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Dissemination of a Carbapenem-Resistant *Acinetobacter baumannii* Strain Belonging to International Clone II/Sequence Type 2 and Harboring a Novel AbaR4-Like Resistance Island in Latvia

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An outbreak of hospital-acquired *Acinetobacter baumannii* infections, caused by a bla_{OXA-23}-positive carbapenem-resistant strain belonging to international clone II/ST2, was detected in Latvia. The strain was partially equipped with the *armA* gene and the *intI1-aacA4-catB8-aadA1-qacEΔ1* class 1 integron. In addition, the strain carried AbaR25, a novel AbaR4-like resistance island of ~46,500 bp containing structures similar to the previously described AbaR22 and Tn*6167* islands. AbaR25 was characterized by the occurrence of a second copy of Tn*6022a* interrupted by Tn*2006* carrying the bla_{OXA-23} gene.

Acinetobacter baumannii is an aerobic Gram-negative opportunistic pathogen with a remarkable ability to acquire resistance to different classes of antibiotics (1). The increased detection of multidrug-resistant (MDR) and carbapenem-resistant (CR) *A. baumannii* strains in clinical settings is mainly linked to the global dissemination of a number of highly successful clones, such as international clones I and II and multilocus sequence types (STs) 15 and ST25 (2).

Resistance to carbapenems in *A. baumannii* is primarily mediated by the production of carbapenem-hydrolyzing β-lactamases (3). Class B metallo-β-lactamases (MBLs) confer high levels of carbapenem resistance as well as resistance to all other β-lactams except for aztreonam, while the substrate profile of class D OXA-type carbapenemases is commonly diverse, with most of these enzymes showing a limited hydrolytic activity against imipenem and meropenem (3). The occurrence of genes encoding aminoglycoside-modifying enzymes (AME) is the main mechanism of resistance to aminoglycosides in *A. baumannii* (4). However, strains producing the 16S rRNA methylase ArmA have also been identified (5). ArmA has so far been a plasmid-encoded enzyme conferring high levels of resistance to several aminoglycosides (5).

Genomic resistance islands in *A. baumannii* (AbaR), first detected in 2006, can be sorted into two main models based on their genetic structures (6, 7). The first model, AbaR3-like, consists of Tn*6019* as a backbone transposon and has, with the exception of AbaR2, been found only among isolates belonging to international clone I (6–10). The second model, AbaR4-like, consists of Tn*6022* as a backbone transposon and has mainly been identified among isolates from international clone II (11–14). Complex structures of AbaR4-like islands, such as Tn*6167* and AbaR22, have recently been described (15, 16). Importantly, the AbaR4-like islands have repeatedly been found to be interrupted by Tn*2006* carrying the $bla_{OXA-23-like}$ gene (12–15).

The aim of this study was to investigate the molecular epidemiology and antimicrobial resistance characteristics of all the CR *A. baumannii* blood culture isolates ($n = 30$) obtained at the P. Stradins University Hospital (SUH) in Riga, Latvia, between May 2008 and December 2009. The study also included five invasive (cerebrospinal fluid and blood culture) CR *A. baumannii* isolates collected by four hospitals from different cities in Latvia between March and July 2009 (Table 1).

Resistance to carbapenems was confirmed in all the isolates (see Table S1 in the supplemental material). Thirty-four isolates showed high levels of resistance to ciprofloxacin. In addition, high levels of resistance to amikacin, gentamicin, and tobramycin were detected in 24 of these isolates. All isolates were susceptible to colistin. Pulsed-field gel electrophoresis (PFGE), using ApaI-digested genomic DNA, assigned all the 34 ciprofloxacin-resistant isolates to indistinguishable or closely related patterns, showing >80% similarity to each other (Table 1) (17). Only the ciprofloxacin-susceptible isolate belonged to a possibly related PFGE pattern showing 70% to 80% similarity to the other patterns. The isolates belonged to international clone II ($n = 33$) and PCR-based group 4 ($n = 2$), using two multiplex PCRs targeting the *ompA*, *csuE*, and $bla_{OXA-51-like}$ genes (18). Of note, the band pattern of PCR-based group 4 differs from that of international clone II only in the negative result for the *csuE* allele, which could simply be due to a single polymorphism in the primer annealing regions (19). Multilocus sequence typing (MLST) was performed on eight isolates with different PFGE patterns, including the two isolates from PCR-based group 4 (http://www.pasteur.fr/recherche/genopole/PF8/mlst/). The isolates were all sorted into ST2 (Table 1).

PCR assays were used to detect antimicrobial resistance genes encoding the OXA carbapenemases ($bla_{OXA-51-like}$, $bla_{OXA-23-like}$, $bla_{OXA-24-like}$, and $bla_{OXA-58-like}$), metallo-β-lactamases (bla_{VIM}, bla_{GIM}, bla_{IMP}, and bla_{SPM}), and 16S rRNA methylases (*armA*,

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TABLE 1 Molecular detection of particular antimicrobial resistance genes and elements in 35 OXA-23-producing *A. baumannii* isolates collected in Latvia[a]

Isolate	Date of isolation (mo/yr)	Hospital	*armA*	*aacA4-catB8-aadA1*	AbaR	Clonal lineage	PFGE	MLST
K51-65	5/2008	PSCUH	+	+	AbaR25	Int. II	A1	ST2
K51-66	6/2008	PSCUH	+	+	AbaR25	Int. II	A2	ST2
K51-67	7/2008	PSCUH	−	−	AbaR4	Int. II	B	ST2
K51-68	9/2008	PSCUH	+	−	AbaR25	Group 4	A3	ST2
K51-69	10/2008	PSCUH	+	+	AbaR25	Int. II	A1	ND
K51-70	10/2008	PSCUH	+	+	AbaR25	Int. II	A1	ND
K51-71	11/2008	PSCUH	+	+	AbaR25	Int. II	A1	ND
K51-72	12/2008	PSCUH	+	+	AbaR25	Int. II	A1	ST2
K51-73	12/2008	PSCUH	+	+	AbaR25	Int. II	A1	ND
K51-74	1/2009	PSCUH	+	+	ΔAbaR25	Int. II	A4	ND
K51-75	1/2009	PSCUH	+	+	AbaR25	Int. II	A1	ND
K51-76	1/2009	PSCUH	+	+	AbaR25	Int. II	A5	ND
K51-77	1/2009	PSCUH	+	−	AbaR25	Int. II	A6	ND
K51-78	3/2009	PSCUH	+	+	AbaR25	Int. II	A7	ND
K51-79	3/2009	PSCUH	+	+	AbaR25	Int. II	A6	ND
K51-80	3/2009	PSCUH	+	+	AbaR25	Int. II	A6	ND
K51-81	4/2009	PSCUH	+	−	AbaR25	Int. II	A1	ND
K70-64	6/2009	PSCUH	+	+	AbaR25	Int. II	A8	ND
K70-65	6/2009	PSCUH	+	+	AbaR25	Int. II	A8	ND
K70-66	6/2009	PSCUH	−	−	AbaR25	Int. II	A9	ND
K70-67	8/2009	PSCUH	+	+	AbaR25	Int. II	A10	ND
K70-68	9/2009	PSCUH	−	−	AbaR25	Int. II	A9	ND
K70-69	9/2009	PSCUH	−	−	AbaR25	Int. II	A9	ND
K70-70	10/2009	PSCUH	+	+	AbaR25	Int. II	A10	ND
K70-71	10/2009	PSCUH	−	−	AbaR25	Int. II	A9	ND
K70-72	10/2009	PSCUH	−	−	AbaR25	Int. II	A9	ND
K70-73	11/2009	PSCUH	−	−	AbaR25	Int. II	A9	ND
K70-74	12/2009	PSCUH	−	−	AbaR25	Int. II	A9	ST2
K70-75	12/2009	PSCUH	−	−	AbaR25	Int. II	A4	ND
K70-76	12/2009	PSCUH	−	−	AbaR25	Int. II	A9	ND
K70-77	7/2009	DRH	+	+	AbaR25	Int. II	A2	ND
K70-78	3/2009	VzH	+	−	AbaR25	Int. II	A1	ND
K70-79	3/2009	VH	+	+	AbaR25	Int. II	A2	ND
K70-80	6/2009	R1H	+	+	AbaR25	Group 4	A3	ST2
K70-81	6/2009	R1H	−	−	AbaR25	Int. II	A1	ST2

[a] Abbreviations: PSCUH, P. Stradins Clinical University Hospital, Riga; DRH, Daugavpils Regional Hospital, Daugavpils; VzH, Vidzemes Hospital, Valmiera; VH, Venstpils Hospital, Ventspils; R1H, Riga 1st Hospital, Riga; Int., international clone; ST, sequence type; ND, not determined.

rmtA, *rmtB*, *rmtC*, *rmtD*, and *npmA*) (20). All the isolates were positive for $bla_{\text{OXA-51-like}}$ and $bla_{\text{OXA-23-like}}$, while none of them carried the $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-58-like}}$, or metallo-β-lactamase genes. The *armA* gene was detected in all the isolates ($n = 24$) showing high levels of resistance to amikacin, gentamicin, and tobramycin (Table 1). Furthermore, sequence analysis detected the occurrence of the *intI1-aacA4-catB8-aadA1-qacEΔ1* class 1 integron in isolate K51-65. Subsequent PCR assays (see Tables S2 to S4 in the supplemental material) confirmed the occurrence of this integron in 20 isolates (Table 1). Interestingly, all the class 1 integron-positive isolates carried the *armA* gene, indicating the succeeding acquisition of *armA* first and the *intI1-aacA4-catB8-aadA1-qacEΔ1* integron second.

The *comM* gene was interrupted in all the isolates, indicating the occurrence of AbaR islands. AbaR25, a novel AbaR4-like island of 46,469 bp, was detected and fully sequenced in isolate K51-65 (Fig. 1; see also Table S2 in the supplemental material). AbaR25 was most similar to Tn*6167* and successively consisted of (i) ΔTn*6022a* (9,148 bp) at the left-hand end; (ii) zone 1 (6,205 bp), including seven conserved open reading frames of unknown function and a proposed tyrosine integrase gene; (iii) Δ1Tn*6022b* (2,892 bp); (iv) zone 2 (9,049 bp), including the IS*Aba1-sul2*-ΔCR2-*tetA*-*tetR*-CR2-*strB*-*strA* configuration; and (v) Δ2Tn*6022b* (2,262 bp), containing *orf4b*, at the right-hand end (15). Interestingly, the *tetA* gene of zone 2 was interrupted by a sequence of 11,998 bp, representing a complete second copy of Tn*6022a*. In addition, the *sup* gene of this Tn*6022a* was interrupted by a sequence of 4,805 bp (Tn*2006*). Transposition of Tn*6022a*/Tn*2006* into *tetA* was associated with the standard 5-bp target duplication (11). PCR assays (see Tables S3 and S4 in the supplemental material) confirmed the occurrence of AbaR25 in 33 isolates and detected the occurrence of ΔAbaR25, a variant form of AbaR25, in one additional isolate (Table 1). ΔAbaR25 was fully sequenced in isolate K51-74 and found to be identical to AbaR25 except for the occurrence of an internal deletion of 5,822 bp (Fig. 1). The deletion included ~2,750 bp of Δ1Tn*6022b*, IS*Aba1-sul2*-ΔCR2-Δ*tetA* of zone 2, and ~150 bp of Tn*6022a*/Tn*2006*. The deletion was most likely due to a single intramolecular recombination event between Δ1Tn*6022b* and the corresponding region of Tn*6022a*/Tn*2006*. This was indicated by the occurrence of Tn*6022a′*/Tn*2006*, a novel structure character-

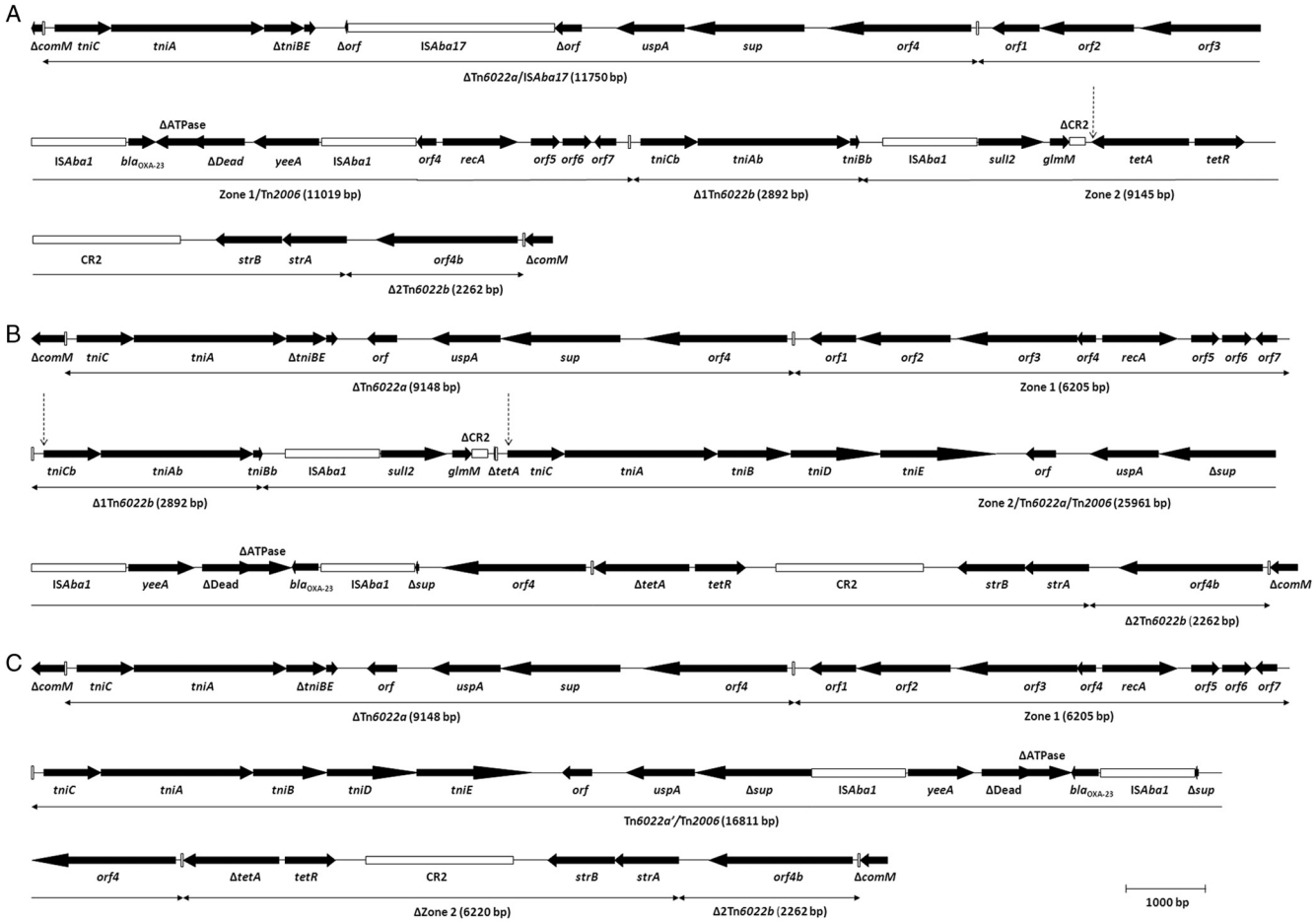

FIG 1 Structures of three different *Acinetobacter baumannii* resistance islands: Tn*6167* (A), AbaR25 (B), and ΔAbaR25 (C) (GenBank accession numbers JN968483, JX481978, and JX481979, respectively). The genes and open reading frames (*orf*) are shown by labeled arrows, with the arrowhead indicating the direction of transcription. The mobile elements IS*Aba17*, IS*Aba1*, and CR2 are shown as labeled open boxes. Inverted repeats are shown as vertical bars. The vertical dashed arrow in panel A defines the transposition site of the second copy of Tn*6022a* in AbaR25. The vertical dashed arrows in panel B define the deletion in ΔAbaR25. The genes and genetic structures are drawn to scale.

ized by a mosaic sequence derived from the two recombined segments. On the other hand, sequence analysis of the AbaR island in the ciprofloxacin-susceptible isolate (K51-67) detected an island of 16,808 bp showing 99.9% similarity with AbaR4 (GenBank accession numbers JN107991 and CP001182) (7, 12).

Overall, our results detected the occurrence of a strain, representing 34 out of 35 isolates, responsible for a prolonged ongoing outbreak/endemic status of hospital-acquired infections in Latvia. The strain was linked to international clone II/ST2 and carried the $bla_{\text{OXA-23-like}}$ carbapenemase gene within a novel AbaR4-like island. The strain was partially equipped with the *armA* gene, and the *armA*-positive subdivision of this strain has subsequently acquired the *aacA4-catB8-aadA1* class 1 integron. The occurrence of minor differences among the PFGE patterns and limited variations in the phenotypic and genotypic resistance characteristics was anticipated since the isolates were collected over a prolonged period of more than 1 year (17). As previously described, a linkage was detected between international clone II/ST2 and the *armA*, *aacA4-catB8-aadA1*, and AbaR4-like antimicrobial resistance elements (2, 14). Further studies are required in order to determine the evolution and geographical and clonal distribution of the different AbaR islands in *A. baumannii*.

Nucleotide sequence accession numbers. The nucleotide sequences of AbaR25 and ΔAbaR25 were deposited in the GenBank nucleotide database under accession numbers JX481978 and JX481979, respectively.

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