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First Occurrence of OXA-72-Producing *Acinetobacter baumannii* in Serbia

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Here, we characterized the first OXA-72-producing *Acinetobacter baumannii* isolate (designated MAL) recovered from a urine sample from a Serbian patient. Antimicrobial susceptibility testing, plasmid analysis, and whole-genome sequencing (WGS) were performed to fully characterize the resistome of the *A. baumannii* MAL clinical isolate. The isolate was multidrug resistant and remained susceptible only to colistin and tigecycline. PCR analysis revealed the presence of the carbapenemase OXA-72, an OXA-40 variant. Extraction by the Kieser method revealed the presence of two plasmids, and one of these, a ca. 10-kb plasmid, harbored the bla_{OXA-72} gene. WGS revealed 206 contigs corresponding to a genome of 3.9 Mbp in size with a G+C content of 38.8%. The isolate belonged to sequence type 492 and to worldwide clone II (WWCII). Naturally occurring β-lactamase-encoding genes (bla_{ADC-25} and bla_{OXA-66}) were also identified. Aminoglycoside resistance genes encoding one aminoglycoside adenyltransferase (*aadA2*), three aminoglycoside phosphatases (*strA*, *strB*, *aphA6*), and one 16S RNA methylase (*armA*) conferring resistance to all aminoglycosides were identified. Resistance to fluoroquinolones was likely due to mutations in *gyrA*, *parC*, and *parE*. Of note, the resistome matched perfectly with the antibiotic susceptibility testing results.

The circumstances that allow *Acinetobacter* to assume a pathogenic role are not well understood. Accordingly, most *Acinetobacter* species are considered opportunistic pathogens responsible for nosocomial infections, mostly in debilitated patients and/or external device carriers (1, 2). *Acinetobacter baumannii* was frequently involved in nosocomial outbreaks that occurred mostly in intensive care units (2). The *A. baumannii*-related infections ranged from septicemia to pneumonia and urinary tract infections, often associated with catheters (2). During the last 3 decades, multidrug resistance has increasingly been reported in *A. baumannii*, resulting in carbapenems being considered the last-resort antibiotic for the treatment of infections with this pathogen (3).

Unfortunately, the overuse of carbapenems has rapidly resulted in the worldwide dissemination of carbapenem-resistant *A. baumannii* strains, raising serious concerns about antimicrobial treatment options. In *Acinetobacter* spp., the most prevalent mechanism responsible for carbapenem resistance is the production of a carbapenem-hydrolyzing Ambler class D β-lactamase (CHDL) (3). These CHDLs include both (i) acquired carbapenemases of the OXA-23, OXA-24/40, OXA-58, OXA-143, and OXA-235 types (4) and (ii) enzymes with the IS*Aba1*-enhanced expression of the intrinsic chromosomal $bla_{OXA-51-like}$ gene (3). Except for their detection in a few isolates of OXA-23-producing *Proteus mirabilis* (5, 6), CHDLs are currently restricted to *Acinetobacter* species.

Aminoglycosides are also currently used in the treatment of severe infections. In *A. baumannii*, aminoglycoside resistance might rely on (i) decreased outer membrane permeability, (ii) active efflux, (iii) modifications in ribosomal proteins, and (iv) most often, enzymatic modification of the drug. Recently, methylation of 16S rRNA has emerged in Gram-negative organisms, leading to methylation of the 16S rRNA target, which subsequently confers a high level of resistance to all clinically useful aminoglycosides (i.e., amikacin, gentamicin, and tobramycin) (7). Among the currently described 16S rRNA methylases, the ArmA enzyme is predominant in *A. baumannii*. In addition, it is often associated with the carbapenemase OXA-23 (3).

Here, using a whole-genome sequencing (WGS) approach, we have characterized an OXA-72-producing *A. baumannii* isolate recovered from a urine sample from a Serbian patient.

MATERIALS AND METHODS

Antimicrobial susceptibility testing. Antimicrobial susceptibility was determined by the disk diffusion method and interpreted according to EUCAST guidelines, as updated in 2015 (www.eucast.org). The MICs were determined using Etest (bioMérieux, La Balme-les-Grottes, France) on Mueller-Hinton agar at 37°C.

Plasmid identification and transformation. Plasmid DNA of the *A. baumannii* MAL strain was extracted using the Kieser method, as previously described (8). Transfer of the β-lactam resistance markers was attempted by electroporation of the plasmid DNA suspension into electrocompetent *A. baumannii* CIP 70.10 cells. Transformants were selected on Trypticase soy agar supplemented with ticarcillin at 100 μg/ml. Plasmids

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TABLE 1 MICs of β-lactams for *A. baumannii* MAL, *A. baumannii* CIP 70.10 transformed with the natural plasmid carrying bla_{OXA-72} (pAB-MAL-1), and the *A. baumannii* CIP 70.10 reference strain

β-Lactam	MIC (mg/liter)		
	A. baumannii MAL	*A. baumannii* CIP 70.10(pAB-MAL-1)	*A. baumannii* CIP 70.10
Ticarcillin	>256	>256	8
Ticarcillin + CLA[a]	>256	>256	8
Piperacillin	>256	>256	4
Piperacillin + TZB[b]	>256	128	4
Cefotaxime	>64	32	32
Ceftazidime	>64	4	4
Cefepime	64	16	16
Imipenem	>32	>32	0.25
Meropenem	>32	>32	0.5
Doripenem	>32	>32	0.25

[a] CLA, clavulanic acid (4 mg/liter).
[b] TZB, tazobactam (4 mg/liter).

of ca. 154, 66, 48, and 7 kb from *Escherichia coli* NCTC 50192 were used as plasmid size markers. Plasmid DNAs of *A. baumannii* MAL and its transformants were analyzed by agarose gel electrophoresis. In addition, the bla_{OXA-72}-harboring plasmid, named pAB-MAL-1, was extracted from the transformant using a QIAprep spin miniprep kit (Qiagen, Courtaboeuf, France) and sequenced separately, as described below.

Detection of carbapenemase activity. The carbapenemase activity was searched for using the CarbAcineto NP test as previously described (9). The CarbAcineto NP test, which detects imipenemase activity, was performed using a bacterial inoculum taken directly from the antibiogram on Mueller-Hinton medium.

WGS and sequence analysis. Briefly, total DNA was isolated using an UltraClean microbial DNA isolation kit (Mo Bio Laboratories) from overnight cultures on blood agar (Bio-Rad, Marnes-la-Coquette, France). Genomic DNA quantifications were performed using a Qubit fluorometer (Life Technologies, Carlsbad, CA), and DNA quantities were adjusted to 0.2 ng/μl. Library preparation was performed using a Nextera XT DNA sample preparation kit (Illumina, San Diego, CA). Sequencing was performed on an Illumina MiSeq 2000 sequencer with v3 chemistry using two 75-bp paired-end reads at a raw cluster density of ~1,300,000 clusters/mm^2.

The contigs were fully annotated using the RAST server (http://rast.nmpdr.org/), which predicted 3,710 coding sequences in the genome.

Total raw data sequences of the *A. baumannii* MAL were subjected to analysis with the ResFinder (v2.1) server (http://www.genomicepidemiology.org/), which is dedicated to the identification of acquired antimicrobial resistance genes (10). Genes conferring resistance to aminoglycosides, β-lactams, fluoroquinolones, fosfomycin, macrolides, lincosamide, streptogramin B, phenicol, rifampin, sulfonamide, tetracycline, and trimethoprim were sought using a 98% threshold for nucleotide sequence identity and 60% for the minimum length coverage.

Multilocus sequence typing (MLST) was performed using the MLST (v1.8) server (http://www.genomicepidemiology.org/) as previously described (11). The two different MLST schemes (the Oxford and Pasteur schemes) available for *A. baumannii* were tested (http://pubmlst.org/abaumannii/) (12, 13).

Accession number(s). The *A. baumannii* MAL genome sequence corresponds to GenBank accession number LKIB00000000 (14). GenBank accession numbers for plasmids pAB-MAL-1 and pAB-MAL-2 are KX230793 and KX230794, respectively.

RESULTS

Clinical history and characteristics of the *A. baumannii* MAL clinical isolate. A man in his 70s living in Serbia was admitted to a Serbian hospital for acute urinary retention. An indwelling long-term urethral catheter was inserted, and the patient was discharged. One month later, he was diagnosed with a urinary tract infection and treated with cefixime for 2 weeks; the urethral catheter was not removed. Two weeks after the end of the antimicrobial treatment, the patient was admitted to the emergency unit of a hospital in the suburbs of Paris, France, because of high fever, perineal pain, and dysuria. The urethral catheter was removed, and ceftriaxone and gentamicin were administered. Culture of the urine grew an *Enterococcus faecalis* isolate (10^5 CFU/ml) and an *A. baumannii* isolate named MAL (10^6 CFU/ml). Antimicrobial susceptibility testing revealed that the *E. faecalis* isolate possessed a wild-type phenotype and that *A. baumannii* MAL was resistant to all tested antimicrobial molecules except colistin and tigecycline (Table 1). The diagnosis of *E. faecalis* prostatitis and catheter-associated asymptomatic bacteriuria with *A. baumannii* MAL was made. Amoxicillin and gentamicin treatment was initiated, and this was followed by sulfamethoxazole-trimethoprim treatment. The fever rapidly disappeared, and the urinary flow was completely restored.

In order to characterize the underlying mechanism responsible for carbapenem resistance in *A. baumannii* MAL and rapidly implement infection control measures (including placement of the patient in an individual room and the use of dedicated medical staff), the CarbAcineto NP test was performed directly on the antibiogram as previously described (9). This test confirmed in less than 2 h the presence of a carbapenem-hydrolyzing enzyme; thus, proper infection control measures could be implemented rapidly, preventing the further spread of this highly drug-resistant bacterium.

Genomic features of *A. baumannii* MAL. WGS of the *A. baumannii* MAL isolate gave a total of 2,611,036 reads with an average length of 75.17 bp (14). Two hundred six contigs were obtained using the CLC genomic workbench (v8.5), corresponding to a total of 3,914,647 bp, including the chromosome and plasmids, with a G+C content of 38.8%, which is in accordance with that for *A. baumannii* species.

The global analysis of *A. baumannii* MAL sequences identified the bla_{OXA-72} gene encoding the OXA-72 carbapenemase, which is responsible for carbapenem hydrolysis and which was detected with the CarbAcineto NP test (Table 2). The resistance to all β-lactams was completed by the overexpression of the intrinsic chromosomally encoded ADC-25 cephalosporinase due to the insertion of IS*Aba1* upstream of the bla_{ADC-25} gene. No insertion

TABLE 2 WGS-detected resistance genes and *in silico*-deduced antimicrobial resistance phenotype

Antibiotic family	Resistance gene	% identity	Localization	Enzyme		GenBank accession no.	Antimicrobial resistance[c]	
				Name	Function		*In silico* deduced	Observed
Aminoglycosides	*aadA2*	100	Chromosome	AAD	Aminoglycoside adenylase	JQ364967	GEN, TOB	GEN, TOB, NET, AMK
	strA	100	Chromosome	APH(3″)-1b	Aminoglycoside phosphatase	M96392	TOB, AMK	GEN, TOB, NET, AMK
	strB	100	Chromosome	APH(6)-1d	Aminoglycoside phosphatase	M96392	STR	GEN, TOB, NET, AMK
	aphA6	100	Chromosome	APH(3′)-VIa	Aminoglycoside phosphatase	X07753	AMK	GEN, TOB, NET, AMK
	armA	100	Chromosome	ArmA	16S RNA methylase	AY220558	GEN, TOB, NET, AMK	GEN, TOB, NET, AMK
β-Lactams	$bla_{\text{ADC-25}}$	99.91[a]	Chromosome	ADC-25	Cephalosporinase	EF016355	AMX, AMC, cephalosporins	All β-lactams
	$bla_{\text{OXA-66}}$	100	Chromosome	OXA-66	CHDL[b]	FJ360530	None[d]	All β-lactams
	$bla_{\text{OXA-72}}$	100	Plasmid	OXA-72	CHDL	GU199039	All β-lactams except cephalosporins	All β-lactams
Macrolides, lincosamide, and streptogramin	*mphE*	100	Chromosome	MphE	Macrolide phosphatase	EU294228	ERY	ERY
	msrE	100	Chromosome	MsrE	Macrolide efflux protein	EU294228	ERY	ERY
Sulfonamide	*sul1*	100	Chromosome	Sul1	Alternate dihydropteroate synthetase	CP002151	SUL	SUL
	sul2	100	Chromosome	Sul2	Alternate dihydropteroate synthetase	GQ421466	SUL	SUL
Trimethoprim	*dfrA12*	100	Chromosome	DfrA12	Alternate dihydropteroate reductase	AB571791	TMP	TMP
Tetracycline	*tetB*	100	Chromosome	TetB	Efflux pump	AP000342	TET	TET

[a] This 99.91% nucleotide identity with $bla_{\text{ADC-25}}$ corresponds to 100% amino acid similarity with ADC-25.
[b] CHDL, carbapenem-hydrolyzing Ambler class D β-lactamase.
[c] GEN, gentamicin; TOB, tobramycin; AMK, amikacin; NET, netilmicin; STR, streptomycin; AMX, amoxicillin; AMC, amoxicillin-clavulanic acid; ERY, erythromycin; SUL, sulfonamide; TMP, trimethoprim; TET, tetracycline.
[d] OXA-66 is a chromosomally encoded CHDL which is normally not expressed. If $bla_{\text{OXA-66}}$ is associated with an upstream insertion sequence (IS), it leads to the expression of the CHDL, resulting in decreased susceptibility to carbapenems.

sequence was identified upstream of the intrinsic chromosomally encoded OXA-66 oxacillinase, suggesting a likely basal expression. Five genes encoding aminoglycoside resistance determinants were identified: one aminoglycoside adenylase (*aadA2*), three aminoglycoside phosphatases (*strA*, *strB*, *aphA6*), and one 16S RNA methylase (*armA*) responsible for resistance to all aminoglycosides (Table 2). Although resistance to macrolides was mediated by the impermeability of Gram-negative rods, two macrolide resistance genes were identified, namely, *mph*(E) and *msr*(E). The products of the *sul1*, *sul2*, and *dfrA12* genes caused resistance to sulfamethoxazole-trimethoprim, and tetracycline resistance was mediated by *tet*(B). The localization of all acquired resistance genes is shown in Table 2. No acquired genes encoding resistance to quinolones were found in *A. baumannii* MAL. However, after comparison of the sequence of *A. baumannii* MAL with that of the *A. baumannii* ATCC 19606 reference strain, we identified substitutions in GyrA (Ser-81-Leu), ParC (Ser-84-Leu, Ser-467-Gly), and ParE (V-237-Ala). These mutations in GyrA and ParC have previously been shown to be responsible for resistance to fluoroquinolones in *Acinetobacter* species (15–17).

Using the MLST (v1.8) server, we found that *A. baumannii* MAL belongs to sequence type (ST) 492 (ST492), according to the Pasteur scheme, and to a novel ST, according to the Oxford database.

The genome of *A. baumannii* MAL was further analyzed, especially in respect to the sequences of different porins, which were compared to the sequences of porins encoded by the *A. baumannii* ATCC 19606 reference genome. The sequence of the *carO* gene exhibited a low percent identity with the sequence of *carO* from *A. baumannii* ATCC 19606 (73% amino acid identity), with 57 different substitutions and a deletion of 3 amino acids in the protein. Three substitutions (Ala-137-Glu Cys-163-Arg, and Asp-172-Glu) were identified in OmpA, and two substitutions (Leu-47-Iso and Thr-48-Ser) were identified in OprD. A last porin, namely, the 33- to 36-kDa Omp, was analyzed and showed a perfect identity with that of *A. baumannii* ATCC 19606.

The regulatory efflux pumps AdeR and AdeS of *A. baumannii* MAL showed 99% (3 substitutions) and 97% (10 substitutions) amino acid sequence identities, respectively, with those of *A. baumannii* ATCC 19606, whereas AdeN and AdeB of *A. baumannii* MAL showed 99% amino acid sequence identities with those of *A. baumannii* ATCC 19606.

A. baumannii MAL possesses an AbaR element within the *comM* gene but an intact *uspA* gene, giving rise to the minimal form of the AbaR variant, which might be involved in the acquisition of several antibiotic resistance genes identified by WGS.

Plasmid characterization. Using the Kieser extraction method (8), two distinct plasmids of ca. ~10 kb and ~67 kb were identified (data not shown). Transformation of the Kieser extracts into *A. baumannii* CIP 70.10 revealed that the bla_{OXA-72} gene encoding the carbapenemase was located on the ca. ~10-kb plasmid, named pAB-MAL-1 (Table 1). WGS revealed that plasmid pAB-MAL-1 was 9,810 bp in size and belonged to the GR2 plasmid family. This plasmid was 99% identical to the recently published pA105-2 plasmid (GenBank accession number KR535993.1) recovered from an *A. baumannii* strain of ST636 (18). It showed strong similarity with plasmid p2ABST25 (GenBank accession number AEPA01000396.1) (19), differing only by the insertion of IS*Aba31* upstream of the bla_{OXA-72} gene (Fig. 1). In addition, these two plasmids were highly similar to plasmid pAB0057 (GenBank accession number NC_011585.1) (20). The major difference with plasmid pAB0057 consisted of the replacement of the XerC/XerD flanking sequence by a nucleotide sequence containing the bla_{OXA-72} gene and IS*Aba31* (Fig. 1). Another similar plasmid is pAB120 (GenBank accession number NC_019359.1), recovered from a carbapenem-resistant *A. baumannii* isolate recovered in Lithuanian hospitals (Fig. 1) (21). The main differences between pAB-MAL-1 and pAB120 reside in the number of bla_{OXA-72} gene copies (two in pAB120) and the inversion of a ca. ~4.4-kb-long sequence containing the septocolysin (*sep*) and the TonB-dependent receptor genes (Fig. 1).

The second plasmid, named pAB-MAL-2 (67,025 bp), was related to two previously described *repAci6* plasmids named pD72-2 and pD46-3 (GenBank accession number KM977710) (Fig. 2) (22). Since no antibiotic resistance genes are located on this plasmid, no transformant could be obtained. As shown in Fig. 2, pAB-MAL-2 and pD72-2 differed by only 61 single nucleotide polymorphisms (SNPs) and a composite transposon Tn*aphA6* containing the *aphA6* gene flanked by two copies of IS*Aba125* inserted at nucleotide position 21,406 bp. Plasmids pD46-3 and pD72-2 shared 99.99% sequence identity with only five SNPs in their backbone and differed only by the insertion of Tn*2006*, which includes the bla_{OXA-23} gene.

DISCUSSION

To the best of our knowledge, this is the first description of an OXA-72-producing *A. baumannii* isolate from Serbia. OXA-72 is a single-amino-acid variant of OXA-24/40 (G224D). In contrast to OXA-23, which is the most common acquired carbapenemase in *A. baumannii* worldwide, OXA-72-producing isolates have been reported in only a few countries, including Brazil, Colombia, Taiwan, France, Croatia, Poland, Italy, Lithuania, and Sweden (19, 21, 23–30). The clonal spread of OXA-72-producing *A. baumannii* isolates was previously reported in the Balkan region, in a Croatian university hospital (24). Although this outbreak was related to a WWCII of *A. baumannii*, the plasmidic location of the bla_{OXA-72} was not evidenced. In addition, we found that the *A. baumannii* MAL belonged to a novel ST, ST492. This ST is a single-locus variant (SLV) of ST2 (with a single mutation in the *fusA* allele), indicating that the *A. baumannii* MAL isolate belongs to worldwide clone II (or global clone GC-2) (31). This clone is widely distributed and responsible for the dissemination of carbapenemases, including OXA-23, and OXA-24/40-like enzymes, including OXA-72 (31). Interestingly, bla_{OXA-72} was mostly identified in GC-2 isolates and was rarely reported from non-GC-2 *A. baumannii* isolates (32, 33). Our findings suggest that *A. baumannii* ST492 may possess all the features necessary for the efficient dissemination of OXA-72.

In *A. baumannii*, permeability defects, such as porin modification of efflux system overexpression, might be involved in antimicrobial resistance. As an example, the CarO porin has been advocated to play a role in carbapenem resistance (34). Since the CarO porin of *A. baumannii* MAL shares only 73% amino acid sequence identity with wild-type CarO, we could not exclude the possibility that it might also add to carbapenem resistance. In addition, although no direct evidence of the role of the porins OmpA and OprD in antimicrobial resistance has been demonstrated, the observed changes may act on their functionality and, thus, on membrane permeability to several antimicrobial compounds. Similarly, we have analyzed the sequence of efflux pump regulatory

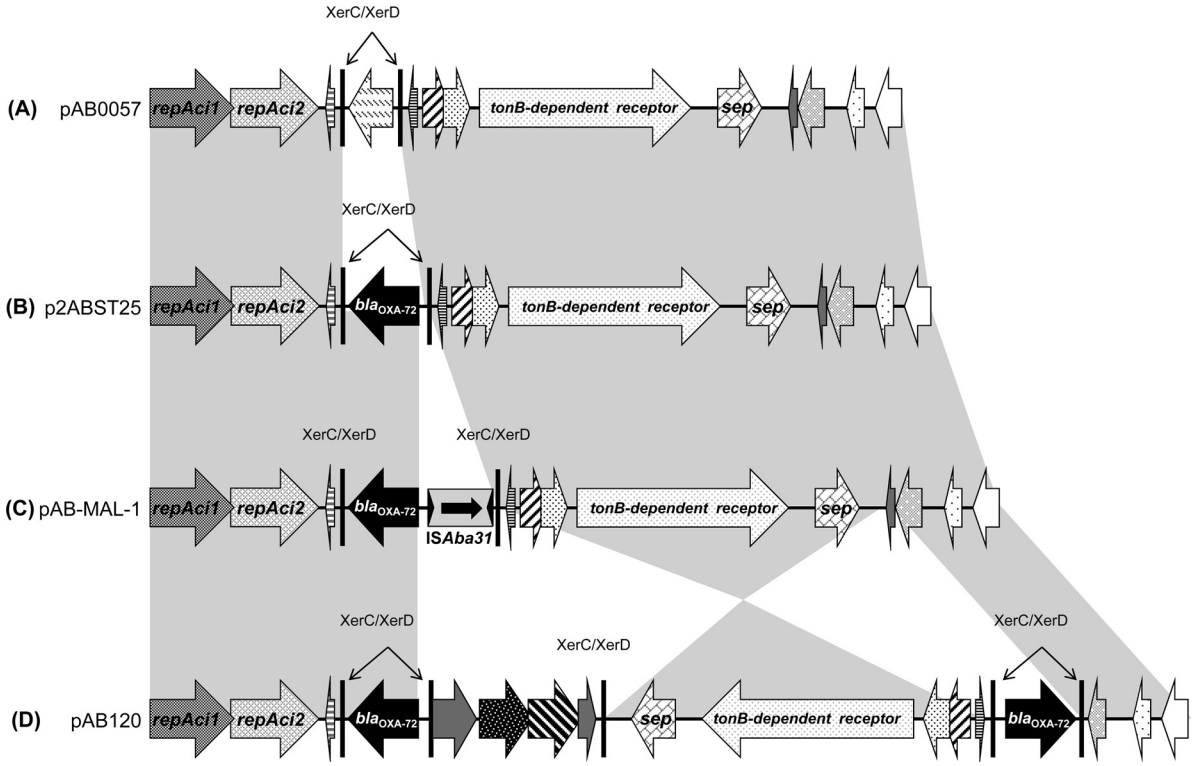

FIG 1 Schematic representation of pAB-MAL-1, the bla_{OXA-72} gene-harboring plasmid of *A. baumannii* MAL, and of its related plasmids. Common features are highlighted with gray shading. (A) Plasmid pAB0057 from *A. baumannii* AB0057 (GenBank accession number NC_011585.1) (20); (B) plasmid p2ABST25 (GenBank accession number AEPA01000396.1) (19); (C) plasmid pAB-MAL-1; (D) plasmid pAB120 from a carbapenem-resistant *A. baumannii* isolate from Lithuania (GenBank accession number NC_019359.1) (21). Gene names are as follows: *repAci*, replicase gene; *sep*, septicolysin-encoding gene; IS*Aba31*, insertion sequence of *A. baumannii* 31; XerC/XerD, specific site of the tyrosine recombinases XerC and XerD.

genes, which are known to be involved in antibiotic resistance, namely, the AdeR/S two-component system and AdeN and AdeB, which regulate the AdeIJK and AdeAB efflux pumps, respectively (35–37). Although only a few substitutions were found in efflux pump regulatory genes, further investigations using reverse transcription-PCR will be necessary to correlate these substitutions with overexpression of these efflux pumps.

The AbaR variant element is a putative class II transposon involved in the acquisition of resistance genes in *A. baumannii*, as exemplified by the discovery that AbaR1 possesses more than 14 resistance genes inserted within the *comM* gene (38). More than 20 different AbaR variants have been characterized to date (39). Two different AbaR variants were described: either the AbaR3-like variant, a large genetic element carrying numerous resistance genes, or its minimal form (40). These two forms differ by the acquisition of a large composite transposon within the *uspA* gene (40). *A. baumannii* MAL possesses the minimal form of AbaR, which might be involved in the acquisition of antibiotic resistance genes.

Globally, the *in silico* antimicrobial susceptibility profile, predicted using WGS, perfectly correlates with the observed phenotype of the *A. baumannii* MAL isolate.

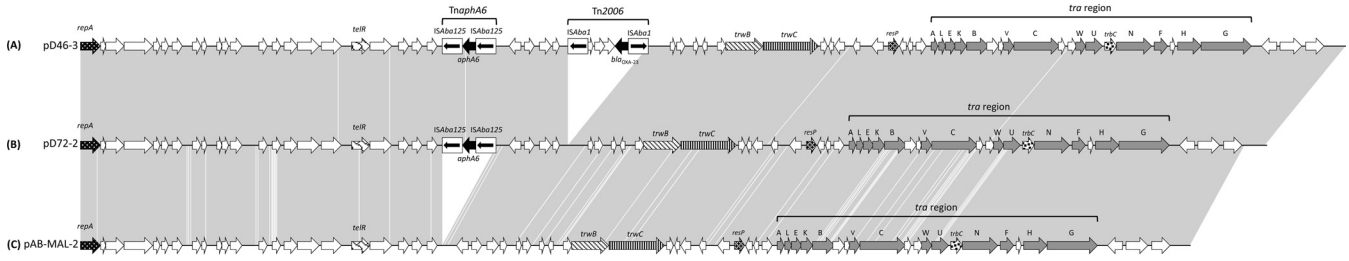

FIG 2 Schematic representation of pAB-MAL-2 of *A. baumannii* MAL and of its closely related plasmids. Common features are highlighted with gray shading. (A) Plasmid pD46-3 plasmid from *A. baumannii* D46 (GenBank accession number KM977710) (22); (B) plasmid pD72-2 from *A. baumannii* D72 (GenBank accession number NC_025111) (22); (C) plasmid pAB-MAL-2. Gene names are as follows; *repA*, replicase gene; *telR*, tellurite resistance gene; IS, insertion sequence; Tn, composite transposon; *aphA6*, aminoglycoside phosphotransferase A6; *trwB*, gene encoding an inner membrane nucleoside triphosphate-binding protein involved in plasmid transfer; *trwC*, gene encoding a helicase/relaxase involved in plasmid transfer; *resP*, gene encoding a putative resolvase; *tra*, plasmid transfer genes; *trbC*, gene involved in mating pair formation; white arrows, putative open reading frames with an unknown function.

Two plasmids were identified in our isolate. The first one carried the bla_{OXA-72} gene and shared similarity with the pAB120 plasmid, which was recovered from a carbapenem-resistant *A. baumannii* isolate recovered in Lithuanian hospitals and which carried two copies of the bla_{OXA-72} gene (21). Our study suggests that our plasmid carrying bla_{OXA-72} (pAB-MAL-1) might be derived from the p2ABST25 plasmid (only with an insertion of IS*Aba31* upstream of the bla_{OXA-72} gene), which itself might be considered an intermediate between pAB0057, which does not carry bla_{OXA-72} but with which p2ABST25 shares a common backbone, and pAB120, which carries two copies of the bla_{OXA-72} gene with an inversion in the common backbone (Fig. 1). The second plasmid (pAB-MAL-2) was found to share high similarity and a common backbone with the recently described pAD46-3 and pA105-2 plasmids, recovered from an *A. baumannii* isolate collected in Australia in 2010 (Fig. 2) (22) and Sweden in 2013 (18), respectively. As shown in Fig. 2, the pAB-MAL-2 plasmid might be considered the progenitor of pD72-2, in which a composite transposon (Tn*aphA6*) has been inserted. Plasmid pD72-2 might itself be considered the progenitor of plasmid pD46-3, in which Tn*2006* containing the bla_{OXA-23} gene has been inserted (Fig. 2) (22). Sixty-one SNPs were identified between pAB-MAL-2 and pD72-2, suggesting an ancient acquisition of Tn*aphA6* by pAB-MAL-2. In comparison, only five SNPs were found in the plasmid backbones of pD46-3 and pD72-2, suggesting a very recent acquisition of Tn*2006* by pD72-2 (Fig. 2). The pAD46-3 plasmid has been reported to be a conjugative plasmid, which highlights the possible role of such *repAci6* plasmids in the spread of antimicrobial resistance determinants worldwide. Hence, further studies are needed to evaluate the exact role of these types of plasmids in the global spread of antibiotic resistance genes in *A. baumannii*.

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We have no conflicts to declare.

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