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Comparative Genome Sequence Analysis of Multidrug-Resistant *Acinetobacter baumannii*▽†

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The recent emergence of multidrug resistance (MDR) in *Acinetobacter baumannii* has raised concern in health care settings worldwide. In order to understand the repertoire of resistance determinants and their organization and origins, we compared the genome sequences of three MDR and three drug-susceptible *A. baumannii* isolates. The entire MDR phenotype can be explained by the acquisition of discrete resistance determinants distributed throughout the genome. A comparison of closely related MDR and drug-susceptible isolates suggests that drug efflux may be a less significant contributor to resistance to certain classes of antibiotics than inactivation enzymes are. A resistance island with a variable composition of resistance determinants interspersed with transposons, integrons, and other mobile genetic elements is a significant but not universal contributor to the MDR phenotype. Four hundred seventy-five genes are shared among all six clinical isolates but absent from the related environmental species *Acinetobacter baylyi* ADP1. These genes are enriched for transcription factors and transporters and suggest physiological features of *A. baumannii* that are related to adaptation for growth in association with humans.

Among gram-negative pathogens that are reported as "multidrug resistant" (MDR), *Acinetobacter baumannii* is rapidly becoming a focus of significant attention (1, 10, 35, 56). *A. baumannii*, a pleomorphic, gram-negative coccobacillus, is currently recognized by the Infectious Diseases Society of America as one of the most important pathogens threatening our health care delivery system (48). Global surveillance programs conducted over the last decade show an unparalleled increase in resistance rates among clinical *Acinetobacter* isolates (26). *Acinetobacter* spp. are now the third leading cause of respiratory tract infections among patients in intensive care units, and *A. baumannii* is responsible for up to 10% of hospital-acquired infections (26). These nosocomial infections are typically found in immunocompromised patients and are associated with an increased length of stay and excess morbidity (13, 25, 38, 47). In intensive care units, up to 30% of *A. baumannii* clinical isolates are resistant to at least three classes of antibiotics (26).

A significant nosocomial outbreak of MDR *A. baumannii* in the United States occurred in the period 2003–2005 in a military treatment facility caring for injured service personnel and civilians (Walter Reed Army Medical Center [WRAMC]) (19, 40). Molecular typing of isolates from this outbreak revealed eight major clone types, and about 60% of the isolates were related to three pan-European types (5, 52), suggesting multiple independent origins (40). Examination of specific resistance determinants in the WRAMC isolates demonstrated considerable variability in the composition of resistance genes within each clone type and similar patterns across certain divergent clone types. Thus, genetic relatedness was a poor predictor of the MDR phenotype. This led to the hypothesis that there exist multiple independent genetic mechanisms leading to MDR in *A. baumannii*. A large cluster of antibiotic resistance genes and mobile genetic elements is present as an 86-kb "resistance island" (RI) in the *A. baumannii* AYE genome (15). The RI is not present in the genomes of the drug-susceptible *A. baumannii* isolates ATCC 17978 (43) and SDF (51), suggesting that it is an important contributor to the MDR phenotype.

In an effort to understand the significance of the RI and the integration of genetic factors that permit *A. baumannii* to successfully infect patients in multiple clinical settings, we undertook a whole-genome sequence analysis of six clinical isolates in comparison to a nonhuman *A. baumannii* isolate (SDF) and an environmental *Acinetobacter* strain, *Acinetobacter baylyi* ADP1. We compared three MDR isolates to three drug-susceptible isolates in order to explore common features of their evolution and the diversity of mechanisms by which resistance is demonstrated. Our goals were (i) to determine the repertoire and phylogeny of resistance determinants among recent clinical isolates, (ii) to see how these "successful" clinical iso-

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TABLE 1. Selected genetic and phenotypic properties of *A. baumannii* genomes

Genome	Accession no.	Genome size (bp)	No. of genes	Source (reference)	Size of RI (kb)	Beta-lactamase gene(s)			Plasmid-carried beta-lactamase gene	Resistance gene(s)			*gyrA*/*parC* QRDR[a]
						Class A	Class C	Class D		Tetracycline	Chloramphenicol	Trimethoprim-sulfa	
AB0057	ABJM01000001	4,050,513	3,853	WRAMC	42 + 18	bla_{TEM-1}	Two copies of *ampC*	bla_{OXA-69}, bla_{OXA-23}		*tetA*	*cat*	Two copies of *sulI*, *dhfrX*	RR
AB307-0294	CP001172	3,760,981	3,458	Buffalo, NY			*ampC*	bla_{OXA-69}				*dhfrX*	RS
AYE	CU459141	3,936,291	3,607	France (51)	86	bla_{VEB-1}	*ampC*	bla_{OXA-69}, bla_{OXA-10}		*tetA*	*cat*, *cmlA*	Four copies of *sulI*, *dhfrX*, *dhfrI*	RR
AB900	ABXK00000000	3,913,289		WRAMC			*ampC*	$bla_{OXA-51-like}$				*dhfrX*	SS
ATCC 17978	CP000521	3,976,747	3,791	ATCC (43)			*ampC*	bla_{OXA-51}				*sulI*, *dhfrX*	SS
ACICU	CP000863	3,904,116	3,667	Rome, Italy (20)	17		*ampC*	bla_{OXA-66}, bla_{OXA-20}	Two copies of bla_{OXA-58}			*sulI*, *dhfrX*	RS
SDF	CU468230	3,421,954	2,913	Louse in France (51)				bla_{OXA-75}				*dhfrX*	SS
ADP1	CR543861	3,598,621	3,325	Soil (3)			*ampC*					*dhfrX*	

[a] RR, resistance-associated Ser83Leu mutation found in *gyrA* and Ser80Ile mutation found in *parC*; SS, susceptibility-associated alleles in both *gyrA* and *parC*; RS, resistance-associated mutation found only in *gyrA*.

[b] R, resistant. Numbers in bold indicate resistance, and numbers in italics indicate intermediate susceptibility. Resistance data were published previously for AYE (15, 36), SDF (14, 24), and ACICU (20).

lates differ from pathogenic and nonpathogenic strains/species already examined, (iii) to assess the function and purpose of the RI, and (iv) to evaluate the importance and impact of insertion sequences (IS) in the evolution of the resistance phenotype. Our results show that the RI is variable in composition and is only one contributor to the MDR phenotype. Based on closely matched MDR and drug-susceptible isolates, it appears that the MDR phenotype is largely due to the acquisition of a series of genes encoding drug-inactivating enzymes that are located on mobile genetic elements. Moreover, we identified a series of genes that may define the ability of *A. baumannii* to live in association with the human host and describe extensive diversity in genes that contribute to the lipopolysaccharide barrier.

MATERIALS AND METHODS

***A. baumannii* isolates.** The origins and certain genetic and phenotypic properties of the *A. baumannii* isolates studied here are presented in Table 1. *A. baumannii* ATCC 17978 was obtained from a case of fatal meningitis in 1951. Its antibiotic susceptibility profile has not previously been reported. *A. baumannii* ACICU was isolated from the cerebrospinal fluid of a patient in an intensive care unit in Rome, Italy, in 2005 (20). *A. baumannii* AYE was cultured from a patient with pneumonia and a urinary tract infection in France in 2001 (36). *A. baumannii* SDF was recovered from a human body louse (24). *A. baumannii* isolate AB307-0294 was obtained from the blood of a patient hospitalized in Buffalo, NY, in 1994. *A. baumannii* strain AB900 is a perineal isolate obtained in 2003 from an active duty military patient at WRAMC. *A. baumannii* AB0057 is an MDR bloodstream isolate collected in 2004 from a patient at WRAMC (19).

Resistance profiles. Resistance profiles reported for AYE, SDF, and ACICU are given in Table 1. Profiles for AB0057, AB307-0294, AB900, and ATCC 17978 were determined using Vitek2 and interpreted by Clinical Laboratory Standards Institute standards (7); these are also given in Table 1. Results for AB0057 are consistent with those reported previously (19).

Genome sequencing and annotation. AB307-0294 and AB900 were sequenced by pyrosequencing, and AB0057 was sequenced by a combination of pyrosequencing and traditional Sanger shotgun sequencing. Finished genome sequences with no gaps were completed for AB307-0294 and AB0057. Genome annotation was performed at The Institute for Genomic Research (TIGR) and later at the J. C. Venter Institute, Rockville, MD, and the Institute for Genome Sciences, Baltimore, MD, using the TIGR Annotation Engine (www.jcvi.org/ems/research/projects/annotation_service/overview/) followed by manual curation. Details of the sequencing, assembly, gap filling, and annotation methods are given in the supplemental material.

PCR. PCR was performed using the primers listed in Table S1 in the supplemental material and as described by Hujer et al. (19).

Nucleotide sequence accession numbers. The genome sequences of AB0057, AB307-0294, and AB900 have been deposited in the DDBJ/EMBL/GenBank databases under the accession numbers CP001182-3, CP001172, and ABXK00000000, respectively.

RESULTS

Genomes. We have completed the genome sequences of three recent clinical isolates, the MDR isolate AB0057 and two drug-susceptible isolates, AB307-0294 and AB900 (Table 1). Within the past year, genome sequences of four *A. baumannii* isolates have been published (20, 43, 51). Two of these, AYE and ACICU, are MDR, while ATCC 17978 is susceptible to many standard antibiotics. SDF was isolated from a human body louse, and its status as a human pathogen has not been determined.

Despite acquisition from geographically diverse regions, AYE, AB0057, and AB307-0294 are remarkably similar at the DNA level, averaging over 99.9% nucleotide sequence identity in orthologous regions (Fig. 1). The other isolates are each ~98% identical to one another. Interestingly, the >50-year-old isolate (ATCC 17978) and the louse isolate (SDF) are not more divergent than any of the other isolates at the sequence identity level (Fig. 1A). The relationship of isolates to one another based on shared gene content differs from the relationships inferred based on analysis of conserved genes, reflecting a considerable contribution of lateral gene transfer in the evolution of *A. baumannii* (Fig. 1B). SDF has a much smaller genome than the other *A. baumannii* isolates, presumably as a result of extensive IS-mediated deletion events (51). The extent of divergence in gene content in SDF and its questionable role as a human pathogen have prompted us to focus most of our analysis on comparisons among the six human clinical isolates.

Comparison between ADP1 and *A. baumannii* isolates. Based on reciprocal best BLAST matches, there are 2,688 genes shared among the six clinical isolates; these define the core

TABLE 1—*Continued*

Resistance gene(s)			MIC[b]												
AME gene	Ampicillin	Ampicillin-sulbactam	Piperacillin-tazobactam	Cefazolin	Ceftriaxone	Cefepime	Meropenem	Imipenem	Tigecycline	Aztreonam	Gentamicin	Amikacin	Tobramycin	Trimethoprim-sulfamethoxazole	Ciprofloxacin
aphA1, *aacC1*, *aadA1*	**≥32**	**≥32**	**≥128**	**≥64**	**≥64**	**≥64**	**≥16**	**≥16**	*4*	**≥64**	**≥16**	≤2	≤1	**160**	**≥4**
	≥32	≤2	≤4	**≥64**	*16*	8	≤0.25	≤1	≤0.5	**≥32**	*2*	≤2	≤1	≤20	*2*
aphA1, *aadB*, two copies of *aadA1*, *aacC1*	**R**		*8*			**512**	1	1		**≥512**	**R**	**R**	**R**	**R**	**R**
	16	≤2	≤4	**≥64**	*16*	2	≤0.25	≤1	≤0.5	**≥32**	≤1	≤2	≤1	≤20	≤0.25
	16	≤2	≤4	**≥64**	*16*	2	≤0.25	≤1	≤0.5	**≥64**	≤1	≤2	≤1	**160**	≤0.25
aac3	**R** ND		**R**			**R**	**R**	**R**		**R**		**R**	**R**	**R**	**R** ND

genome of *A. baumannii*. These core genes encode basic functions involved in DNA replication, transcription, and translation, as well as many metabolic pathways (Fig. 2). Eighty-two percent of the core genes have orthologs in the environmental organism *A. baylyi* ADP1 (3, 53). For comparison, only 75% of the core genes are present in the louse isolate *A. baumannii* SDF.

The average percent identity between presumed orthologs in the *A. baumannii* isolates and ADP1 is 71%, but the range of identities among reciprocal best BLAST matches is 21% to 100%. This variation indicates a wide range of selective pressures acting on different genes since the split of these two

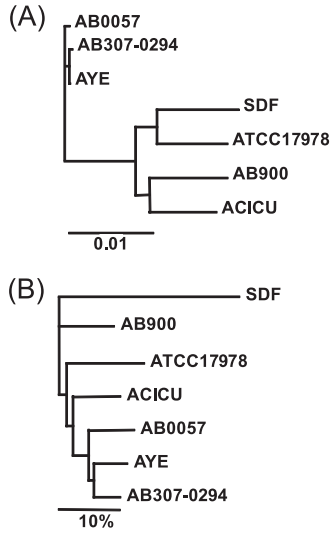


FIG. 1. Phylograms of *A. baumannii* isolates. The relationships of *A. baumannii* isolates were inferred based on the similarity of shared sequences that have most likely been retained in each genome from a common ancestor (A) and based on the extent of unique gene content in each isolate (B). (A) A maximum likelihood tree was constructed using *dnaml* from the PHYLIP package, based on the concatenated DNA sequences of 1,942 ORFs that were reciprocal best matches in each genome. Bar, 0.01 substitution per nucleotide. (B) A tree based on the shared content of accessory genes was constructed as described previously (44). Bar, 10% difference in gene content.

Acinetobacter lineages. An important feature of the ADP1 genome is a series of islands carrying genes related to the catabolism of a diverse array of complex organic compounds. Interestingly, three of the four catabolic islands are largely intact in the *A. baumannii* isolates, although they are rearranged so they are distributed over a larger genomic region rather than grouped into an archipelago as in ADP1 (3). The cluster that is missing carries the *ssu*, *sox*, *ats*, and *nth* genes, encoding products that metabolize alkanesulfonates, dibenzothiophene, sulfuric esters, and nitriles. Genes that encode proteins involved in catabolism of betaine, vanillate, urea, benzoate, catechol, protocatechuate, quinate, and several other complex organic compounds are retained in the *A. baumannii* isolates.

Adaptation to the human host. *A. baylyi* ADP1 is a naturally transformable soil isolate. We hypothesize that genes found in the *A. baumannii* isolates but not in ADP1 may contribute to features of growth in association with a human host. We found 475 genes that are shared by all six *A. baumannii* clinical isolates and that are missing from the ADP1 genome (pan-*A. baumannii* accessory genes [see Table S2 in the supplemental material]). Hence, we propose that these genes may play an important role in human adaptation. Reinforcing the significance of these genes for growth in association with humans, 279 (59%) have been lost in the SDF strain, which was isolated from a human body louse.

Gene annotations for each genome were classified using gene ontology (GO) terms to group related genes by molecular function, biological role, and cellular compartment (2). The pan-*A. baumannii* accessory genes are enriched in the categories of transport activity and transcription factor activity compared with core genes shared with ADP1 (Fig. 2). In contrast, GO categories for metabolism and cellular processes were overrepresented among core genes compared with pan-*A. baumannii* accessory genes. Remarkably, 59 of the pan-*A. baumannii* accessory genes are predicted to be transcription factors based on sequence similarity. This suggests a more extensive regulatory program in *A. baumannii* than in ADP1. Transporter genes comprise 22% of the pan-*A. baumannii* accessory genes, compared to 10% of all genes. Transport systems are identifiable for urea, taurine, oligopeptides, and

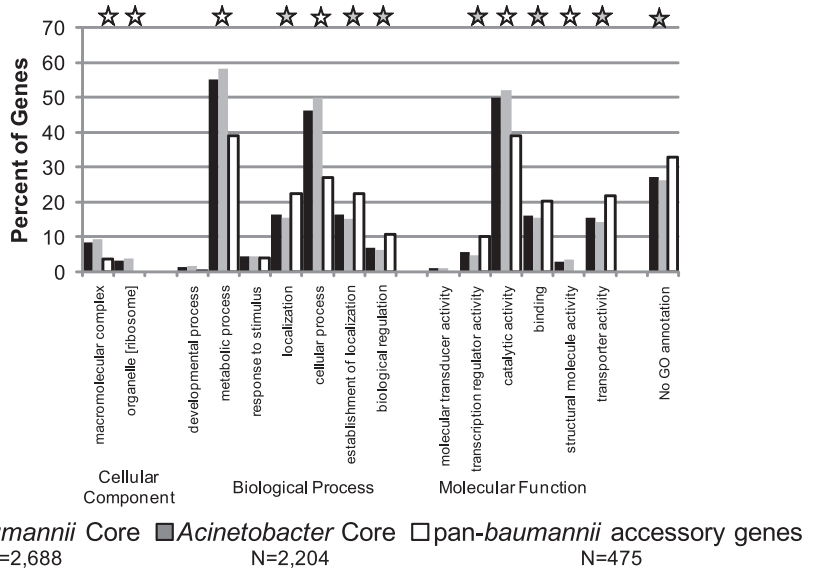


FIG. 2. GO categories of core and *A. baumannii*-specific genes. GO categories were inferred for AB0057 genes by using the JCVI Annotation Engine. The percentage of genes in each GO category was determined for core *A. baumannii* genes (those present in all six clinical isolates), *Acinetobacter* core genes (*A. baumannii* core genes that are also present in ADP1), and pan-*A. baumannii* accessory genes (core genes absent from ADP1), using WEGO (58). Percentages indicate the fractions of gene groups annotated with each GO category. Stars indicate GO categories that are significantly underrepresented (open) or overrepresented (filled) among pan-*A. baumannii* accessory genes compared to core genes ($P < 0.05$).

histidine. The presence of additional transport systems for amino acids and other nitrogen-containing compounds presumably reflects their greater availability in humans than in soil. Several amino acid utilization enzymes were also found, including arginine *N*-succinyltransferase and histidine decarboxylase.

Two hundred sixty-one of the 475 pan-*A. baumannii* accessory genes (55%) are present in clusters of at least four adjacent genes. As noted above, not all clusters should be presumed to be present due to lateral transfer into the *A. baumannii* ancestor. Although by definition none have orthologs in ADP1, 40% have orthologs in *Pseudomonas aeruginosa* PAO1 and may have been lost in the ADP1 lineage.

Two clusters are worthy of further note. One is a cluster of genes responsible for the biosynthesis of a homoserine lactone that is involved in quorum sensing (AB57_0151 to AB57_0161). The AbaI protein (AB57_0151) has been shown to be a homoserine lactone synthase which is involved in quorum sensing and is required for biofilm formation (33). The second cluster (AB57_2565 to AB57_2574) is a chaperone/usher pilus assembly gene cluster that has also been shown to be involved in adherence to mammalian cells and in biofilm formation (50).

Variation among *A. baumannii* isolates. As shown for *Haemophilus influenzae*, *P. aeruginosa*, *Streptococcus pneumoniae*, and other bacterial pathogens, there is considerable variation in the DNA content of each isolate (18, 29, 41, 42). The accessory genome of *A. baumannii* comprises 2,649 annotated genes that are present in one to five of the six isolates. This number will surely grow as additional isolates are characterized. The number of unique genes ranges from 80 (AB307-0294) to 528 (ATCC 17978).

Genomic regions of unique content (islands) have been noted in each of the reported *A. baumannii* genomes, but the availability of seven complete genomes enables a much more rigorous evaluation of the contents and distribution of these islands. A variety of terms have been applied to clustered groups of genes in *A. baumannii*, including (putative) alien islands, pathogenicity islands (PAIs), and RIs (11, 15, 23, 39, 43). Based on the gene content, the latter term seems appropriate for a subset of islands with a clear concentration of genes associated with resistance to several classes of antibiotics. However, the function of most islands is not proven, so we prefer the simple term genomic islands.

In AB0057, 60% of lineage-specific genes are within clusters of at least 10 genes located adjacent to one another. Some genomic islands are likely the result of lateral gene transfer, such as prophage insertions, which in general are the largest genomic islands in each isolate. The RI described below is comprised largely of genes that were likely acquired by lateral transfer. It is also clear that not all genomic islands were derived by lateral gene transfer, however. Some likely represent regions of an ancestral *Pseudomonadales* genome that have been lost in ADP1, for example, a leucine metabolism cluster at AB57_1597 to AB57_1604 that has orthologs in both *P. aeruginosa* and another closely related organism, *Psychrobacter cryohalolentis* (AB0057 numbers are given, but this cluster is shared by all of the *A. baumannii* isolates except for SDF).

Each genome contains a considerable number of unique genes, as 46% of accessory genes are isolate specific. A disproportionate number of these are hypothetical and are likely cryptic prophage regions. Of the 1,228 total genes that are annotated for only one of the six clinical isolates, 412 fall within prophage regions and 487 others are annotated as encoding hypothetical proteins (73% in the two categories combined). The phage regions are not orthologous, suggesting recent insertion and/or rapid loss and a large pool of potential bacteriophage genomes. One prophage was found in both AB0057

and ACICU, albeit at different chromosome locations in the two genomes (AB57_1230 to AB57_1300 and ACICU_02150 to ACICU_02228). The significance of these two independent insertion events in the context of the natural history of these isolates is unclear.

The excess of hypothetical proteins in the accessory genome could be due to several causes. First, incorrectly annotated (wrong frame, strand, or region) open reading frames (ORFs) that do not code for bona fide proteins will not match ORFs from the other isolates. Second, incomplete or inaccurate annotation can result in the unintended omission of identification of a set of orthologous proteins. Finally, frameshift errors in the DNA sequence can result in unpaired orthologs. The first two groups of hypothetical proteins are difficult to quantify. The number of frameshifted annotations was examined by comparison of the predicted protein set from each isolate to the genomes of the other isolates, using TBLASTN. Instances where the alignment was apportioned across two different frames were counted. The number of potentially frameshifted annotations varied from 21 in AYE to 162 in ATCC 17978 (see Table S3 in the supplemental material).

A. baumannii is thought of as a cousin of another human pathogen, *P. aeruginosa*, and 65% of the core genes have orthologs in *P. aeruginosa* PAO1. The accessory genome of *P. aeruginosa* has been shown to be inserted into limited locations around the genome, defined as regions of genome plasticity (29). In contrast, there does not seem to be a similar preference for strain-specific insertion locations in *A. baumannii*, with the exception of the location of the RI.

RIs of variable composition. An interesting feature of many drug-resistant genomes is the presence of a genomic island or RI containing a collection of genes encoding proteins related to antibiotic inactivation and efflux. RIs can be plasmid carried (20, 22) or located on the bacterial chromosome (15), and antibiotic resistance genes are usually interspersed with mobile genetic elements (11).

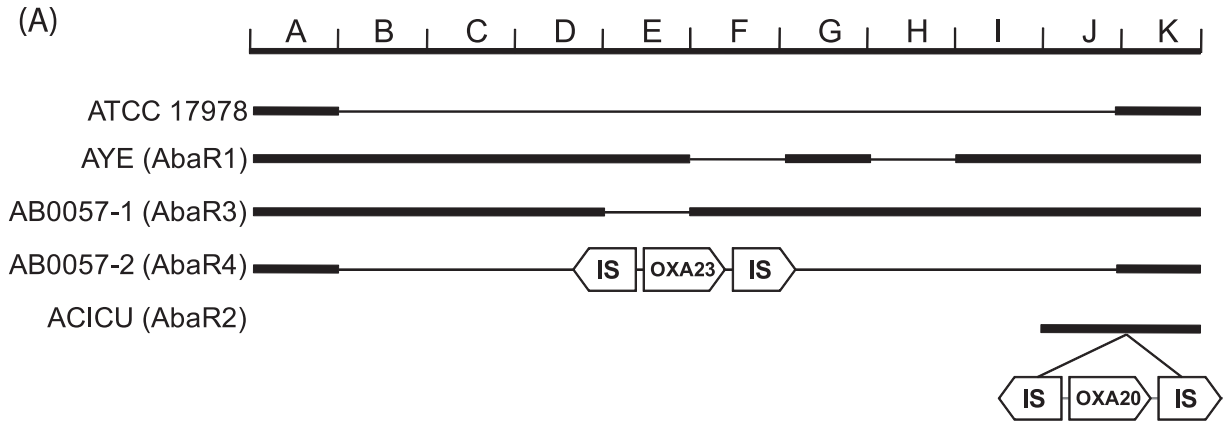
Comparison of the sequence and structure of the genomic island in each *A. baumannii* isolate revealed a series of insertion/deletion events at this locus (Fig. 3A). The 86-kb island in AYE (AbaR1) is the largest, with 90 annotated genes, including genes related to the inactivation of β-lactams, aminoglycosides, chloramphenicol, rifampin, and tetracycline. These resistance determinants are intermixed with a complex set of partial and complete integrons and transposons (15). The 49-kb AB0057 RI (AbaR3) is largely a subset of AbaR1. It contains eight genes associated with antibiotic resistance. Unique sequences in AbaR3 include a bla_{TEM} gene that is associated with a Tn*3* transposon and a small cluster of genes, including two that encode a DNA topoisomerase and a single-strand binding protein that are most similar to proteins from a broad-host-range plasmid (49). The ACICU RI contains the distal portion of the AYE/AB0057 RI, into which an ISAba1-flanked $bla_{\mathrm{OXA\text{-}20}}$ gene has been inserted (AbaR2). In ATCC 17978, only the initial and terminal segments are present, and these do not contain resistance determinants. An unrelated 37.7-kb insertion is present at the location of the RI in AB900. The AB900 island appears to have been transferred laterally based on the source of the best protein database matches. It contains a copper resistance operon and a high concentration of hypothetical proteins. ADP1 and AB307-0294 preserve the ancestral gene order across the location of the RI insertion. Given the close relationship between AYE and AB307-0294, it is likely that the larger AYE RI is more like an original insertion, with AB0057 and ACICU derived by series of subsequent deletion, insertion, and rearrangement events. Another interpretation, however, is that the RI represents a series of independent insertion events; this view is supported by the fact that ACICU and AYE/AB0057 are representatives of divergent European clone types II and I, respectively.

Supporting the notion that the RI may be mobile, a second copy of the resistance island is present in the AB0057 genome (AbaR4). AbaR4 is the location of a 4.9-kb ISAba1-flanked gene cassette including $bla_{\mathrm{OXA\text{-}23}}$, reinforcing the idea that the environment is permissive for resistance gene accumulation. The other five clinical isolates do not have a second copy of sequences related to the RI, and only two other closely related isolates amplified sequences related to AbaR4 by PCR (Fig. 3B).

A distinguishing feature of AbaR1 and AbaR3 in the MDR isolates AYE and AB0057 is the presence of a pair of ISPpu12 IS elements that flank the resistance gene portion of the island (Fig. 3A, fragments D to J). In the second AB0057 island (AbaR4), the *uspA* gene, encoding a universal stress protein, is present intact, while this gene is interrupted at the corresponding position in the main RI (AbaR3) by the ISPpu12-flanked insertion. The average G+C content of ORFs between the ISPpu12 elements is 57%, compared to 39% for segments A to C and K of the RI and the rest of the genome, suggesting a recent acquisition of the ISPpu12-flanked cassette by lateral transfer. Many of the predicted proteins in segments E to I are nearly identical to segments of a broadly distributed integron (45). In addition, as noted above, AbaR3 in AB0057 contains genes for a plasmid-derived DNA topoisomerase that may contribute to RI mobility. Only one ISPpu12 element is present in ACICU, perhaps reflecting deletion of one element along with a significant fraction of the 5′ end of the island, including the 5′-flanking sequence.

How much of the MDR phenotype can be explained by the RI? The RI is a dramatic feature of the MDR isolates AB0057 and AYE. A significant concentration of genes involved in antibiotic resistance is also present in an orthologous location in ACICU. It is tempting to speculate that the MDR phenotype is correlated with the presence of some variant of the RI. To test this hypothesis, we attempted to amplify segments of the RI from several drug-susceptible and MDR isolates (Fig. 3B). The basic structure of the RI, as found in AB0057 and AYE, appears to be restricted to closely related clones, as defined by PCR-electrospray ionization mass spectrometry (PCR/ESI-MS) typing (12). In other PCR/ESI-MS types, however, MDR isolates typically exhibit the presence of some of the resistance genes but not other markers of the RI per se, such as the ISPpu12 elements that flank the resistance gene region. It is possible that these genes either are not associated with an RI or are associated with islands with an alternate structure and/or location. One example is the presence of *aadB* in several isolates despite the absence of $bla_{\mathrm{VEB\text{-}1}}$, which is adjacent to *aadB* in the AYE RI, indicating that this segment of the RI is not widespread (Fig. 3C, RIN primers). The RI is completely missing in AB307-0294 and a subset of other isolates that are largely susceptible (Fig. 3C, RIH primers). An

(A)



(B)

A	transposon
B	*trkA, trxB*
C	arsenate resistance operon
D	ISPpu12
E	AYE-specific sequence including *strA, strB, dfrI, cmlA, arr-2, tetR, tetA, aadA1, aadB, aac6,* bla_{VEB-1}, bla_{OXA-10}
F	AB0057-specific sequence including *topA, ssb*
G	including mercury resistance operon, *tetR, tetA, cat*
H	Tn3 including bla_{TEM}
I	including *aphA1, aac3, aadDA1*
J	ISPpu12
K	sulfate permease

(C)

Fig Label		A	C	D/J	E	F	G	H	I	I	I												
Primers		RIC	RIA	RIG	RIN	RID	RIE	Hujer	Hujer	Hujer	Hujer	RIH	RIK										
Isolate	PCR/ ESI-MS type	Trans-posase	Ars	ISPpu12	*aadB*	*topA*	*tetA*	*bla*-TEM	Int1	*aadA1*	*aacC1*	Island Presence[a]	2nd Island Presence[a]	AMP	CIP	CAZ	FEP	AMK	TOB	SAM	MEM	IPM
AB056	15	+	+	+	-	+	-	-	+	+	+	-	-	+	+	+	+			+	+	+
AB0057	15	+	+	+	-	+	+	+	+	+	+	-	-	+	+	+	+			+	+	+
AB058	15	+	+	+	+	+	-	-	-	-	-	-	+	+	+	+	+	+	+			
AB059	15	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+		+	+	+
AB060	3	-	+	-	+	-	-	-	+	-	-	-	+	+	+	+	+		+	+	+	+
AB061	3	-	+	-	+	-	-	-	+	-	+	-	+	+	+	+	+		+	+	+	+
AB062	3	-	+	-	+	-	-	-	+	-	+	-	+	+	+	+	+		+	+	+	+
AB063	3	-	+	-	+	-	-	-	+	-	-	-	+	+	+	+	+	+		+		
AB064	3	-	+	-	+	-	-	-	-	-	+	-	+	+								
AB065	3	-	+	-	+	-	-	-	-	-	+	-	+	+	+		+		+	+	+	+
AB070	16	-	-	-	+	-	-	-	+	+	+	-	+	+	+	+	+		+			
AB074	39							-	-	-	-	+		+								
AB069	1							-	+	+	+	+		+	+	+	+		+			
AB072	48							-	-	-	-	+		+								
AB068	9							-	-	-	-	+		+		+		+	+			
ATCC17978*	ND	-	-	-	-	-	-	-	-	-	-	-	+	+								
AYE*	15	+	+	+	+	-	+	-	+	+	+	-	+	+	+		+	+	+			
AB307-0294*	46	-	-	-	-	-	-	-	-	-	-	+	+	+								
AB900*	19	-	-	-	-	-	-	-	-	-	-	-	+	+								
SDF*	ND	-	-	-	-	-	-	-	-	-	-	-	+									
ACICU*	ND	-	-	+	-	-	-	-	+	-	+	-	+	+	+		+	+	+		+	+

FIG. 3. RI composition. (A) Modules that comprise components of the RI in sequenced *A. baumannii* genomes are designated by the letters A to J. A bold bar indicates that the region is present in an isolate. (B) Representative genes present in each module are listed. (C) The presence of various RI components in additional *A. baumannii* isolates was tested by PCR. *, PCR results were inferred based on a BLAST search of each genome with primer sequences; [a], these primer pairs flank the island location, so successful PCR amplification (+) indicates that the island, an insertion that is too long for PCR amplification, is absent. AB0057 was reported previously as AB057 (19). Abbreviations: AMP, ampicillin; CIP, ciprofloxacin; CAZ, ceftazidime; FEP, cefepime; AMK, amikacin; TOB, tobramycin; SAM, ampicillin-sulbactam; MEM, meropenem; IPM, imipenem. Hujer, data obtained from reference 19.

insertion is present at this genomic location in a large majority of isolates. The genes present may be quite different, though, since completely unrelated sequences are present at this genome location in AB900 and SDF.

In the three MDR isolates, AB0057, AYE, and ACICU, a significant fraction of genes responsible for antibiotic resistance are located in mobile genetic elements, including the RIs in each isolate and a plasmid in ACICU. In AB0057, a second gene encoding ADC ($bla_{\text{ADC-40}}$) is present, flanked by ISAba1 elements. The $bla_{\text{ADC-40}}$ gene was likely obtained by lateral transfer because it contains a 15-bp insertion relative to most other bla_{ADC} genes as well as four amino acid substitutions and several silent changes compared with the chromosomal copy.

The corollary issue, i.e., how many resistance genes are not associated with mobile genetic elements, is equally compelling because it may provide a sense of the innate resources in the genome that are available to counter the antibiotic threat. Resistance to fluoroquinolones is mediated by mutations in *gyrA* and *parC*, which are not present on mobile elements. Resistance to aminoglycosides and β-lactams may be mediated by a combination of drug-inactivating enzymes, efflux pumps, and changes in the bacterial outer membrane that reduce drug uptake. Three multidrug efflux pumps, encoded by *adeABC*, *adeIJK*, and *abeM*, have been shown to contribute to drug resistance in *A. baumannii* (9, 27, 32, 46).

The very close relationship of the drug-susceptible isolate AB307-0294 with the MDR isolates AB0057 and AYE enables another perspective on the acquisition of resistance determinants. Virtually all of the AB307-0294 genome aligns with the AB0057 and AYE genomes, and the average percent identity between orthologs is >99.7%. The genes that are present in AYE and AB0057 but not in AB307-0294 are almost exclusively related to the RIs or to prophage or IS element insertions (see Table S4 in the supplemental material). Only ~3% of the AB307-0294 genome is not represented in AB0057 or AYE; this is virtually all in two prophage clusters. The significance of the genetic similarity among these three isolates rests with the observation that AB307-0294 retains all of the transport, metabolism, and regulatory capacities of the MDR isolates and yet is drug susceptible.

The *adeABC* and *adeIJK* genes encoding drug efflux pumps are present and the encoded proteins are 100% identical in sequence in all three isolates. The two-component regulatory proteins AdeR and AdeS are also present in AB307-0294, with only a single conservative amino acid substitution in each protein differing from the AB0057 sequence. In fact, the entire *adeRSABC* and *adeIJK* gene regions together have only three nucleotide differences in 12,436 bp between AB307-0294 and AB0057 or AYE, suggesting that both their regulation and function are similar among the three isolates. It has been suggested that the AdeIJK proteins contribute to intrinsic but not acquired antibiotic resistance in *A. baumannii* (9), and these proteins are also identical in AB307-0294, AB0057, and AYE. Regulators of AdeIJK expression have not been described, and it is likely that additional proteins that control the *adeABC* and *adeIJK* operons remain to be found.

There are 15 genomic islands in ACICU annotated as being involved in drug resistance (20). For most of these islands, the designation is based on the presence of one or more genes annotated as transporters of the DMT superfamily (21), and their substrates have not been verified experimentally. Most of these genes are also present in the drug-susceptible isolates AB307-0294, AB900, and ATCC 17978 and thus do not seem to be indicative of antibiotic susceptibility status.

In addition to the RIs and prophage regions, there are two additional clusters of genes in the MDR isolates AB0057 and AYE that are absent from AB307-0294: these are a copper resistance cluster and a cluster that includes a glycine/betaine transport operon. Betaines serve as organic osmolytes for protection against osmotic stress, drought, high salinity, or high temperature (57). At least some portion of the copper resistance cluster is present in ATCC 17978 and AB900, and the cluster is absent from ACICU, so these genes are unlikely to contribute directly to antibiotic resistance. The role, if any, of the cluster including the glycine/betaine transport genes in resistance and/or pathogenicity remains to be determined.

IS elements represent another potential source of variability between AB307-0294 and the MDR isolates. AB307-0294 does not contain ISAba1 elements, while AB0057 carries 9 copies and AYE carries 21 copies. Four of the nine ISAba1 elements in AB0057 flank β-lactamase genes that appear to have been transferred laterally into this isolate ($bla_{\text{ADC-40}}$ and $bla_{\text{OXA-23}}$). These ISAba1 elements are presumably involved in overexpression of the *bla* genes from outward-facing promoters in the IS element (17). Two AB0057 ISAba1 insertions are polymorphic and are thus unlikely to be related to drug resistance. One ISAba1 element appears to inactivate the *uup* gene, in which mutations have been reported to increase the precise excision of transposons (31). The final two insertions are in or adjacent to hypothetical protein genes. None of the ISAba1 insertion locations are shared between AB0057 and AYE, suggesting that any impact on expression of adjacent genes at the insertion sites is not a primary feature of drug resistance.

Other features of antibiotic resistance. The RI carries genes that encode resistance to chloramphenicol, an antibiotic that is not commonly used in contemporary acute care settings in developed countries. This suggests that the RI is not a recent acquisition. Further work will be required to assess the genetic stability of the RI.

All tested isolates of *A. baumannii* are resistant to aztreonam, which specifically targets penicillin-binding protein 3 (PBP-3) (8). The basis for this resistance has yet to be determined but could be related to the considerable divergence of PBP-3 in *Acinetobacter*. The AB0057 PBP-3 protein is only 40% identical to the orthologous proteins from the closest non-*Acinetobacter* relative, *Psychrobacter cryohalolentis* K5, and to *P. aeruginosa* PAO1.

All isolates of *A. baumannii* displayed high-level resistance to nitrofurantoin. Nitrofurantoin is a prodrug that is reduced by the bacterial enzyme nitrofuran reductase to form highly reactive intermediates that in turn produce DNA damage. Although present in the close *Acinetobacter* relative *Psychrobacter* sp. strain PRwf-1 and many other gammaproteobacteria, this enzyme is absent from ADP1 and all *A. baumannii* isolates examined to date.

Plasmids were identified during genome sequencing of AB0057 (1), ATCC17978 (2), AYE (4), SDF (3), and ACICU (2), but only in ACICU is there a direct contribution to antibiotic resistance. Two copies of $bla_{\text{OXA-58}}$ are present on a plasmid in ACICU and are flanked by insertion sequences. No

TABLE 2. Distribution of O-antigen genes with first primer set

Isolate[a]	PCR/ESI-MS type[a]	PCR result with primer									
		AB57_0094	AB57_0095	AB57_0096	AB57_0097	AB57_0099	AB57_0100	AB57_0102	AB57_0103	AB57_0104	AB57_0105
ATCC 17978	ND	−	−	−	−	−	−	−	−	−	−
AB0076	15	+	+	+	+	+	+	+	+	+	+
AB0077	15	+	+	+	+	+	+	+	+	+	+
AB0078	15	+	+	+	+	+	+	+	+	+	+
AB0079	15	+	+	+	+	+	+	+	+	+	+
AB0080	15	+	+	+	+	+	+	+	+	+	+
AB0057	15	+	+	+	+	+	+	+	+	+	+
AB056	15	+	+	+	+	+	+	+	+	+	+
AB058	15	−	−	−	−	−	−	−	−	−	−
AB059	15	+	+	+	+	+	+	+	+	+	+
AB070	16	−	−	−	−	−	−	−	−	−	−
AB900	19	−	−	−	−	−	−	−	−	−	−
AB307-0294	46	−	−	−	−	−	−	−	−	−	−
AB066	46	−	−	−	−	−	−	−	−	−	−
AB067	46	−	−	−	−	−	−	−	−	−	−

[a] Isolate and PCR/ESI-MS type information is from reference 19.

plasmids were found after accounting for all genome sequence reads from AB900 and AB307-0294.

Evaluation of *esv* genes. Smith et al. identified 16 genes that, when mutated, resulted in attenuation of the virulence of *A. baumannii* ATCC 17978 in two invertebrate models of infection (43). They also identified 28 potential PAIs based on analysis of base composition and gene content. Surprisingly, only three of the attenuated virulence mutations mapped to PAIs, and the two PAIs containing mutations are prophage regions. Eleven of the 16 so-called *esv* genes (for *e*thanol-*s*timulated *v*irulence) have orthologs in the nonpathogenic organism *Acinetobacter baylyi* ADP1, and 4 of the remaining 5 are absent from all five recent clinical isolates of *A. baumannii* discussed here and from six additional tested isolates (data not shown). This suggests that they are not ubiquitous contributors to clinical infections. The one remaining *esv* gene (*esvB*; A1S_1232) encodes an HxlR family helix-turn-helix transcription factor of unknown specificity. *esvB* is also absent from the SDF strain. Further work to determine the role of this gene in pathogenicity of *A. baumannii* will be required, preferably with a mammalian model system.

Diversity at the O-antigen biosynthetic cluster. The outer surfaces of gram-negative bacteria are comprised of a lipopolysaccharide layer that serves as a barrier between the outer membrane and the environment. Considerable intraspecies variability has been observed in the composition of the O-antigen side chains of the lipopolysaccharide, and this contributes to antigenic variability among isolates. For *P. aeruginosa*, at least 11 highly divergent gene clusters encoding proteins involved in O-antigen biosynthesis at a conserved genome location have been reported (37). A similar arrangement is found in *A. baumannii.* Among the seven isolates analyzed here, there are six highly divergent sets of genes located at the O-antigen cluster. In each genome, the cluster is located at an orthologous position (between ACIAD0069 and ACIAD1016 in ADP1) and contains 17 to 30 genes. The sequences of AB307-0294 and AYE are identical across this region. Testing of the O-antigen gene content of other isolates by PCR showed that a subset of PCR/ESI-MS type 15 isolates share the AB0057 gene set, while other isolates match the AB307-0294/AYE gene set (Table 2). Among the closely related isolates defined by PCR/ESI-MS type 15, the amplification pattern is quite consistent across the gene cluster, and all type 15 isolates match either the AB0057 or AYE/AB307-0294 pattern (Table 2). With more diverse isolates, it is less common to see amplification with both of the tested primer pairs for each O-antigen cluster type, implying that the entire cluster is not always present intact (Table 3). Nonetheless, 18 of 20 isolates in the diverse set of isolates tested by PCR were able to amplify at least one of the O-antigen cluster genes (Table 4). Thus, it remains a possibility that a relatively small set of O-antigen gene sets is present among *A. baumannii* isolates. Previous characterization of the O-antigen diversity among isolates from the *A. baumannii-A. calcoaceticus* complex by use of monoclonal antibodies showed considerable variation in reactivity, indicating multiple distinct lipopolysaccharide modifications, even among closely related isolates (34).

Two-component regulatory systems. Bacterial two-component systems, consisting of a sensor histidine kinase and a response regulator, enable changes in gene expression in response to external stimuli (6, 16). Nineteen two-component systems were identified in *A. baumannii* AB0057 (see Table S5 in the supplemental material). Most of these are also present in the other clinical isolates. The downstream target genes for most of these remain unknown, but some are likely to play important roles in infection. The AdeRS pair regulates a multidrug efflux pump, contributing to resistance to aminoglycosides, tetracycline, erythromycin, chloramphenicol, trimethoprim, and fluoroquinolones (27, 28). AdeRS proteins are not present in AB900, SDF, or ADP1.

Two two-component systems that are likely to be involved in controlling resistance to heavy metals were found, including *cusSR* (30) (AB57_0660-1) and a less-well-characterized pair encoded by AB57_2550-1. The *cusSR* genes are absent from AB307-0294 and ACICU, along with the likely target genes involved in copper resistance. The *cusSR* genes are also missing from ADP1, while the likely target genes involved in copper resistance are present. In ATCC 17978, the regulatory and

TABLE 2—*Continued*

PCR result with primer[b]												
AB57_0106	AB57_0107	AB57_0108	AB57_0109	ABAYE3806	ABAYE3807	ABAYE3808	ABAYE3809	ABAYE3810	ABAYE3811	ABAYE3812	ABAYE3813	ABAYE3814
−	−	−	−	−	−	−	−	−	−	−	−	−
+	+	+	+	−	−	−	−	−	−	−	−	−
+	+	+	+	−	−	−	−	−	−	−	−	−
+	+	+	+	−	−	−	−	−	−	−	−	−
+	+	+	+	−	−	−	−	−	−	−	−	−
+	+	+	+	−	−	−	−	−	−	−	−	−
+	+	+	+	−	−	−	−	−	−	−	−	−
+	+	+	+	−	−	−	−	−	−	−	−	−
−	+	−	−	+	+	+	+	+	+	+	+	+
+	+	+	+	−	−	−	−	−	−	−	−	−
−	−	−	−	+	−	+	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−	−	−	−	+
−	+	−	−	+	+	+	+	+	+	+	+	+
−	−	−	−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−	−	−	−	−

likely target genes are present adjacent to one another, but the gene cluster is located in a nonsyntenic position relative to flanking genes in AB0057. The predicted CusS and CusR proteins have their best (non-*A. baumannii*) matches to proteins from several betaproteobacteria, suggesting that they are derived from a lateral transfer event into an *A. baumannii* ancestor following the split from *A. baylyi* ADP1.

At least one component of 11 of the 19 two-component systems that are present in the clinical isolates is absent from SDF, suggesting a reduced capacity to respond to environmental changes in this organism.

DISCUSSION

MDR in *A. baumannii* has been defined operationally as resistance to representatives of three or more of the following classes of antibiotics: quinolones (e.g., ciprofloxacin), extended-spectrum cephalosporins (e.g., ceftazidime and cefepime), β-lactam–β-lactamase inhibitor combinations (e.g., piperacillin-tazobactam), aminoglycosides (amikacin, gentamicin, and tobramycin), and carbapenems (e.g., imipenem and meropenem) (19). *A. baumannii* uses several strategies to evade antibiotics, including multiple detoxifying enzymes, efflux

TABLE 3. Distribution of O-antigen genes with second primer set

Isolate[a]	PCR/ESI-MS type[a]	PCR result with primer[b]											
		AB57_0094	AB57_0095	ABAYE3806	ABAYE3807	AB900_1	AB900_2	A1S_0053	A1S_0057	ACICU_00077	ACICU_00080	ABSDF0078	ABSDF0066
ATCC 17978		−	−	−	−	−	−	+	+	−	−	+	−
AB069	1	−	−	−	−	−	−	−	−	+	+	+	+
AB063	3	−	−	−	−	−	−	−	−	+	+	−	+
AB061	3	−	−	−	−	−	−	−	−	−	−	−	+
AB068	9	−	−	−	−	−	−	−	−	−	−	−	−
AB039	10	−	−	−	−	−	−	−	−	+	+	+	−
AB033	10	−	−	−	−	−	−	−	−	+	+	+	−
AB021	11	−	−	−	−	−	−	−	−	−	−	−	+
AB024	11	−	−	−	−	−	−	−	−	−	−	−	+
AB054	12	−	−	−	−	−	−	−	−	−	−	−	−
AB002	14	−	+	+	−	−	−	−	−	−	−	−	−
AB009	14	−	+	+	−	−	−	−	−	+	−	−	−
AB058	15	−	+	+	+	−	−	−	−	−	−	−	−
AB0057	15	+	+	−	−	−	−	−	−	−	−	−	−
AB070	16	−	−	+	−	+	−	+	−	−	−	−	−
AB900	19	−	−	−	−	+	+	−	−	−	−	−	−
AB051	24	+	+	−	−	+	−	−	−	−	−	+	−
AB041	24	−	-	−	−	+	−	−	−	−	−	+	−
AB074	39	−	+	+	−	+	−	−	−	−	−	−	−
AB307-0294	46	−	+	+	+	−	−	−	−	−	−	−	−
AB066	46	−	−	−	−	−	−	−	−	−	−	−	+
AB071	47	+	+	−	−	−	−	−	−	+	+	−	+
AB072	48	−	−	−	−	+	−	−	−	+	+	−	+

[a] Isolate and PCR/ESI-MS type information is from reference 19.
[b] Primer set prefixes indicate primers designed from O-antigen genes in the following isolates: AB57, AB0057; ABAYE, AYE; A1S, ATCC 17978; ABSDF, SDF; ACICU, ACICU; and AB900, AB900.

TABLE 4. O-antigen genes tested by PCR

ORF	Description
AB57_0094	VI polysaccharide biosynthesis protein VipA/TviB
AB57_0095	VI polysaccharide biosynthesis protein VipB/TviC
AB57_0096	Polysaccharide biosynthesis protein
AB57_0097	Conserved hypothetical protein
AB57_0099	Glycosyltransferase, group 1
AB57_0100	Hypothetical protein
AB57_0102	Putative glycosyltransferase family 1
AB57_0103	Glycosyltransferase, group 1
AB57_0104	UDP-glucose 4-epimerase
AB57_0105	Polyprenol phosphate:*N*-acetyl-hexosamine 1-phosphate transferase
AB57_0106	Acetyltransferase
AB57_0107	Nucleotide sugar epimerase/dehydratase
AB57_0108	UDP-glucose 4-epimerase
AB57_0109	Conserved hypothetical protein
ABAYE3806	Putative perosamine synthetase (WeeJ/Per)
ABAYE3807	Putative acetyltransferase (WeeI)
ABAYE3808	Putative UDP-galactose phosphate transferase (WeeH)
ABAYE3809	Putative glycosyltransferase family 1
ABAYE3810	Conserved hypothetical protein; putative polysaccharide polymerase
ABAYE3811	Putative polysaccharide polymerase
ABAYE3812	Putative glycosyltransferase family 1
ABAYE3813	Putative polysaccharide biosynthesis protein
ABAYE3814	Putative NAD-dependent epimerase/dehydratase (WbpP)
A1S_0053	MviM protein
A1S_0057	Capsular polysaccharide synthesis enzyme
ABSDF0066	Putative UDP-glucose/GDP-mannose dehydrogenase
ABSDF0078	Putative UDP-galactose phosphate transferase (WeeH)
ACICU_00077	CMP-*N*-acetylneuraminic acid synthetase
ACICU_00080	Sialic acid synthase

pumps, target site mutations, and gene regulatory changes. AB307-0294 is largely drug susceptible yet highly similar to the MDR strains AYE and AB0057. This prompted us to seek to explain the resistance phenotype on the basis of genetic differences between AB307-0294 and the MDR strains. Resistance to each tested antibiotic (Table 1) could be accounted for by the acquisition of a specific resistance determinant in AYE and AB0057. AB307-0294 has orthologs of the transporters and efflux pumps that are present in AYE and AB0057, but it appears that these alone are not sufficient to confer resistance to β-lactam and aminoglycoside antibiotics.

Among 74 isolates from WRAMC, resistance to the aminoglycosides amikacin and tobramycin was always associated with the presence of at least one aminoglycoside-modifying enzyme (AME) (19). In addition, a recent study of *A. baumannii* isolates in New York City showed that resistance to cefepime and tigecycline could be ascribed to increased expression of AdeABC in some isolates, while resistance to other cephalosporins and aminoglycosides was mediated by antimicrobial drug-inactivating enzymes and fluoroquinolone resistance was always associated with gyrase gene mutations (4). It is possible that upregulation of efflux pumps may be found to be sufficient to confer resistance to certain classes of antibiotics in other isolates. For example, one WRAMC isolate is ciprofloxacin resistant, despite carrying *gyrA* and *parC* alleles associated with drug susceptibility (54, 55), suggesting an alternative mechanism of resistance. Taken together, the extensive similarities between AB307-0294 and the MDR isolates imply that while drug efflux may be a contributor to clinically relevant antibiotic resistance, it is not the primary mechanism of MDR.

The RI is a dominant genomic feature in AYE and AB0057, exhibiting a large concentration of laterally transferred genes associated with antimicrobial resistance intermixed with a diverse set of transposon- and integron-related sequences that have presumably facilitated the transfer of this region of the genome into *A. baumannii.* The importance of the RI in contributing to the MDR phenotype is considerable in these isolates, with genes encoding resistance to cephalosporins, carbapenems, and aminoglycosides. In each of the MDR isolates, however, additional loci contribute to drug resistance. IS-flanked β-lactamase genes are found outside the RIs in AB0057 and ACICU, and cephalosporin resistance in AYE is likely due to an ISAba1 insertion adjacent to the $bla_{\text{ADC-9}}$ gene. Furthermore, the RI found in AYE and AB0057, and to a lesser extent, ACICU, does not appear to be present in all MDR isolates based on PCR amplification of several genes located throughout the RI. Thus, it is likely that alternative genetic arrangements of resistance genes will be found as additional isolates are sequenced.

Quinolone resistance is primarily mediated by mutations in the housekeeping genes *gyrA* and *parC*, which are not RI associated. The *bla* genes (e.g., encoding TEM, OXA, VEB, and CTX-M) are frequently found in locations outside the RI, even in isolates that contain the RI. In ACICU, two copies of $bla_{\text{OXA-58}}$ are present on a plasmid, and in AB0057, $bla_{\text{OXA-23}}$ is located on the second island copy, ISAbaR4. In the MDR isolates analyzed to date, AMEs are associated with the RI. It remains to be determined whether AMEs are associated with RIs of novel structure in isolates that do not contain an RI analogous to those described in AYE, AB0057, and ACICU. In summary, evidence to support the notion that the RI is the sole, or even primary, genetic basis for clinically relevant MDR is still needed.

It is curious that the sequence of the >50-year-old isolate ATCC 17978 is not more divergent than those of other isolates, suggesting that although *A. baumannii* isolates differ by ~2% at the sequence level, there may be considerable stability in each clade. AYE and AB0057 were isolated more than a decade after AB307-0294 yet have diverged <0.1% at the nucleotide level compared with the older isolate. SDF also differs from the other *A. baumannii* isolates by ~2% at the DNA level, although it has also undergone a considerable reduction in gene number, presumably driven by the action of a large number of IS elements (51). It was argued that SDF was acquired by the louse directly from the bloodstream of an infected human in the course of a blood meal, since the louse gut is sterile (24). An alternative explanation is that SDF represents an *A. baumannii* lineage that is adapted for growth in the louse rather than in humans. Additional epidemiological surveys will be necessary to determine whether SDF-like *A. baumannii* isolates can be found in humans.

Antimicrobial resistance in *A. baumannii* arises by a combination of genetic changes and selective pressure. The following three classes of genetic alteration contribute to the acquisition

of a resistance phenotype: lateral gene transfer, gene amplification (by duplication and/or increased expression), and mutation of genes or their promoters (resulting in loss of function, enhancement of function, or gain of novel function). Each of these mechanisms is represented in *A. baumannii*. Further studies that address the balance among these strategies, the genetic and genomic architectures that support them, the role of mobile genetic elements, and the pace of genetic change will be facilitated by the sequences presented here and those of additional isolates that represent further examples of MDR and susceptible isolates of clinical significance.

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