* pKPN5	92 (YP_001338806)	Pos	GR3	This study
Ab203	P203 (GU978997; partial)	Aci3	Rep-3 pfam01051	*Klebsiella pneumoniae* pKPN5	85 (YP_001338806)	Pos	GR3	This study
Ab844	p844 (GU978998; partial)	Aci4	Rep-3 pfam01051	*Klebsiella pneumoniae* pKPN5	85 (YP_001338806)	Pos	GR4	This study
Ab537	p537 (GU978999; partial)	Aci5	Rep-3 pfam01051	*Klebsiella pneumoniae* pKPN5	72 (YP_001338806)	Pos	GR5	This study
Ab11921	p11921 (GU979000; partial)	Aci8	Rep-3 pfam01051	*Klebsiella pneumoniae* pKPN5	46 (YP_001966359)	Pos	GR8	This study

[a] This comparison was performed by a BLASTP search excluding the sequence of the *Acinetobacter* spp.
[b] Pos, positive; Neg, negative.

described below, from plasmids carrying the bla_{OXA-58} genes from a collection of *A. baumannii* clinical isolates.

The primers used for AB-PBRT are listed in Table 2. The PCR amplifications were organized into six multiplexes, each recognizing three or four different homology groups (Table 2). Specificity and sensitivity tests were performed for each primer pair in simplex form and in multiplex form with genomic DNA extracted from the respective reference strain (Table 2). The multiplexes were also tested using single and mixed control DNA templates. All the PCRs were highly specific on each template, as expected. The PCRs for GR2, GR3, and GR8 recognize the related replicases Aci1/Aci2, Aci3/Aci7, and Aci8/Aci9, respectively. The replicase variants belonging to GR2, GR3, and GR8 can be recognized by DNA sequencing of the respective amplicon.

Each multiplex reaction mixture contained (final concentrations) 1× Immo-Buffer [16 mM $(NH_4)_2SO_4$, 67 mM Tris-HCl, pH 8.3, 0.01% Tween 20], 4.0 mM $MgCl_2$, 0.4 mM deoxynucleoside triphosphate, 1.0 μM each primer, 5% dimethyl sulfoxide, 0.04 U/μl Immolase DNA polymerase (Bioline, Kondon, United Kingdom), and 200 to 400 ng of DNA template per reaction tube. Template DNA was prepared by total DNA extraction by the Wizard genomic DNA purification kit (Promega, Madison, WI), starting from 2 ml of LB broth cultures. PCR amplifications were performed with the following amplification scheme: 1 cycle of denaturation at 94°C for 7 min, followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 52°C for 30 s, and elongation at 72°C for 1.5 min. The amplification was finished with an extension program of 1 cycle at 72°C for 5 min.

Positive controls. The PCR amplifications were tested with the ACICU, AYE, SDF, ATCC 19798, and Ab135040 reference strains and 20 nonclonally related *A. baumannii* isolates known to possess plasmids carrying the CHDL gene bla_{OXA-58} ($n = 13$, originating from France, Tunisia, Sweden, Turkey, Romania, and Belgium) or bla_{OXA-23} ($n = 7$, originating from Belgium, Monaco, Kingdom of Bahrain, Egypt, Algeria, Libya, and Saudi Arabia). Those carbapenem-resistant *A. baumannii* isolates had been characterized previously (23, 25, 26, 30) and belong to the INSERM U914 strain collection (Table 3). Plasmid typing was also performed with transformants and transconjugants obtained from the bla_{OXA-58}- and bla_{OXA-23}-positive plasmids (Table 3). All the amplicons obtained with the primers listed in Table 2 were cloned into a TA cloning vector (Invitrogen-Life Technologies, Milan, Italy) and transformed into competent *Escherichia coli* DH5α cells (MAX Efficiency DH5α chemically competent cells; Invitrogen-Life Technologies). Selection of the transformants was performed on LB agar plates containing ampicillin (100 μg/ml). The cloned amplicons were fully sequenced and used as positive controls for the multiplex PCRs in the AB-PBRT scheme.

Plasmid transfer by transformation and conjugation. Plasmid DNAs were purified from bla_{OXA-58}-positive *A. baumannii* isolates by using an Invitrogen PureLink HiPure plasmid filter midiprep kit and electrotransformed into recip-

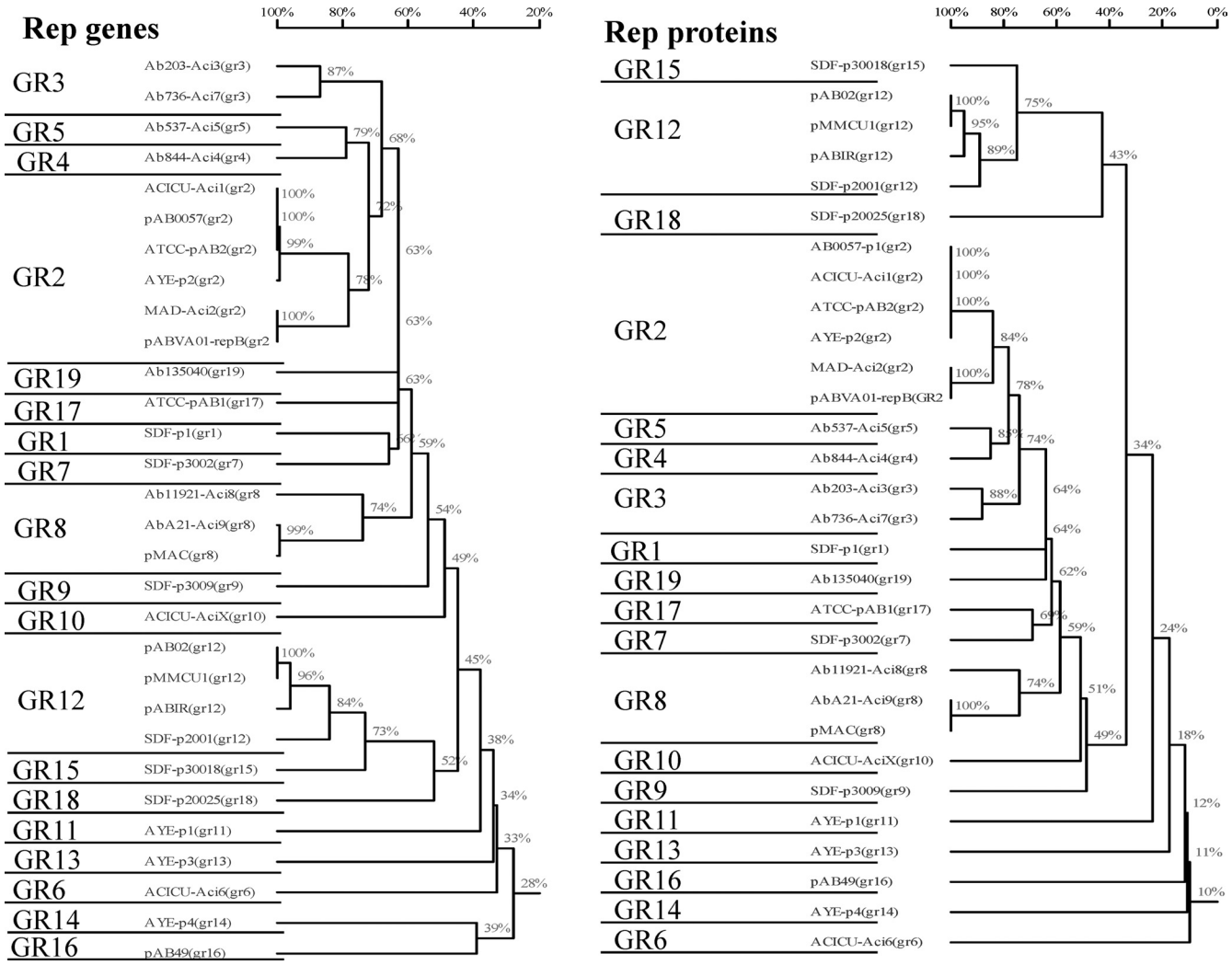

FIG. 1. Multiple-sequence alignments and groups of homology of the replicase genes and their deduced amino acid protein sequences from *A. baumannii* plasmids.

ient strain *A. baumannii* BM4547 (22), and transformants were selected on ticarcillin-containing plates (50 μg/ml). Mating-out assays were performed by using isolates harboring bla_{OXA-58} and bla_{OXA-23} plasmids as donors and rifampin-resistant recipient strain *A. baumannii* BM4547, as described previously (26). Briefly, one colony of each of the donor and recipient strains obtained after 24 h of growth was cultured separately under weak agitation in 1 ml tryptic soy broth at 37°C, and they were then used in the mating-out assays. Conjugation was done by incubating 800 μl of the recipient strain with 200 μl of the donor strain under low agitation at 37°C for an additional 3-h step. The transconjugants were then selected by plating 200 μl of that mixture on agar plates containing ticarcillin (100 μg/ml) and rifampin (50 μg/ml).

The bla_{OXA-58} and bla_{OXA-23} genes were detected by PCR using previously described primer pairs (2, 7).

Identification and cloning of novel replicase genes from *A. baumannii* plasmids. Plasmid DNAs were purified from the *A. baumannii* transformants by the Invitrogen PureLink HiPure plasmid filter midiprep kit. EcoRI-restricted fragments were separated by 0.8% agarose gel electrophoresis. Plasmid DNA was transferred to a Hybond-N^+ membrane (Roche Diagnostics, Monza, Italy) by standard methods (31). Southern blot hybridization was carried out under low-stringency conditions (58°C) using the *aci1* amplicon from the pACICU1 plasmid as the probe, and the amplicon was labeled with digoxigenin (DIG)-11-dUTP by PCR using a DIG PCR probe synthesis kit (Roche Diagnostics, Monza, Italy). After hybridization with the probe, the hybridized DNA was detected with Nitro Blue Tetrazolium–5-bromo-4-chloro-3-indolylphosphate using a DIG nucleic acid detection kit (Roche Diagnostics).

The EcoRI-restricted fragments identified by cross-reaction with the *aci1* probe were separated by and eluted from the agarose gel by a Qiagen (Courtaboeuf, France) gel extraction kit and cloned into the EcoRI cloning site of the pUC18 vector, and the vector was transformed into competent *E. coli* DH5α cells (MAX Efficiency DH5α chemically competent cells; Invitrogen, Milan, Italy). Selection of the transformants was performed on LB agar plates containing ampicillin (100 μg/ml). The inserts were fully sequenced using standard and walking primers. The DNA sequences were determined by used of fluorescent dye-labeled dideoxynucleotides and an AB3730 automatic DNA sequencer (Perkin-Elmer, Foster City, CA).

Nucleotide sequence accession numbers. The DNA sequences of the *aci3*, *aci4*, *aci5*, *aci7*, and *aci8* replicase genes and the *aci9* replicase gene from plasmid AbA21 have been deposited in the EMBL GenBank under accession numbers GU978996 to GU979001, respectively.

RESULTS AND DISCUSSION

Detailed analysis and definitions of *A. baumannii* replicons. The nucleotide and deduced protein sequences of 18 *A. baumannii* plasmids available in GenBank were analyzed. Twenty-

TABLE 2. Primers used to detect the replicase gene groups in the *A. baumannii* PCR-based replicon typing scheme

Multiplex	Group	Primer name	Primer sequence	Amplicon size (bp)	Replicase name (short name)	Reference strain/plasmid
1		gr1FW gr1RV	5′-CATAGAAATACAGCCTATAAAG-3′ 5′-TTCTTCTAGCTCTACCAAAAT-3′	330	p1ABSDF001 (p1S1)	SDF-p1ABSDF
	GR2	gr2FW gr2RV	5′-AGTAGAACAACGTTTAATTTTATTGGC-3′ 5′-CCACTTTTTTTAGGTATGGGTATAG-3′	851	Aci1 Aci2	ACICU-pACICU1 MAD
	GR3	gr3FW gr3RV	5′-TAATTAATGCCAGTTATAACCTTG-3′ 5′-GTATCGAGTACACCTATTTTTTGT-3′	505	Aci3 Aci7	Ab599 Ab736
2	GR5	gr5FW gr5RV	5′-AGAATGGGGAACTTTAAAGA-3′ 5′-GACGCTGGGCATCTGTTAAC-3′	220	Aci5	Ab537
	GR18	gr18FW gr18RV	5′-TCGGGTTATCACAATAACAA-3′ 5′-TAGAACATTGGCAATCCATA-3′	676	p2ABSDF00025 (p2S25)	SDF-p2ABSDF
	GR7	gr7FW gr7RV	5′-GAACAGTTTAGTTGTGAAAG-3′ 5′-TCTCTAAATTTTTCAGGCTC-3′	885	p3ABSDF002 (p3S2)	SDF-p3ABSDF
3	GR9	gr9FW gr9RV	5′-GCAAGTTATACATTAAGCCT-3′ 5′-AAAAATAAACGCTCTGATGC-3′	191	p3ABSDF0009 (p3S9)	SDF-p3ABSDF
	GR4	gr4FW gr4RV	5′-GTCCATGCTGAGAGCTATGT-3′ 5′-TACGTCCCTTTTTATGTTGC-3′	508	Aci4	Ab844
	GR11	gr11FW gr11RV	5′-GGCTATTCAAAACAAAGTTAC-3′ 5′-GTTTCCTCTCTTACACTTTT-3′	852	p1ABAYE0001 (p1AYE)	AYE-p1ABAYE
4	GR12	gr12FW gr12RV	5′-TCATTGGTATTCGTTTTTCAAAACC-3′ 5′-ATTTCACGCTTACCTATTTGTC-3′	165	p2ABSDF0001 (p2S1)	SDF-p1ABSDF
	GR10	gr10FW gr10RV	5′-TTTCACTAGCTACCAACTAA-3′ 5′-ACACGTTGGTTTGGAGTC-3′	371	AciX	ACICU-pACICU1
	GR13	gr13FW gr13RV	5′-CAAGATCGTGAAATTACAGA-3′ 5′-CTGTTTATAATTTGGGTCGT-3′	780	p3ABAYE0002 (p3AYE)	AYE-p3ABAYE
5	GR8	gr8FW gr8RV	5′-AATTAATCGTAAAGGATAATGC-3′ 5′-GACATAGCGATCAAATAAGC-3′	233	Aci8 repM (Aci9)	Ab11921 pMAC02
	GR14	gr14FW gr14RV	5′-TTAAATGGGTGCGGTAATTT-3′ 5′-GCTTACCTTTCAAAACTTTG-3′	622	p4ABAYE0001 (p4AYE)	AYE-p4ABAYE
	GR15	gr15FW gr15RV	5′-GGAAATAAAAATGATGAGTCC-3′ 5′-ATAAGTTGTTTTTGTTGTATTCG-3	876	p3ABSDF0018 (p3S18)	SDF-p3ABSDF
6	GR16	gr16FW gr16RV	5′-CTCGAGTTCAGGCTATTTTT-3′ 5′-GCCATTTCGAAGATCTAAAC-3′	233	repApAB49 (pAB49)	pAB49
	GR17	gr17FW gr17RV	5′-AATAACACTTATAATCCTTGTA-3′ 5′-GCAAATGTGACCTCTAATATA-3′	380	A1s_3471 (A1S3471)	ATCC 17978-pAB1
	GR6	gr6FW gr6RV	5′-AGCAAGTACGTGGGACTAAT-3′ 5′- AAGCAATGAAACAGGCTAAT-3′	662	Aci6	ACICU-pACICU2
	GR19	gr19FW gr19RV	5′- ACGAGATACAAACATGCTCA-3′ 5′- AGCTAGACATTTCAGGCATT-3′	815	rep135040	Ab135040

two intact replicons were identified *in silico* (Table 1). Each replicon included the origin of replication (*ori*) and the replicase gene (*rep*). *A. baumannii* replicons differ from all those previously described in other prokaryotic species, indicating that *A. baumannii* possesses its own plasmid types. For 17 out of the 22 replicons, the *rep* genes were preceded by four direct and perfectly conserved repeats that, in analogy with the basic replicons of plasmids, may be defined as "iterons" (Table 1 and

TABLE 3. AB-PBRT applied to the collection of bla_{OXA-58}- and bla_{OXA-23}-positive strains and their respective transformants/transconjugants

Strain	OXA	Multiplex 1			Multiplex 2			Multiplex 3			Multiplex 4			Multiplex 5			Multiplex 6			
		GR2 (851 bp)	GR3 (505 bp)	GR1 (330 bp)	GR7 (885 bp)	GR18 (676 bp)	GR5 (220 bp)	GR11 (852 bp)	GR4 (508 bp)	GR9 (191 bp)	GR13 (780 bp)	GR10 (371 bp)	GR12 (165 bp)	GR15 (876 bp)	GR14 (622 bp)	GR8 (233 bp)	GR6 (662 bp)	GR17 (425 bp)	GR16 (233 bp)	GR19 (815 bp)
ACICU	58	Aci1										AciX					Aci6			
AYE		p2AYE (Aci1)						p1AYE			p3AYE				p4AYE					
SDF			Aci3[b]	p1S1	p3S2	p2S1				p3S9			p2S25	p3S18						
ATCC		A1S_3472 (Aci1)																A1S3471		
135040T	143																			Rep
A21	58	Aci1											p2S25			Aci9	Aci6		pAB49	
A21T[a]	58						[Aci5][c]		[Aci4]							Aci9				
Ab11921	58	Aci1											p2S25			Aci8				
11921T[a]	58						[Aci5]		[Aci4]							Aci8				
Ab203	58		Aci3																	
203T[a]	58		Aci3				[Aci5]		[Aci4]											
Ab844	58	Aci2							Aci4				p2S25				Aci6			
844T[a]	58						[Aci5]		Aci4											
Ab537	58		Aci3				Aci5		Aci4											
537T[a]	58		Aci3				[Aci5]		[Aci4]											
MAD	58	Aci2															Aci6			
MADT[a]	58	Aci2					[Aci5]		[Aci4]											
Ab736	58		Aci7														Aci6			
736T[a]	58		Aci7				[Aci5]		[Aci4]											
Ab727	58	Aci1															Aci6		pAB49	
727T[a]	58	Aci1					[Aci5)]		[Aci4]											
AbA22	58	Aci1															Aci6		pAB49	
A22T[a]	58	Aci1					[Aci5)]		[Aci4]											
Ab120066	58	Aci1										AciX					Aci6			
Ab587	58		Aci3																	
587T[a]	58		Aci3				[Aci5]		[Aci4]											
Ab692	58		Aci3																	
692T[a]	58		Aci3				[Aci5]		[Aci4]											
Ab599	58	Aci2	Aci3										p2S25							
599T[a]	58		Aci3				[Aci5]		[Aci4]											
AbAS3	23												p2S25				Aci6			
AS3TcJ[a]	23						[Aci5]		[Aci4]								Aci6			
Ab1190	23												p2S25				Aci6			
1190Tcj[a]	23						[Aci5]		[Aci4]								Aci6			
Ab614	23												p2S25				Aci6			
614Tcj[a]	23						[Aci5]		[Aci4]								Aci6			
Ab861	23	Aci1															Aci6			
861Tcj[a]	23						[Aci5]		[Aci4]								Aci6			
Ab877	23								Aci4								Aci6			
Ab14	23																Aci6			
AbBel	23	Aci1															Aci6			
Recipient							[Aci5]		[Aci4]											

[a] bla_{OXA}-positive transformants (T) and transconjugants (Tcj) were obtained from the respective donor strain indicated on the line above the transformant or transconjugant designation using *A. baumannii* BM4547 as the recipient.

[b] The *aci3* gene was not identified in the whole-genome sequencing of the SDF strain.

[c] Brackets indicate that Aci4 and Aci5 replicases are present in the recipient strain.

TABLE 4. Iterons in *A. baumannii* replicons

Replicon(s)	Iteron sequence	No. of direct repeats	Distance from iteron to *rep* start codon (bp)
p1ABSDF0001	5′-CAATAAGTACACCTTTATCTTG-3′	4	50
pACICU1-Aci1, p2ABAYE0001, A1S_3472 pAB0057	5′-ATATGTCCACGTTTACCTTGCA-3′	4	53
pABVA01-Aci2	5′-TTTACCTTGCAATATGACACCG-3′	3	66
Ab203-Aci3 Ab736-Aci7	5′-TAAAACGAGGTTTACCTTGCAT-3′	4	57
Ab844-Aci4	5′-ATATGACTACGTTTACCTACCA-3′	4	107
Ab537-Aci5	5′-ATATGACTACGTTTACCTACCA-3′	4	105
Ab11921-Aci8	5′-TAGGTTTATCGACCCATAAAAT-3′	4	91
pA21-Aci9	5′-TAAAACTAGGTTTATCGACCCT-3′	4	96
pMAC-Aci9	5′-ATAAAACTAGGTTTATCGACCC-3′	4	97
p3ABSDF0009	5′-TATCTATACGTTTATGCAGTCT-3′	4	60
pACICU1-AciX	5′-CATTCAATCACAGATTCCATTC-3′	4	80
p1ABAYE0001	5′-AAAGGGTACAAATAGCATGAT-3′	4[a]	90
p2ABSDF0001, pAB02	5′-GGATTGACTACTAACTATGAC-3′	4	41
pABIR	5′-CTAACTATGACGGATTGACTA-3′	4	55
p3ABSDF0018	5′-TATGAGGGATTGACTACTAAC-3′	4	32
pAB1	5′-ATTTCTTTGCATTTGACTACA-3′	4	10
p2ABSDF0025	5′-TAACTATGAGGGATTGACGCA-3′	5	15
p135040	5′-CATAT CTATACGTTTATCGACC-3′	4	89

[a] Imperfect.

Table 4). Iterons have been identified not only on many prokaryotic plasmids but also on chromosomes, phages, and eukaryotic *ori* genes (6, 27). In enterobacterial plasmids, each replicase protein binds to the reiterated iterons at the *ori* site and stimulates DNA replication by interacting with the host proteins (DNAK, DNAJ, RNApol) required for replication initiation. However, no iterons were identified in association with the replicase genes for five plasmids. For these plasmids, one can speculate about alternative mechanisms of replication control, presumably based on regulation of *rep* translation mediated by an inhibitory antisense RNA, as previously described for IncI1 and IncF plasmids (14).

Similar to plasmids described from other species (33), *A. baumannii* pACICU1, p2ABSDF, and p3ABSDF were multireplicon plasmids, since they carried more than one replicon (Table 1). Interestingly, the iterons and replicase genes of replicons from given plasmids showed weak sequence identities, likely to minimize the effect of competition of the replicase protein on its relative binding sites (6, 27). Plasmids pABIR and pABVA01 also showed two replicons, one that was functional, which was considered in this study, and one whose replicase gene was truncated by an insertion sequence (EMBL accession no. EU294228).

On the basis of the nucleotide sequence identities deduced from the *in silico* analysis, the 22 replicase genes were grouped into homology groups. Each group showed replicase genes showing less than 74% nucleotide identity (Table 1 and Fig. 1). Plasmids p2ABSDF, pABIR (carrying the $bla_{\text{OXA-58}}$ gene), and pAB02 (carrying the $bla_{\text{OXA-24/OXA-40}}$ gene) carried highly related replicons which were included in the same homology group, designated GR12, showing conserved replicase gene and iteron sequences (>84% nucleotide identity; Fig. 1 and Table 4). This group also contains other plasmids carrying the $bla_{\text{OXA-24/OXA-40}}$ gene that were recently identified and that showed *rep* gene sequences identical to the *rep* gene sequences of pAB02 (pMMCU1 [EMBL accession no. GQ342610], pMMCU2 [EMBL accession no. GQ476987], and pMMD [EMBL accession no. GQ904226]; the sequence of plasmid pMMCU1 is included in the tree in Fig. 1 for comparison).

Conserved replicons were observed for plasmids pACICU1, p2ABAYE, pAB2, pAB0057, pABVA01, and pMAD; and all have been included in GR2. Two variants (*aci1* and *aci2*) showing 78% nucleotide identity were included in this group (Fig. 1). This group also contains plasmid pMMCU3 (EMBL accession no. GQ904227), carrying the $bla_{\text{OXA-24/OXA-40}}$ gene, which had a *aci2 rep* gene sequence identical to that of pABVA01 (9, 24). The *aci1* and *aci2* replicase genes showed different iteron sequences (Table 4).

Most of the replicase proteins belonged to the Rep-3 superfamily, identified by the pfam0151 conserved domain (NCBI nonredundant Clusters of Orthologs [COG]; http://www.ncbi.nlm.nih.gov/COG/), and showed variable amino acid similarities with the replicase proteins of plasmid pKPN5, recently identified in *Klebsiella pneumoniae* strain MGH 78578 (GenBank accession no. CP000650.1), and with plasmids identified in *Moraxella bovis*, *Pasteurella multocida*, and *Neisseria lactamica* (Table 1). It may be hypothesized that these replicase genes actually derive from a common ancestor of the Rep-3 superfamily group (Table 1). Two replicase proteins from plasmids p4ABAYE and pAB49 belonged to the Rep-1 superfamily (pfam01446) and showed significant homologies with plasmids from *Pseudomonas putida* and *Bacillus cereus*. The replicase from plasmid pACICU2 was peculiar since it belonged to an undefined Rep superfamily (pfam03090) whose closest homologous plasmid was identified from a *Pseudoalteromonas* sp. (Table 1).

Setup of a novel *A. baumannii* PCR-based replicon typing scheme. PCR amplifications were devised to recognize the replicase genes identified *in silico* and were successfully tested with the ACICU, AYE, SDF, ATCC 19798, and Ab135040 *A. baumannii* strains (Tables 2 and 3). Those PCRs were then used to test 20 clinical isolates carrying the plasmid-mediated carbapenem-hydrolyzing $bla_{\text{OXA-58}}$ and $bla_{\text{OXA-23}}$ oxacillinase

TABLE 5. Plasmid maintenance and addiction systems identified *in silico* on *A. baumannii* plasmids

Predicted function	Plasmid	CDS[a] protein identifier	Protein name, putative function	Source of homology	% best hit
Plasmid partitioning	pACICU1	P006	ParA, putative partition protein	*Moraxella bovis*	73
	pACICU1	P007	Probable copy no. control protein	*Moraxella bovis*	56
	pACICU2	P0040	ParB, involvement in plasmid partition	*Collimonas fungivorans*	44
	pACICU2	P0047	ParA, putative partition protein	*uncultured bacterium*	34
	pACICU2	P0048	ParB-like nuclease domain	*Caminibacter mediatlanticus*	39
	p3ABAYE	p3ABAYE0112	ParB- nuclease domain	*Ralstonia eutropha*	41
	p3ABAYE	p3ABAYE0113	ParA, putative partitioning protein	*Chromobacterium violaceum*	38
Toxin-antitoxin systems	pACICU1	P009	Antitoxin StbE, prevent-host-death protein	*Burkholderia ubonensis*	57
	pACICU1	P0010	Toxin RelE/StbE family	*Burkholderia ubonensis*	61
	p1ABAYE	p1ABAYE0004	Antitoxin, prevent-host-death protein	*Burkholderia cenocepacia*	56
	p1ABAYE	p1ABAYE0005	Toxin, Txe/YoeB family	*Burkholderia ambifaria*	70
	p2ABSDF	p2ABSDF0030	Toxin, RelE family protein	*Haemophilus somnus*	62
	p2ABSDF	p2ABSDF0031	Antitoxin, RelB homolog of RelB/DinJ family	*Haemophilus somnus*	
Restriction and antirestriction systems	pACICU1	P008	Type I site-specific DNase, HsdR family	*Chlorobium limicola*	33
	pACICU2	P0046	Type I restriction enzyme M subunit	*Haemophilus influenzae*	28
	p3ABAYE	p3ABAYE0069	Type II restriction/modification enzyme	*Polaromonas* sp.	52
	p2ABSDF	p2ABSDF0015	HpaII restriction endonuclease	*Flavobacterium psychrophilum*	49
	p2ABSDF	p2ABSDF0016	HpaIIM-like cytosine-specific methyltransferase, modification enzyme	*Haemophilus arainfluenzae*	79
	p3ABSDF	p3ABSDF0013	Type II restriction/modification enzyme	*Bacillus megaterium*	55
	p3ABSDF	p3ABSDF0014	Methyltransferase cytosine, modification enzyme	*Bacillus megaterium*	46
	p3ABSDF	p3ABSDF0015	Bfii restriction endonuclease	*Bacillus firmus*	63

[a] CDS, coding sequence.

genes (Table 3). Mating-out assays were initially performed with several bla_{OXA-58}-positive *A. baumannii* isolates as donors, but no transconjugants were obtained. However, all the bla_{OXA-58}-positive plasmids except one (from strain Ab120066) were successfully transferred by electroporation into *A. baumannii* BM4547 (Table 3). Transformants showed resistance to ticarcillin and reduced susceptibility to carbapenems as a result of bla_{OXA-58} gene expression. Four out of seven bla_{OXA-23}-positive plasmids were successfully transferred by conjugation into *A. baumannii* BM4547 and included in this study (Table 3) (26). All the *A. baumannii* isolates and their respective transformants and transconjugants were previously tested by the PCR-based replicon typing method described for the *Enterobacteriaceae* (5), but all of them gave negative results, indicating that those plasmids were not corresponding to those known to circulate among the *Enterobacteriaceae* (data not shown).

Twelve strains and their respective bla_{OXA-58} or bla_{OXA-23} transformant or transconjugant strains were successfully typed by the PCR amplifications devised with the 22 *A. baumannii* replicase genes identified *in silico* in previously characterized plasmids (Table 1). Four bla_{OXA-58}-positive strains and their respective transformants (strains Ab203, Ab537, Ab587, and Ab692) were negative by all these PCRs. Furthermore, strains Ab11921, Ab844, Ab736, and Ab599 were positive by the GR12 and/or GR6 PCR, but their respective transformants, carrying the bla_{OXA-58} gene, were negative for all the *A. baumannii* replicase genes identified *in silico*, suggesting that other replicons were present on these bla_{OXA-58}-positive plasmids.

Five novel replicase genes (*aci3*, *aci4*, *aci5*, *aci7*, and *aci8* [Table 1]) were identified and subsequently cloned and sequenced from the bla_{OXA-58}-positive strains: the *aci8 rep* gene from plasmid p11921 was 74% homologous to the *repM* replicase gene from plasmid pMAC02 and was included in GR8; *aci3* and *aci7* corresponded to novel replicase genes identified in plasmids from isolates Ab203, Ab537, Ab587, Ab599 (*aci3*), and Ab736 (*aci7*). The *aci3* and *aci7 rep* genes showed 87% nucleotide identity with each other and identical iteron sequences and were grouped into a novel homology group designated GR3; the *aci4* and *aci5* replicase genes from isolates Ab844 and Ab537, respectively, were classified in the novel groups GR4 and GR5, respectively, being highly divergent from all the other replicase genes (Fig. 1; Table 1). Noteworthy is the finding that the BM4547 strain used as the susceptible recipient for transformation and conjugation was positive for the *aci4* and *aci5* replicase genes, probably due to the integration of a multireplicon plasmid within the bacterial chromosome, since no extrachromosomal plasmids were identified for that strain (data not shown). Southern blot hybridization experiments performed with plasmid DNA purified from the 844T transformant confirmed that this bla_{OXA-58} plasmid possessed the *aci4* replicase gene (data not shown).

In conclusion, the AB-PBRT scheme for *A. baumannii* plasmid typing showed that donor strains often carried more than one plasmid type. However, each transformant or transconjugant carrying the bla_{OXA-58} or bla_{OXA-23} gene carried only a single replicon that was also identified from its corresponding donor strain by the AB-PBRT scheme.

AB-PBRT applied to our collection of *A. baumannii* strains

TABLE 6. Conjugal transfer and mobilization systems identified *in silico* on *A. baumannii* plasmids

Plasmid	CDS protein identifier	Conjugal transfer or mobilization protein name and function	Source of homology	Amino acid identity (% best hit)
pACICU2	P0058	Type IV secretory pathway, VirD4, TraD component	*Burkholderia cenocepacia*	40
	P0059	TraI, relaxase-helicase for conjugative transfer	*Pseudomonas* sp.	40
	P0070	TraA, conjugal transfer protein	*Acidithiobacillus ferrooxidans*	36
	P0071	TraL, putative membrane protein	*Acidovorax* sp.	40
	P0072	TraE, conjugative transfer protein	*Burkholderia cenocepacia*	30
	P0074	TraB, pilus assembly family protein	*Acidovorax* sp.	33
	P0075	DsbC precursor protein, disulfide isomerase	*Burkholderia thailandensis*	53
	P0076	TraV, membrane lipoprotein lipid attachment site	*Acidovorax* sp.	44
	P0077	TraC, conjugative transfer protein	*Burkholderia cenocepacia*	41
	P0079	TraW, conjugative transfer protein precursor	*Burkholderia cenocepacia*	46
	P0080	TraU, conjugative transfer protein precursor	*Burkholderia cenocepacia*	65
	P0081	Conjugative transfer protein	*Burkholderia cenocepacia*	43
	P0082	TraN, conjugal transfer mating pair stabilization	*Acidovorax* sp.	37
	P0083	TraF, conjugative transfer protein	*Burkholderia cenocepacia*	48
	P0085	TraH, conjugative transfer protein	*Burkholderia cenocepacia*	70
	P0086	TraG, domain containing protein	*Burkholderia thailandensis*	32
	P0088	DNA-directed DNA polymerase UmuC	*Acinetobacter* sp. strain ATCC 27244	57
	P0089	DNA-directed DNA polymerase RumB	*Acinetobacter baumannii* ATCC 17978	56
p1ABAYE	p1ABAYE0006	Putative mobilization protein, MobS-like	*Rhizobium leguminosarum*	40
	p1ABAYE0007	TraA, putative mobilization protein, MobL-like	*Sinorhizobium meliloti*	43
p1ABSDF	p1ABSDF0002	Putative mobilization protein, MobS-like	*Psychrobacter psychrophilus*	69
p2ABSDF	p2ABSDF0026	Putative mobilization protein, MobS-like	*Polaromonas naphthalenivorans*	46
	p2ABSDF0028	Putative mobilization protein, MobL-like	*Agrobacterium tumefaciens*	45
p3ABSDF	p3ABSDF0010	Putative mobilization protein, MobS-like	*Psychrobacter psychrophilus*	69
	p3ABSDF0011	Putative mobilization protein, MobL-like	*Agrobacterium tumefaciens*	46
pMAC02	pMAC_11	Putative mobilization protein, MobA-like	*Escherichia coli*	43
pMMCU1, pMMD	pMMCU1p5	Putative mobilization protein, MobA-like	*Escherichia coli*	49
pMMCU2	pMMCU2_06	Putative mobilization protein, MobA-like	*Escherichia coli*	49

demonstrated that the bla_{OXA-58}-positive plasmids differed, with six of them showing replicons belonging to GR3, including both the *aci3* and *aci7* replicase genes. A previously unidentified replicase of GR3 was also identified in the SDF strain. Three bla_{OXA-58}-positive plasmids carried the *aci1* or *aci2* replicase gene, belonging to GR2, and two plasmids carried the *aci8* or *aci9* gene, belonging to GR8. Interestingly, the AbA21 plasmid showed a replicase 99% homologous to the *repM-aci9* gene of pMAC02 but carried a different iteron sequence (Table 4). All isolates carrying the bla_{OXA-23} gene showed a positive PCR result for the *aci6* replicase gene, which was originally identified on plasmid pACICU2.

***In silico* analysis of *A. baumannii* plasmid maintenance and inheritance.** As extrachromosomal elements, plasmids bear the burden of ensuring their own segregation at cell division and employ various strategies, such as active partition systems and postsegregational killing mechanisms. These systems have never been described for *A. baumannii* plasmids. A careful annotation of the coding sequences from fully sequenced plasmids allowed the identification of putative ParA and ParB (3) partitioning proteins on plasmids pACICU1, pACICU2, and p3ABAYE (Table 5).

Plasmids pACICU1, p1ABAYE, and p2ABSDF encoded putative postsegregational killing systems. In particular, the orthologs of the RelBE toxin-antitoxin system of plasmids from *Escherichia coli* (20) were identified on pAUCU1 and p2ABSDF, while the orthologs of the Txe system of plasmid pRUM of *Enterococcus faecium* (16) were identified on the p1ABAYE plasmid (Table 5).

Plasmids pACICU1, pACICU2, p3ABAYE, p2ABSDF, and p3ABSDF also encoded putative restriction and antirestriction systems, including type I and type II restriction/modification enzymes, the HpaII and Bfii endonucleases, and their specific antirestriction methyltransferases (Table 5).

***A. baumannii* plasmid transferability.** Bacterial conjugation is one of the fundamental processes used for gene dissemination in nature. A putative conjugative system was identified only for plasmid pACICU2 (Table 6). This system is homologous to a conjugative system identified for uncharacterized plasmids of *Burkholderia cenocepacia* and *Burkholderia thailandensis*, suggesting a potential common origin of ancestor plasmids among these bacteria. The conjugative system of plasmid pACICU2 also showed a protein equivalent to the relaxase-helicase (TraI) belonging to a novel clade of the MOB_F family of relaxase proteins previously described for other transmissible plasmids from the prokaryotic kingdom (15, 21).

Even if plasmid pACICU2 was a unique plasmid endowed

with a conjugative apparatus, plasmids p1ABAYE, p1ABSDF, p2ABSDF, p3ABSDF pMMCU1, pMMCU2, pMAC02, and pMMD showed some orthologs of the MobS-MobL or MobA mobilization proteins that are characteristic of a number of small plasmids that are mobilizable by self-transmissible plasmids. These proteins are required for recognizing and cleaving the *nic* site, directing the complex to the transferosome determined by the conjugative element (14).

The transconjugants obtained from the bla_{OXA-23}-positive isolates harbored the *aci6* replicase gene of pACICU2 that was confirmed to be located on the bla_{OXA-23}-positive plasmid by Southern blot hybridization (data not shown). These results clearly indicate that plasmids similar to pACICU2 are present in those isolates and are able to self-conjugate. These pACICU2-related plasmids harbored the carbapenem resistance gene bla_{OXA-23}, which, however, was absent from the original fully sequenced pACICU2 plasmid (19). Noteworthy is the fact that the *aci6* replicase gene was also identified from 7 out of 13 bla_{OXA-58}-positive isolates but did not correspond to the replicon associated with this resistance gene. These findings open a new and interesting scenario describing the transmission of resistance plasmids into *A. baumannii*, since the pACICU2-like plasmids seem to be widely diffused and are likely responsible for both bla_{OXA-58} plasmid mobilization and bla_{OXA-23} plasmid self-conjugation.

Conclusion. The present study is the first to characterize the main features of the plasmids circulating among *A. baumannii* strains. Through an *in silico* analysis complemented by several experimental cloning experiments, 27 replicase genes have been identified. Primer sequences have been defined in order to characterize those 27 replicase genes, and a PCR-based methodology has been proposed to detect them in a convenient way. A multiplex approach has been set up by defining 19 distinct groups in 6 multiplexes, each of them grouping either three or four primer pairs that may allow faster and cheaper screening. Indeed, plasmid typing is a useful tool for studying their respective circulation and spread among members of the *Acinetobacter* genus and eventually among isolates of other genera. Through the epidemiological survey that has been conducted here, we exemplified what kind of approach that methodology can deserve. Here, we traced the diffusion of the carbapenem-hydrolyzing oxacillinase genes bla_{OXA-23} and bla_{OXA-58}, known to be the sources of resistance to carbapenems in *A. baumannii* worldwide. Interestingly, we showed that the current worldwide diffusion of the bla_{OXA-23} gene was mainly related to a single plasmid type and, conversely, that the diffusion of the bla_{OXA-58} gene was related to several unrelated plasmid types.

We aim to provide an easy, rapid, and reliable tool for investigating the plasmid epidemiology of *A. baumannii*. That kind of approach of performing plasmid typing will be useful and informative when studies focus on dissemination of specific markers only, such as a given antibiotic resistance gene, contributing to the better tracing of specific plasmids among a diversity of *A. baumannii* genetic backgrounds. This can be done in a way similar to that previously set up for the *Enterobacteriaceae* family that is now applied worldwide, and the corresponding so-called PBRT method is nowadays the main technique used to trace resistance plasmids among strains belonging to that family and improve knowledge of the evolution of drug resistance (4).

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REFERENCES

1. **Adams, M. D., K. Goglin, N. Molyneaux, K. M. Hujer, H. Lavender, J. J. Jamison, I. J. MacDonald, K. M. Martin, T. Russo, A. A. Campagnari, A. M. Hujer, R. A. Bonomo, and S. R. Gill.** 2008. Comparative genome sequence analysis of multidrug-resistant *Acinetobacter baumannii*. J. Bacteriol. **190:** 8053–8064.
2. **Bertini, A., L. Poirel, S. Bernabeu, D. Fortini, L. Villa, P. Nordmann, and A. Carattoli.** 2007. Multicopy bla_{OXA-58} gene as a source of high-level resistance to carbapenems in *Acinetobacter baumannii*. Antimicrob. Agents Chemother. **51:**2324–2328.
3. **Bignell, C., and C. M. Thomas.** 2001. The bacterial ParA-ParB partitioning proteins. J. Biotechnol. **91:**1–34.
4. **Carattoli, A.** 2009. Resistance plasmid families in *Enterobacteriaceae*. Antimicrob. Agents Chemother. **53:**2227–2238.
5. **Carattoli, A., A. Bertini, L. Villa, V. Falbo, K. L. Hopkins, and E. J. Threlfall.** 2005. Identification of plasmids by PCR-based replicon typing. J. Microbiol. Methods **63:**219–228.
6. **Chattoraj, D. K.** 2000. Control of plasmid DNA replication by iterons: no longer paradoxical. Mol. Microbiol. **37:**467–476.
7. **Corvec, S., L. Poirel, T. Naas, H. Drugeon, and P. Nordmann.** 2007. Genetics and expression of the carbapenem-hydrolyzing oxacillinase gene bla_{OXA-23} in *Acinetobacter baumannii*. Antimicrob. Agents Chemother. **51:**1530–1533.
8. **Couturier, M., F. Bex, P. L. Bergquist, and W. K. Maas.** 1988. Identification and classification of bacterial plasmids. Microbiol. Rev. **52:**375–395.
9. **D'Andrea, M. M., T. Giani, S. D'Arezzo, A. Capone, N. Petrosillo, P. Visca, F. Luzzaro, and G. M. Rossolini.** 2009. Characterization of pABVA01, a plasmid encoding the OXA-24 carbapenemase from Italian isolates of *Acinetobacter baumannii*. Antimicrob. Agents Chemother. **53:**3528–3533.
10. **Datta, N., and R. W. Hedges.** 1971. Compatibility groups among fi-R factors. Nature **234:**222–223.
11. **Datta, N.** 1975. Epidemiology and classification of plasmids, p. 9–15. *In* D. Schlessinger (ed.), Microbiology. American Society for Microbiology, Washington, DC.
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. **Fondi, M., G. Bacci, M. Brilli, M. C. Papaleo, A. Mengoni, M. Vaneechoutte, L. Dijkshoorn, and R. Fani.** 2010. Exploring the evolutionary dynamics of plasmids: the Acinetobacter pan-plasmidome. BMC Evol. Biol. **10:**59.
14. **Frost, L. S., K. Ippen-Ihler, and R. A. Skurray.** 1994. Analysis of the sequence and gene products of the transfer region of the F sex factor. J. Microbiol. Rev. **58:**162–210.
15. **Garcillán-Barcia, M. P., M. V. Francia, and F. de la Cruz.** 2009. The diversity of conjugative relaxases and its application in plasmid classification. FEMS Microbiol. Rev. **33:**657–687.
16. **Grady, R., and F. Hayes.** 2003. Axe-Txe, a broad-spectrum proteic toxin-antitoxin system specified by a multidrug-resistant, clinical isolate of *Enterococcus faecium*. Mol. Microbiol. **47:**1419–1432.
17. **Helinski, D. R.** 2004. Introduction to plasmids: a selective view of their history, p. 1–21. *In* B. E. Funnell and G. J. Philips (ed.), Plasmid biology. ASM Press, Washington, DC.
18. **Higgins, P. G., L. Poirel, M. Lehmann, P. Nordmann, and H. Seifert.** 2009. OXA-143, a novel carbapenem-hydrolyzing class D beta-lactamase in *Acinetobacter baumannii*. Antimicrob. Agents Chemother. **53:**5035–5038.
19. **Iacono, M., L. Villa, D. Fortini, R. Bordoni, F. Imperi, R. J. 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* strain belonging to the European clone II group. Antimicrob. Agents Chemother. **52:**2616–2625.
20. **Kim, Y., X. Wang, Q. Ma, X. S. Zhang, and T. K. Wood.** 2009. Toxin-antitoxin systems in *Escherichia coli* influence biofilm formation through YjgK (TabA) and fimbriae. J. Bacteriol. **191:**1258–1267.
21. **Lawley, T., B. M. Wilkins, and L. S. Frost.** 2004. Bacterial conjugation in Gram-negative bacteria, p. 203–226. *In* B. E. Funnell and G. J. Philips (ed.), Plasmid biology. ASM Press, Washington, DC.
22. **Marchand, I., L. Damier-Piolle, P. Courvalin, and T. Lambert.** 2004. Ex-

pression of the RND-type efflux pump AdeABC in *Acinetobacter baumannii* is regulated by the AdeRS two-component system. Antimicrob. Agents Chemother. **48:**3298–3304.
23. **Marqué, S., L. Poirel, C. Héritier, S. Brisse, M. D. Blasco, R. Filip, G. Coman, T. Naas, and P. Nordmann.** 2005. Regional occurrence of plasmid-mediated carbapenem-hydrolyzing oxacillinase OXA-58 in *Acinetobacter* spp. in Europe. J. Clin. Microbiol. **43:**4885–4888.
24. **Merino, M., J. Acosta, M. Poza, F. Sanz, A. Beceiro, F. Chaves, and G. Bou.** 2010. OXA-24 carbapenemase gene flanked by XerC/XerD-like recombination sites in different plasmids from different *Acinetobacter* species isolated during a nosocomial outbreak. Antimicrob. Agents Chemother. **54:**2724–2727.
25. **Mugnier, P. D., K. M. Bindayna, L. Poirel, and P. Nordmann.** 2009. Diversity of plasmid-mediated carbapenem-hydrolysing oxacillinases among carbapenem-resistant *Acinetobacter baumannii* isolates from Kingdom of Bahrain. J. Antimicrob. Chemother. **63:**1071–1073.
26. **Mugnier, P. D., L. Poirel, T. Naas, and P. Nordmann.** 2010. Worldwide dissemination of the bla_{OXA-23} carbapenemase gene of *Acinetobacter baumannii*. Emerg. Infect. Dis. **16:**35–40.
27. **Paulsson, J., and D. K. Chattoraj.** 2006. Origin inactivation in bacterial DNA replication control. Mol. Microbiol. **61:**9–15.
28. **Peleg, A. Y., H. Seifert, and D. L. Paterson.** 2008. *Acinetobacter baumannii*: emergence of a successful pathogen. Clin. Microbiol. Rev. **21:**538–582.
29. **Poirel, L., T. Naas, and P. Nordmann.** 2010. Diversity, epidemiology, and genetics of class D beta-lactamases. Antimicrob. Agents Chemother. **54:**24–38.
30. **Poirel, L., W. Mansour, O. Bouallegue, and P. Nordmann.** 2008. Carbapenem-resistant *Acinetobacter baumannii* isolates from Tunisia producing the OXA-58-like carbapenem-hydrolyzing oxacillinase OXA-97. Antimicrob. Agents Chemother. **52:**1613–1617.
31. **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.
32. **Smith, M. G., T. A. Gianoulis, S. Pukatzki, J. J. Mekalanos, L. N. Ornston, M. Gerstein, and M. Snyder.** 2007. New insights into *Acinetobacter baumannii* pathogenesis revealed by high-density pyrosequencing and transposon mutagenesis. Genes Dev. **21:**601–614.
33. **Sykora, P.** 1992. Macroevolution of plasmids: a model for plasmid speciation. J. Theor. Biol. **159:**53–65.
34. **Vallenet, D., P. Nordmann, V. Barbe, L. Poirel, S. Mangenot, E. Bataille, C. Dossat, S. Gas, A. Kreimeyer, P. Lenoble, S. Oztas, J. Poulain, B. Segurens, C. Robert, C. Abergel, J. M. Claverie, D. Raoult, C. Médigue, J. Weissenbach, and S. Cruveiller.** 2008. Comparative analysis of *Acinetobacters*: three genomes for three lifestyles. PLoS One **19:**e1805.
35. **Zarrilli, R., D. Vitale, A. Di Popolo, M. Bagattini, Z. Daoud, A. U. Khan, C. Afif, and M. Triassi.** 2008. A plasmid-borne bla_{OXA-58} gene confers imipenem resistance to *Acinetobacter baumannii* isolates from a Lebanese hospital. Antimicrob. Agents Chemother. **52:**4115–4120.