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Genomewide Analysis of Divergence of Antibiotic Resistance Determinants in Closely Related Isolates of *Acinetobacter baumannii*▽

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Multidrug resistance has emerged as a significant concern with infections caused by *Acinetobacter baumannii*. Ample evidence supports the involvement of mobile genetic elements in the transfer of antibiotic resistance genes, but the extent of variability and the rate of genetic change associated with the acquisition of antibiotic resistance have not been studied in detail. Whole-genome sequence analysis of six closely related clinical isolates of *A. baumannii*, including four from the same hospital, revealed extensive divergence of the resistance genotype that correlated with observed differences in antimicrobial susceptibility. Resistance genes associated with insertion sequences, plasmids, and a chromosomal resistance gene island all showed variability. The highly dynamic resistance gene repertoire suggests rapid evolution of drug resistance.

Acinetobacter baumannii has garnered significant attention as a cause of infection among military personnel injured in Iraq and Afghanistan and is also a rapidly emerging nosocomial pathogen in hospitals throughout the world (1, 8, 24, 28, 29, 38). In many health care facilities, more than two-thirds of *A. baumannii* infections are resistant to at least three chemical classes of antimicrobial compounds and are considered multidrug resistant (MDR), leaving patients and doctors few options for therapy (18, 28). Better understanding of the mechanisms of acquisition of multidrug resistance and the rate and nature of genetic change is essential for developing strategies to combat the spread of MDR *A. baumannii.*

Comparative analysis of genome sequences of *A. baumannii* isolates revealed considerable genetic variability among recent isolates, with poor correlation between genetic relatedness and pattern of antimicrobial susceptibility (2, 14, 39). Mobile genetic elements are known to contribute to antibiotic resistance by facilitating resistance gene transfer and by upregulating transcription (7). A "resistance island" (RI) of variable composition is present in the MDR *A. baumannii* strains analyzed to date (2, 11, 15, 31). The RI is a complex composite transposon that carries laterally transferred genes related to antibiotic and heavy metal resistance. In addition, plasmid-borne and insertion sequence (IS)-associated resistance genes are common (3, 4, 19, 36). Several families of insertion sequences in *A. baumannii* have been described, including IS*Aba1*, which is frequently associated with β-lactamase genes and has intrinsic mobilization capability (23, 33).

In general, outbreaks occurring in hospitals are presumed to be clonal, with patient-to-patient transmission of essentially identical strains. Treatment decisions are based on a combination of *in vitro* susceptibility assays and empirical results based on patient outcomes. Molecular strain typing methods such as pulsed-field gel electrophoresis (PFGE) and PCR combined with electrospray ionization-mass spectrometry (PCR/ESI-MS) are used to assess the relatedness of isolates (8). Based on PFGE and PCR/ESI-MS sequence typing, the large outbreak at Walter Reed Army Medical Center (WRAMC) was clearly polyclonal (14, 39). Furthermore, a diversity of resistance phenotypes was observed, even among isolates with identical molecular typing signatures. This suggests that either the molecular typing methods lack the resolution necessary to distinguish closely related strains or the resistance gene repertoire can change rapidly by lateral gene transfer (or both).

Recently, genomewide studies enabled by high-throughput DNA sequencing have enhanced our ability to evaluate the microevolution of bacterial pathogens (9), for example, by characterizing the appearance of point mutations associated with the development of antibiotic resistance over the course of an infection in a single patient (25), the worldwide distribution of pathogens with reasonably stable genomes (13, 22), and the extent of evolution in more highly variable genomes (17). These and other studies have led to the pan-genome concept, in which "core" genes are shared by all isolates of a species and "accessory" genes are present in a subset of isolates (12, 21). Here we describe a genomewide analysis of six closely related *A. baumannii* isolates belonging to European clone type I (27), including four from WRAMC. Our results illustrate the extent to which mobile genetic elements have caused a rapid divergence of resistance genotype and phenotype.

MATERIALS AND METHODS

Bacterial strains and DNA sequencing. The complete genome sequences of *A. baumannii* AYE, AB307-0294, and AB0057 have been determined (2, 37). The MDR isolates AB056, AB058, and AB059 have been previously described (14). A summary of relevant antibiotic resistance traits is given in Table 1. Assignment to strain types was performed by PCR/ESI-MS for AB056, AB0057, AB058, and AB059 (14) and by comparison of the genome sequence to the sequence type

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TABLE 1. Summary of antimicrobial resistance of WRAMC isolates

Characteristic	AB307-0294	AYE	AB058	AB059	AB056	AB0057
Source	Buffalo, NY	France	WRAMC	WRAMC	WRAMC	WRAMC
Culture date	1994	2001	May 2003	January 2004	February 2004	August 2004
Resistance phenotype[a]						
AMP	+	+	+	+	+	+
CIP		+	+	+	+	+
CAZ		+	+	+	+	+
FEP		+	+	+	+	+
AMK		+	+	+		
TOB		+	+			
SAM		ND[b]		+	+	+
MEM				+	+	+
IPM				+	+	+
PCR-positive genes						
Tn*6018*	−	+	+	+	+	+
aadB	−	+	+	+	−	−
topA	−	−	+	+	+	+
tetA	−	+	−	+	−	+
merAC	−	+	−	+	−	+
merE-R	−	+	−	+	−	+
cat	−	+	−	+	−	+
bla_{TEM}	−	−	−	+	−	+
int1	−	+	−	+	+	+
aadA1	−	+	−	+	+	+
aacC1	−	+	−	+	+	+
aac/aad1	−	+	−	+	+	+
bla_{OXA-23}	−	−	−	+	+	+

[a] AMP, ampicillin; CIP, ciprofloxacin; CAZ, ceftazidime; FEP, cefepime; AMK, amikacin; TOB, tobramycin; SAM, ampicillin-sulbactam; MEM, meropenem; IPM, imipenem.
[b] ND, not determined.

patterns (14) for AYE and AB307-0294. Genomic DNA was prepared using a DNeasy kit from Qiagen, and libraries were prepared for sequencing using kits supplied by Illumina (San Diego, CA), exactly as described by the manufacturer. Illumina sequencing was performed in the Genomics Core Facility at Case Western Reserve University. Single-end 36-base reads were obtained for each genome.

Sequence data analysis. The alignment program Maq (16) was used to align the Illumina reads to the sequence of each published genome. This information was used to determine the presence and absence of specific open reading frames (ORFs) in each draft genome sequence. Each genome set of reads was also assembled independently using the *de novo* sequence assembly program Velvet (41). A series of values was used for the word size parameter *k*, with the setting that resulted in the smallest number of contigs used for further analysis. Sequences and sequence assemblies were compared using Mummer (6). The percentage of each genome represented in the assembled contigs was estimated based on the Mummer alignments of the draft genomes with AB0057.

Phylogenetic analysis. AB0057 ORFs were aligned to each genome. DNA sequences from a set of 1,927 ORFs with alignments at ≥95% nucleotide identity and spanning the full length of the ORF in all six genomes were aligned using ClustalW (5). The concatenated alignment was analyzed in PHYLIP (10), and an unrooted tree was inferred using the neighbor-joining algorithm with 1,000 bootstrap replicates.

Verification of assembly structure by PCR. Eight primer pairs (Table 2) spanning key repeat regions or gaps between contigs were used to amplify segments of the RI and *aphA6* region to confirm the sequence structures hypothesized based on computational analysis.

Nucleotide sequence accession numbers. The Whole Genome Shotgun projects described in "Sequence data analysis" above have been deposited at DDBJ/EMBL/GenBank under accession numbers ADGZ00000000, ADHA00000000, and ADHB00000000.

RESULTS AND DISCUSSION

Our goal is to understand the highly dynamic resistance gene repertoire of *A. baumannii*. To that end, we performed

TABLE 2. PCR primers

Primer names	Primer sequences (5′ to 3′)	Purpose
pACICU2A, pACICU2B	TTTAAAACCCATTTACATCTCCTT, AAAACCGCTCGAAAAGATCA	Determine presence of IS*Aba125* at 34.9–36.0 kbp
aphA, aphB	TTTGACGTTGCTCTTGTTGC, TGCACAATGTGATGGAGTCT	Test insertion at 10.7 kb of pACICU2
aphC, aphD	TCAAGCTCTAGCCGAAAACAA, TTCATCTATTTCAAATTCATCTTGC	Test insertion at 21.9–22.3 kb of pACICU2
aphE, aphF	CATTTTCTTTCGCATACAGCA, TTTTTGCAATTTCAGCTTGAG	Test assembly across *aphA6* region
aphK, aphJ	CAGGGGTATATGATGCACTTGA, ATCACATATCCCGCTTCTGG	Test assembly across *aphA6* region
aphG, aphL	GAGGCCCGCATTTAATCTCT, ATGGGTGGCAAAGTAAGGTT	Test insertion at 21.9–22.3 kbp of pACICU2
RIB, RI3	AGGTGACGATTTCCGATGAC, CTCAAGTAGATTGCGAATCGTG	Test RI structure in AB056, AB058
RIE, RI6	TTGCTCGGCCTTCAGCCTGC, GCCTTGCTCCGCAGCGGTTA	Test RI structure in AB056, AB058

TABLE 3. Features of draft genome assemblies

Parameter	Value for isolate:		
	AB056	AB058	AB059
No. of reads passing quality filter	5,060,940	6,539,630	4,831,329
No. of reads mapped to AB0057 chromosome	4,615,115	5,527,077	4,369,180
No. of Velvet contigs (>100 bases)	1,354	1,416	1,540
Velvet N50 (kbp)	11.3	8.3	9.8
Assembled length (bp)	3,857,297	3,774,325	3,928,094
Avg fold coverage	45.7	57.4	40.7
Estimated % coverage	99.3	99.5	99.2

genomewide comparative analyses of six closely related strains. Four isolates from an *A. baumannii* outbreak at WRAMC between 2003 and 2004 were identified as belonging to ESI-MS sequence type 15 (ST15), suggesting a high degree of similarity to one another (14, 39) and to *A. baumannii* strain AYE, which was responsible for an outbreak in France in 2001 (11, 30, 37). AB307-0294 belongs to ST46, which differs from ST15 by one nucleotide across 1,679 bp assayed by PCR/ESI-MS; it was included in the analysis since it is closely related to the ST15 strains based on genomewide analysis (2) and because it is largely drug susceptible and so serves as a useful comparison. The antibiotic susceptibility profile among the MDR ST15 isolates has considerable overlap, but differences were observed, and each strain is distinct at the level of resistance genotype based on PCR analysis of specific resistance determinants (Table 1). In order to further characterize the relatedness among these isolates and to assess the context of the genetic differences in resistance, we collected and analyzed genome sequences for the WRAMC strains.

The genome sequences of AB0057, AYE, and AB307-0294 were previously completed to high quality (2, 37) and serve as a reference for comparison. We obtained "draft"-quality genome sequence data from the other three isolates (AB056, AB058, and AB059). Table 3 provides summary statistics on the sequencing and assembly.

The sequence data were analyzed in two ways. First, each read was mapped to the AB0057, AYE, and AB307-0294 genomes, and the coverage of each predicted open reading frame was determined. The number of AB0057 ORFs without sequence coverage ranged from zero (AB059) to 19 (AB056) to 354 (AB058). Second, a *de novo* assembly of each data set was performed using Velvet (41) to characterize unique sequences and sequence organization in each isolate. Genome sequencing results confirmed the presence of specific resistance determinants that had been inferred previously on the basis of PCR amplification (Table 1) and clarified their genomic context. We consider the roles of three classes of mobile genetic elements, i.e., the RI, IS elements, and plasmids, in contributing to antibiotic resistance and the roles of endogenous chromosomal genes. We then address other genomic changes and the broader relationships of the ST15 strains.

Resistance island structure. The AB059 RI is identical to AbaR3 (AB0057). Each other isolate has a distinct genetic complement in the resistance island (Fig. 1). The RIs in AB056 and AB058 have been designated AbaR9 and AbaR10, respectively. AbaR9 is missing 19 RI ORFs relative to AbaR3, including the genes *tetA*, *cat*, and bla_{TEM-1} (AB57_0270 to AB57_0288). AbaR10 is missing 26 ORFs compared with AbaR3. The missing region is a superset of the genes that are

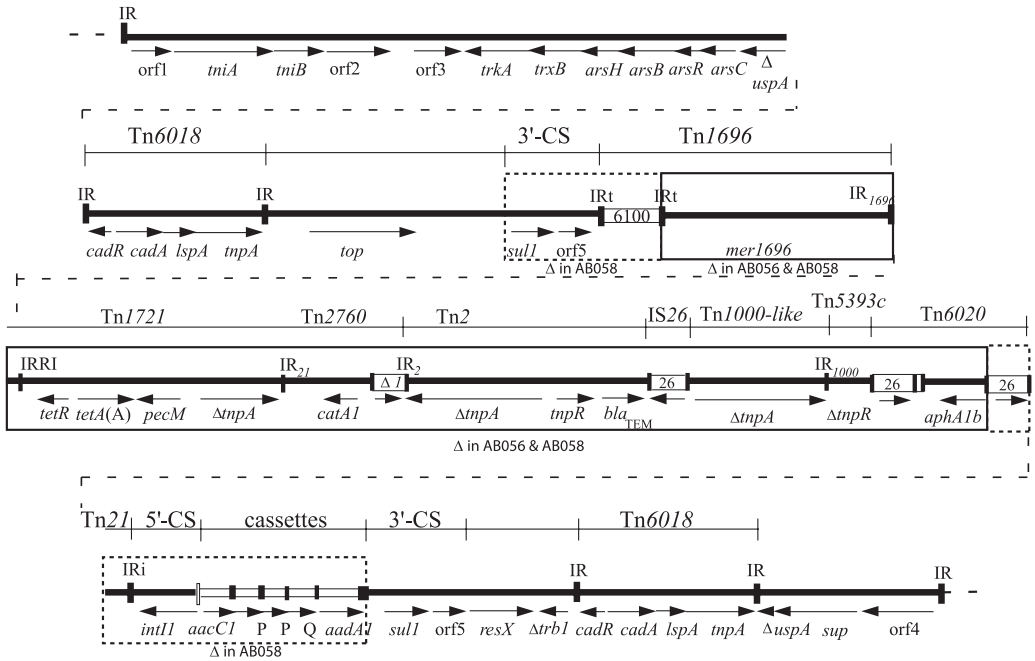

FIG. 1. Diagram of the resistance island. The AB0057 RI is shown, with boxed areas indicating regions that are absent from AbaR9 in AB056 (solid) and from AbaR10 in AB058 (solid and dashed). (Adapted from reference 31 with permission.)

TABLE 4. Insertion sequence locations in ST15 strains: IS*Aba1* locations

AB0057 location (bp)	Present in:						Gene(s)	Knockout	Left	Right
	AB0057	AB056	AB059	AB058	AYE	AB307-0294				
6679	Yes	Yes	Yes				AB57_0006/0013	RND-type efflux pump AdeT		
11014	Yes	Yes	Yes				AB57_0006/0013	RND-type efflux pump AdeT		
553764	Yes	Yes	Yes				AB57_0513/0516	ABC transporter ATP-binding protein Uup		
584630	Yes	Yes	Yes				AB57_0548/0557	Sulfate permease		
588255	Yes	Yes	Yes				AB57_0548/0557	Sulfate permease		
976679	Yes	Yes	Yes				AB57_0915/0918		Hypothetical protein	Hypothetical protein
2837512	Yes	Yes	Yes				ABAYesE1139	Hypothetical protein		
2182134		Yes					AB57_2102/2103		Putative acid phosphatase	Hypothetical protein
567008			Yes				AB57_0529	Monovalent cation transporter		
42837				Yes			AB57_0043/0044		Phosphoribosylaminoimidazole carboxylase, ATPase subunit	Transglycolase
327369				Yes			AB57_0307	Mg chelatase-related protein		
343044				Yes			AB57_0323/0324		Type 4 fimbria expression regulatory protein PilR	Sensor protein histidine kinase
1754856				Yes			AB57_1667/1668		Cytochrome *bd* ubiquinol oxidase, subunit I	Conserved hypothetical protein
1795889				Yes			AB57_1703	Lysine exporter protein		
1891168				Yes			AB57_1804	Type 4 fimbrial biogenesis protein		
1927343				Yes			AB57_1839	Hypothetical protein		
2165299				Yes			AB57_2084	AdeS		
2234941				Yes			AB57_2153/2154		Antibiotic biosynthesis monooxygenase	Conserved hypothetical protein
2873291				Yes	Yes		AB57_2795/2796		GTP cyclohydrolase I	Beta-lactamase AmpC
3100988				Yes			AB57_3010	Hypothetical protein		
3874912				Yes			AB57_3756/3757		Acetate coenzyme A ligase	Conserved hypothetical protein
3932193				Yes			AB57_3812	Hypothetical protein		
29318					Yes		AB57_0028/0029		Cation diffusion facilitator family transporter	Thiol:disulfide interchange protein DsbC
67617					Yes		AB57_0062/0063		Riboflavin biosynthesis protein RibF	C_4-dicarboxylate transporter/malic acid transport protein
601852					Yes		AB57_0567/0568		Alkaline phosphatase	Twin arginine-targeting protein translocase TatC
1219316					Yes		AB57_1148	TonB-dependent siderophore receptor		
1224004					Yes		AB57_1151	Hypothetical protein		
1229984					Yes		AB57_1155-AB57_1187	32 genes		
1502475					Yes		AB57_1426	Transcriptional regulator		
1523389					Yes		AB57_1443	RHS family protein		
1626096					Yes		AB57_1540/1541		Hypothetical protein	Hypothetical protein

1835470	Yes	AB57_1745	Fimbrial biogenesis outer membrane usher protein		
2087097	Yes	AB57_1996/1997		Conserved hypothetical protein	Phospholipase D/ transphosphatidylase
2089231	Yes	AB57_1998	Hypothetical protein		
2221018	Yes	AB57_2137	Conserved hypothetical protein		
2348892	Yes	AB57_2261/2262		Conserved hypothetical protein	Cytochrome *d* ubiquinol oxidase subunit I
2848450	Yes	AB57_2777/2778		Superoxide dismutase [Fe]	Conserved hypothetical protein
2977293	Yes	AB57_2884/2885		Hypothetical protein	Fatty acid desaturase
3540209	Yes	AB57_3433	Conserved hypothetical protein		
3856951	Yes	AB57_3743/3744		GGDEF family protein	Hypothetical protein

absent from AbaR9 and includes the additional loss of the aminoglycoside-modifying enzyme genes *aacC1* and *aadA1*, leaving a sulfonamide-resistant dihydropteroate synthase gene as the only resistance gene located on the RI.

Although it is not possible to firmly establish whether AbaR9 and AbaR10 represent independent or sequential deletions from a common larger progenitor or whether the RIs have been built by independent insertions in a smaller progenitor, the nature of the junction sequences argues for deletions rather than insertions. In AbaR9, a 5′-ward deletion mediated by an IS*26* element is likely. In AbaR10, a deletion mediated by recombination in the two class I integron 3′ conserved sequence (3′CS) regions would explain the observed structure. Structurally, AbaR9 and AbaR10 are more closely related to AbaR3 than to AbaR1 (AYE). Four additional *A. baumannii* RI sequences with distinct structures and gene complements support the dynamic nature of the RI (15, 31, 32).

Mobile genetic elements: insertion sequences. The locations of IS*Aba1* and IS*Aba125* insertion sequences in the draft genomes were determined (Tables 4 and 5). The IS*Aba1* sequence was represented as a single contig in each Velvet assembly, representing a collapsed consensus sequence of multiple IS copies. By using a high E-value cutoff, however, we were able to map short junction fragments located at regions where unique genome segments abut the beginning or end of the IS*Aba1* sequence.

IS*Aba1*-flanked β-lactamase genes $bla_{\text{ADC-7}}$ and $bla_{\text{OXA-23}}$ were found in AB056 and AB059 in the same chromosomal positions as in AB0057, but both of these insertion sequence cassettes were not present in AB058. The presence of $bla_{\text{OXA-23}}$ results in resistance to the carbapenems meropenem and imipenem in AB056 and AB059. Ampicillin/sulbactam resistance in AB0057, AB056, and AB059 may be due to a combination of overexpression of $bla_{\text{ADC-7}}$ driven by the adjacent IS*Aba1* promoter and the $bla_{\text{TEM-1}}$ gene located on the RI; neither of these resistance genes is present in AB058 or AYE.

Seven of eight IS*Aba1* insertion locations in AB0057 are preserved in AB056 and AB059, and each of these three genomes appears to contain one unique insertion site for IS*Aba1*. In contrast, AB058 carries 13 IS*Aba1* elements, and none of them is at the same location as in AB0057. AB058 IS*Aba1* insertion sites also have only a single overlap with the 21 IS*Aba1* insertion sites in the AYE genome: both have an insertion at precisely the same location immediately upstream of the chromosomal $bla_{\text{ADC-7}}$ gene. An IS*Aba1* insertion in the *adeS* gene, which encodes a regulator of the efflux pump AdeABC, may contribute to the resistance phenotype (34).

AB058 has 12 copies of the IS*Aba125* element. As discussed below, two of these flank an *aphA6* gene on the plasmid pAB059, and the remainder are chromosomal. The *aphA6*-associated IS*Aba125* elements are the only two representatives of this IS class found in AB059. The only other sequenced *A. baumannii* genome with IS*Aba125* sequences is ACICU, which belongs to European clone II (15).

Mobile genetic elements: plasmids. A total of nine different plasmids were identified (Table 6) among the five ST15 strains; no plasmids are present in AB307-0294. All four WRAMC isolates carry the plasmid pAB0057. AB056 and AB0057 share the same two plasmids, but the plasmid content is otherwise

TABLE 5. Insertion sequence locations in ST15 strains: IS*Aba125* locations in AB058

AB0057 location (bp)	AYE location	Gene(s)	Knockout	Left	Right
NA[a]				Hypothetical protein	AphA6
NA				AphA6	Hypothetical protein
	3849585	ABAYE3813	Polysaccharide polymerase		
257283		AB57_0237	Outer membrane protein OprE3		
919546		AB57_0855/6		Trehalose-phosphotase	Biotin biosynthesis protein BioH
1559510		AB57_1478	Hypothetical protein		
1836640		AB57_1745/6		Fimbrial biogenesis outer membrane usher protein	Pilus assembly chaperone
2224066		AB57_2143	Hypothetical protein		
2508844		AB57_2422	Conserved hypothetical protein		
2912916		AB57_2829	Hypothetical protein		
3671716		AB57_3564	Hypothetical protein		
3971913-3971985		AB57_3850	Histidine kinase		

[a] NA, not applicable; the IS*Aba125* element is located on a plasmid that is not present in AB0057 or AYE.

distinct in each strain. Three plasmids are worthy of further note.

AB058 and AB059 are known to harbor the aminoglycoside-modifying enzyme gene *aphA6* (14), which confers resistance to amikacin. The *aphA6* gene was found on an ~900-bp contig in each assembly that is flanked by IS*Aba125* sequences. This structure matches the original description of the *aphA6* gene, although the IS elements were not recognized at the time (20). IS*Aba125*-associated sequences adjacent to *aphA6* provide the −35 sequence to drive *aphA6* transcription (26). An IS*Aba125* element is present in the plasmid pACICU2, and a closely related plasmid, termed pAB059, was found in both AB058 and AB059. pACICU2 was originally described in the European clone type II strain ACICU (15). Although an IS*Aba125* element is present in the published pACICU2 sequence at bases 34926 to 35996, our sequence assemblies and directed PCR across this region confirmed that the IS element is not present at that location in pAB059. Further examination of the IS*Aba125* flanking sequences suggested that the 5.7-kbp *aphA6* gene region was located elsewhere in pAB059, and this was confirmed by PCR.

pAB058 is a significantly smaller relative of pABIR (40) that lacks the $bla_{\text{OXA-58}}$ gene for which the plasmid was named (IR stands for imipenem resistance) and also lacks the IS*Aba125* element. The *aadB* gene (conferring resistance to tobramycin) in AB058 is on the plasmid pRAY (35) and is not associated with an IS element or transposon. This is in contrast to the location on AbaR1 in AYE.

Endogenous chromosomal genes associated with antibiotic resistance. Quinolone resistance mutations in *gyrA* and *parC* were as described previously, except that AB058 carries the resistant allele of *parC*. Predicted protein sequences of the penicillin-binding proteins are identical in AYE, AB056, AB057, and AB059; however, differences in the expression of penicillin-binding protein genes could also contribute to the resistance phenotype. As noted above, IS*Aba1* elements have also affected genes associated with resistance, including $bla_{\text{ADC-7}}$ (upregulation) and *adeS* (inactivation).

Unique chromosomal sequences in each isolate. Other than the shorter RI in AB056, the AB056 and AB059 assemblies are consistent with chromosomal sequences that are essentially identical to the AB0057 chromosome. No additional AB0057 gene regions were missing from the assemblies, and there were no contigs that could not be mapped to the AB0057 genome or the plasmids described above.

AB058 has more differences in gene content. In addition to

TABLE 6. Plasmid contents of ST15 isolates

Plasmid	Accession no.	Size (kbp)	Note(s)	Present in:					
				AB0057	AB056	AB058	AB059	AYE	AB307-0294
pAB0057	CP001183	8.7		+	+	+	+		
pAB49	L77992	2.5		+	+				
pAB059		66.5	Related to pACICU2; IS*Aba125-aphA6*			+	+		
pAB058		7.2	Related to pABIR			+			
pRAY	AF003958	6.1	*aadB*			+			
pABAYE1	CU459137	5.6						+	
pABAYE2	CU459138	9.7	Related to pAB0057					+	
pABAYE3	CU459140	94.4						+	
pABAYE4	CU459139	2.7						+	

TABLE 7. Chromosomal gene content differences between AB058 and AB0057/AB056/AB059: AB0057 regions absent from AB058

Region		No. of genes	Present in:		Description
Start	End		AYE	AB307-0294	
AB57_0007	AB57_0012	6	No	No	IS*Aba1*/bla_{ADC} cassette
AB57_0091	AB57_0114	24	No	No	O-antigen biosynthesis
AB57_0268	AB57_0293	26	Yes	No	RI
AB57_0547	AB57_0566	20	No	No	AbaR4
AB57_0985	AB57_0996	12	No	No	Iron uptake operon including FecI, FecR, and heme oxygenase
AB57_1225	AB57_1310	86	No	No	Phage
AB57_1437	AB57_1449	13	Yes	Yes	Rhs operon, hypothetical proteins
AB57_1673	AB57_1686	14	Yes	Yes	Taurine, arsenate
AB57_2010	AB57_2071	62	No	No	Phage
AB57_2397	AB57_2400	4	Yes	Yes	OmpW and 3 hypothetical proteins
AB57_2726	AB57_2739	14	No	No	Partial phage
AB57_3193	AB57_3258	66	No	No	Phage
AB57_3399	AB57_3402	4	No	No	Glycosyltransferase genes

the differences in RI gene content and *bla* gene regions described above, six chromosomal regions that are present in AB0057/AB056/AB059 are absent from AB058 as well as AYE and AB307-0294 (Table 7). A cluster of nine genes, including at least four involved in iron uptake, is missing from AB058. Interestingly, these nine genes are present in *A. baylyi* ADP1 and *A. baumannii* ACICU and SDF but not in ATCC 17978 or A900, suggesting that they are not essential for pathogenicity. The cluster of genes involved in biosynthesis of the O-linked antigen component of lipopolysaccharide has been shown to vary in *A. baumannii*, with one set of genes present in AB0057 and a different set in AYE and AB307-0294 (2); AB058 has the AYE-type O-antigen gene cluster. AB057/AB056/AB059 also share four prophage insertions that are not present in AB058. Three additional regions are absent from AB058 but present in all the other ST15 strains, including a 14-gene block encoding a taurine transporter and arsenate resistance genes and a 17-kbp region containing a recombination hot spot (Rhs) family gene. With the exception of the taurine/arsenate block, each of these regions is part of the "accessory" genome, meaning that it is absent from at least one additional sequenced *A. baumannii* genome.

The AB058 sequence assembly has ~49.5 kbp of chromosomal sequence that is not represented in AB0057. Approximately 10.6 kbp of this sequence is present in the AYE and AB307-0294 genomes in the O-antigen biosynthetic gene cluster. The remaining 38.9 kbp is distributed across one or more novel prophage regions and a novel 5.4-kbp insertion (Table 8).

Phylogenetic relationships. The draft nature of three genomes makes full phylogenetic analysis impractical; however, we identified 1,927 ORFs that are complete in all six ST15/46 genome assemblies. Each was the reciprocal best BLAST match, suggesting that they are orthologous. ClustalW was used to create a multiple-sequence alignment of each gene separately, and the resulting alignments were concatenated and used to build a phylogenetic tree (Fig. 2). The tree clearly groups AB0057, AB056, and AB059 together. The AB0057/AB056/AB059 group has >99.99% intragroup similarity, implying fewer than 300 nucleotide differences genomewide, which is within the expected error rate of the draft genome assemblies. AB307-0294, AYE, and AB058 range from 99.93 to 99.97% identical to one another. The AB0057/AB056/AB059 group is also distinguished from the other ST15 strains by its O-antigen biosynthetic gene cluster (2).

The locations of seven IS*Aba1* insertion sites are identical in AB056, AB059, and AB0057, arguing strongly for a clonal relationship, yet these strains differ in important aspects of their antimicrobial resistance genotype and phenotype. Based on dates of arrival and of culture isolation, it is clear that the AB0057 strain arrived at WRAMC after AB056 and AB059 (Table 1). If AbaR9 (AB056) does in fact represent a deletion relative to an AB0057-like progenitor, then this deletion must have happened prior to the arrival of AB0057 at WRAMC.

Summary of analysis of ST15 strains. All five MDR ST15 strains have a distinct and unique genetic repertoire that includes variation in three classes of mobile element composition: plasmids, IS elements, and the RI. Each class of mobile element has contributed to the development of antimicrobial

TABLE 8. Chromosomal gene content differences between AB058 and AB0057/AB056/AB059: AB058 regions absent from AB0057

Region	Length (bp)	No. of contigs
Putative phage region(s)	33,504	7
5.4 kbp insertion in AB57_3078 (diacylglycerol kinase)	5,408	1
AYE/AB307-0294 O-antigen biosynthetic genes	10,592	29

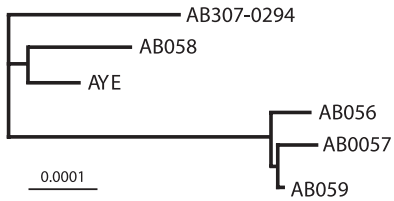

FIG. 2. Phylogenetic tree of ST15 strains. A phylogenetic tree was made using the neighbor-joining method based on an alignment of DNA sequences from 1,972 open reading frames that are present in each genome assembly. Each branch is supported by at least 90% of 1,000 bootstrap replicates.

resistance, either by facilitating the transfer of resistance genes or by upregulation of an endogenous gene. The role of IS-mediated gene knockout in resistance and pathogenicity merits further exploration.

The resistance gene repertoire in the ST15 strains does not strictly conform to the genomewide phylogeny. For example, only AB058 and AB059 carry the plasmid with the *aphA6* gene. Furthermore, one shared gene, *aadB*, is in a completely different genomic context in AYE (RI) and AB058 (plasmid). The pattern of insertion (or deletion) in the RI also cannot be readily explained as a series of stepwise differences consistent with the phylogeny. Multiple independent gene transfer and IS mobilization events have occurred among these closely related strains. In AYE, most of the genes that play a role in antibiotic resistance are located in AbaR1. In the WRAMC strains and in ACICU, the RI plays a significant but less dominant role in resistance to the clinically important classes of antibiotics, including cephalosporins, carbapenems, aminoglycosides, and fluoroquinolones.

An essential assumption of clonality testing is that clonal isolates will have similar antimicrobial susceptibility patterns. The ST15 strains are quite similar genomewide but have considerable divergence in their antibiotic resistance phenotype. Therefore, genomewide assays of relatedness such as PFGE and PCR/ESI-MS are likely to be less effective in monitoring changes in resistance genotype and phenotype. Development of more specific and comprehensive molecular assays for detection of specific resistance genes should prove useful in monitoring changes in resistance pattern and in selection of appropriate therapeutic regimens.

This study has not directly addressed the rate of lateral gene transfer or IS mobilization, and it remains to be determined whether rapid gene gain/loss is common in the context of a clinical outbreak. The diversity of strains present at WRAMC likely derives from multiple founders, and the historical relationships between strains cannot be inferred based on the information available. Therefore, study of more homogenous outbreaks, where most infections are nosocomially transmitted, will be necessary to clarify many aspects of RI- and mobile element-driven evolution. This study highlights the need for further study of IS mobilization, lateral gene transfer, and the kinetics of novel gene acquisition.

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