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Antimicrobial Resistance Determinants in *Acinetobacter baumannii* Isolates Taken from Military Treatment Facilities

Chris Rowe Taitt,[a] Tomasz A. Leski,[a] Michael G. Stockelman,[a] David W. Craft,[b] Daniel V. Zurawski,[b] Benjamin C. Kirkup,[b,c] Gary J. Vora[a]

Center for Bio/Molecular Science and Engineering, Naval Research Laboratory, Washington, DC, USA[a]; Division of Bacterial and Rickettsial Disease, Department of Wound Infections, Walter Reed Army Institute of Research, Silver Spring, Maryland, USA[b]; Department of Medicine, Infectious Diseases Division, Uniformed Services University of the Health Sciences, F. Edward Hebert School of Medicine, Bethesda, Maryland, USA[c]

Multidrug-resistant (MDR) *Acinetobacter baumannii* infections are of particular concern within medical treatment facilities, yet the gene assemblages that give rise to this phenotype remain poorly characterized. In this study, we tested 97 clinical *A. baumannii* isolates collected from military treatment facilities (MTFs) from 2003 to 2009 by using a molecular epidemiological approach that enabled for the simultaneous screening of 236 antimicrobial resistance genes. Overall, 80% of the isolates were found to be MDR, each strain harbored between one and 17 resistant determinants, and a total of 52 unique resistance determinants or gene families were detected which are known to confer resistance to β-lactam (e.g., $bla_{\text{GES-11}}$, bla_{TEM}, $bla_{\text{OXA-58}}$), aminoglycoside (e.g., *aphA1*, *aacC1*, *armA*), macrolide (*msrA*, *msrB*), tetracycline [e.g., *tet*(A), *tet*(B), *tet*(39)], phenicol (e.g., *cmlA4*, *catA1*, *cat4*), quaternary amine (*qacE*, *qacEΔ1*), streptothricin (*sat2*), sulfonamide (*sul1*, *sul2*), and diaminopyrimidine (*dfrA1*, *dfrA7*, *dfrA19*) antimicrobial compounds. Importantly, 91% of the isolates harbored $bla_{\text{OXA-51-like}}$ carbapenemase genes (including six new variants), 40% harbored the $bla_{\text{OXA-23}}$ carbapenemase gene, and 89% contained a variety of aminoglycoside resistance determinants with up to six unique determinants identified per strain. Many of the resistance determinants were found in potentially mobile gene cassettes; 45% and 7% of the isolates contained class 1 and class 2 integrons, respectively. Combined, the results demonstrate a facile approach that supports a more complete understanding of the genetic underpinnings of antimicrobial resistance to better assess the load, transmission, and evolution of MDR in MTF-associated *A. baumannii*.

Acinetobacter baumannii has become a significant concern in hospital settings due to its ability to survive under a wide range of environmental conditions, persist for extended periods on fomites, and, perhaps most importantly, develop or acquire myriad mechanisms to counter a variety of antimicrobial compounds (1–5). In particular, the high rates of antimicrobial resistance among nosocomial isolates and its rising prevalence among hospital-acquired infections have made *A. baumannii* a significant source of morbidity, mortality, and economic loss (6–11). This opportunistic pathogen has been an especially serious threat for personnel hospitalized in military treatment facilities (MTFs), as exemplified by the 2003-2004 outbreak of multidrug-resistant (MDR) *A. baumannii* among personnel injured during combat operations; the subsequent spread of MDR *A. baumannii* to other sites highlighted the role of this organism in the rapid dissemination of resistance genes (12–19). Although the prevalence of *A. baumannii* infections has recently been described as on the decline (15, 18), *A. baumannii* still ranks among the top two MDR organisms colonizing combat-injured personnel at MTFs after evacuation from the combat location where the injury occurred (20).

The level of MDR observed in such *A. baumannii* isolates and the resulting difficulties in making an informed selection of an appropriate chemotherapeutic agent to combat *A. baumannii* infections have prompted researchers to understand the distribution of resistance determinants present in MTF-derived *Acinetobacter* isolates (21–25). While such targeted analyses have provided a baseline for the presence and abundance of a limited number of resistance determinants, a broader spectrum survey of the resistance gene assemblages found in MTF-associated *A. baumannii* can undoubtedly aid in establishing more accurate molecular epidemiological trends and potentially informing refinements of antimicrobial administration policies and infection control measures.

In this study, we describe a broad-spectrum survey of 97 *A. baumannii* isolates from MTFs that were analyzed by using Antimicrobial Resistance Determinant Microarray (ARDM) v.2, a custom DNA microarray capable of detecting 236 different resistance determinants and gene families. Importantly, the content of ARDM v.2 has been refined from previously described ARDM v.1 (26) to minimize unnecessary redundancies, to include probes for *Acinetobacter*-specific genes, and to broaden the represented variety of genes conferring resistance to quaternary amines, streptothricin antibiotics, and 15 other classes of antimicrobial compounds. Due to the breadth of ARDM v.2 and its associated unbiased sample preparation, the results provide information on determinants not included in previous studies of MTF-derived strains as well as those not typically included in routine investigations of *Acinetobacter*. In the process, we present a more comprehensive assessment of *A. baumannii* resistance gene assemblages from MTFs, which in turn may be informative for the civilian hospital environment as well.

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Address correspondence to Chris Rowe Taitt, chris.taitt@nrl.navy.mil.
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MATERIALS AND METHODS

Samples. Ninety-seven *A. baumannii* clinical isolates were obtained from the U.S. Department of Defense (DoD) Multidrug-Resistant Organism Repository and Surveillance Network (MRSN). For each isolate, the associated metadata included source tissue, date of isolation, pulsed-field gel electrophoresis (PFGE) profiles, and resistance phenotypes. The susceptibilities of the isolates to ampicillin-sulbactam (SAM), ceftazidime (CFZ), cefepime (CEF), imipenem (IPM), gentamicin (GEN), tobramycin (TOB), ciprofloxacin (CIP), levofloxacin (LVF), and tetracycline (TET) were determined using the Vitek 2.0 system (bioMérieux Clinical Diagnostics); susceptibility to amikacin (AMI) was determined using the Phoenix Automated Microbiology System (BD Diagnostic Systems). Results were interpreted according to CLSI guidelines (27), and MDR was defined as resistance to three or more classes of antibiotics.

Processing of extracted DNA. Glycerol stocks of the *A. baumannii* isolates were plated on Luria broth agar and incubated overnight at 37°C. Single colonies were picked and replated. Isolated colonies from the second plating were then used to inoculate 5 ml of Luria broth and incubated overnight in a rotary shaker at 37°C and 200 rpm. Two milliliters of each overnight culture was used to extract genomic DNA using the MasterPure DNA and RNA complete purification kit (Epicentre Biotechnologies, Madison, WI). The extracted DNA was quantified using a Qubit fluorometer (Quant-iT double-stranded DNA [dsDNA] BR assay kit; Invitrogen/Life Technologies, Grand Island, NY), and 10 ng of DNA from each sample was amplified using the GenomiPhi v.2 reagent kit (GE Healthcare, Piscataway, NJ) according to the manufacturer's instructions. A total of 2 μg of the resulting high-molecular-weight amplicons was fragmented for 1 min at 37°C using fragmentation reagent and buffer (DNase I; 0.045 units/μl in a total reaction volume of 60 μl; GeneChip resequencing assay kit; Affymetrix, Santa Clara, CA), with subsequent DNase inactivation by incubation at 95°C for 10 min. The resulting fragmented DNA was then purified using the DNA Clean & Concentrator-5 (Zymo Research, Irvine, CA) and labeled and purified using the ULS PlatinumBright biotin nucleic acid labeling kit according to the manufacturer's instructions (Kreatech Diagnostics, Durham, NC; 10-μl reaction volume).

Microarray hybridization and analysis. The content of ARDM v.2 is based on that of ARDM v.1 (26) but was refined to minimize unnecessary redundancy and the number of probes that hybridize nonspecifically, to provide a greater coverage of *Acinetobacter*-associated genes (e.g., $bla_{\text{OXA-51-like}}$, $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-58-like}}$), and to incorporate additional resistance determinants that have been recently identified or that are of emerging importance (e.g., $bla_{\text{NDM-1}}$, *mupA*, bla_{BEL}, bla_{IMI}, $bla_{\text{OXA-48}}$). Probes were selected to target 236 determinants conferring resistance to quaternary ammonium compounds ($n = 2$), streptothricin ($n = 2$), aminoglycosides ($n = 42$), ansamycins ($n = 1$), β-lactams ($n = 46$), phenicol compounds ($n = 10$), diaminopyrimidines ($n = 27$), glycopeptides ($n = 12$), lincosamides ($n = 22$), macrolides ($n = 27$), quinolones ($n = 4$), streptogramins ($n = 18$), sulfonamides ($n = 3$), tetracyclines ($n = 33$), mupirocin ($n = 1$), antimicrobial peptides ($n = 1$), and platensimycin/platencin ($n = 1$). Many of the macrolide, lincosamide, and streptogramin resistance determinants overlap in specificity. Each of the 236 genes is represented in ARDM v.2 by six to 10 probes, with the most frequent representation comprising four or five pairs of duplicate probes per gene. A full listing of the ARDM v.2 content is given in Table S1 in the supplemental material.

Hybridization of the biotinylated DNA fragments to ARDM v.2 and subsequent microarray processing were performed as previously described (26); prehybridizations and hybridizations were performed at 60°C, and a polymeric streptavidin-horseradish peroxidase (S104PHRP; Fitzgerald Industries, North Acton, MA) was used for signal generation. Four samples were analyzed on each ARDM chip (one sample for each subarray; 4 × 2K ElectraSense chips purchased from CustomArray, Inc., Bothell, WA). Data obtained from the ElectraSense reader were analyzed with custom developed Perl scripts. Based on the improved probe selection, increased *A. baumannii*-specific content of ARDM v.2, and results from four sequenced strains (28), a gene was deemed present if at least half of its representative probes had signals above the 95% probe threshold (mean signal from lowest 2,128 probes + 3 standard deviations [SD]) or if >70% of its probes had signals above either of two less stringent thresholds (mean signal from lowest 2,016 probes + 3 SD or mean signal from lowest 2,128 probes + 2 SD) (26).

Confirmation of detected genes. PCR and/or DNA sequence confirmation was performed on select resistance genes detected by ARDM analysis: *aac(6′)-Ib*, *aac(3)-III*, *tet*(A), *tet*(B), *tet*(30), *tet*(39), bla_{GES}, $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-51-like}}$, $bla_{\text{OXA-58-like}}$, *armA*, *dfrA19*, and *cmlA*. PCR amplicons were size confirmed by electrophoresis using 1.2% FlashGel DNA cassettes (Lonza, Walkersville, MD), and all DNA sequencing was performed by Eurofins MWG Operon (Huntsville, AL). Full sequences of all of the $bla_{\text{OXA-51-like}}$ genes detected were submitted to http://www.lahey.org for identification and to assign new variant numbers. All novel $bla_{\text{OXA-51-like}}$ gene sequences were deposited in GenBank under the accession numbers KF057029 to KF057034. The presence of class 1 and class 2 integrons was confirmed by integrase gene-specific PCR (*intI1* and *intI2*, respectively), and where possible, the cassette arrays were amplified using primers complementary to 5′ and 3′ integron-conserved regions. Representative cassette arrays were fully sequenced. The primers used for PCR and DNA sequencing can be found in Table S2 in the supplemental material.

RESULTS

Phenotypic resistance overview. Of the 97 *A. baumannii* clinical isolates that were selected for this study, 78 (80%) were resistant to three or more classes of antimicrobial compounds and thus considered MDR (Table 1). In contrast, eight strains were considered pansusceptible, with no resistance to any of the 10 antimicrobial compounds tested. Two additional isolates were susceptible to nine antimicrobial compounds and intermediate in susceptibility to the tenth antimicrobial agent tested.

ARDM, *Acinetobacter* hybridization controls, and genome-sequenced strains. Control probes targeting the *Acinetobacter folA*, *qacE*, *ampC*, and *uppP* genes were included in ARDM v.2 to assess the quality of the sample, sample processing, and hybridization. Hybridization-positive *qacE*, *ampC*, and *uppP* probes were observed in 89 to 95% of the isolates tested, and the lack of detection of any of these three genes was highly correlated with phenotypic pansensitivity (χ^2 test, $P < 0.005$) (Table 2). Almost 20% of the MDR isolates harboring *ampC* hybridized only weakly to the *ampC* probes but, as they met the minimal criteria for positive *ampC* determination, were declared positive nonetheless. Interestingly, the *uppP* gene product, undecaprenyl-disphosphatase, has been postulated to be essential for cell structure and viability and a promising target for future drugs (29). The most effective positive control, *folA*, was detected in all but a single isolate (no. 51); nevertheless, *qacE* and *uppP* were detected in this sample. The inclusion of four *Acinetobacter*-specific control sequences was therefore necessary to enable accurate detection and analysis of sensitive as well as resistant strains.

Based on the hybridization control probes and thresholding criteria applied, only the classes of antimicrobial resistance determinants that were expected to be observed in *Acinetobacter* were detected within the tested strains: determinants encoding β-lactamases, aminoglycoside- and phenicol compound-modifying enzymes, narrow-spectrum efflux pumps for phenicol compounds and tetracycline, and resistant variants of dihydrofolate reductase and dihydropterate synthase (Table 2). Importantly, although 83% of the isolates were resistant to ciprofloxacin and/or levofloxacin, determinants known to confer resistance to quinolones were not detected. While *qnr*-mediated resistance to fluoroquino-

TABLE 1 Metadata and resistance phenotypes for the *A. baumannii* isolates obtained from the MRSN

Isolate no.	MRSN no.	Source tissue[a]	Date of isolation (mo/day/yr)	Resistance phenotype[b]										ARDM-positive controls
				FEP	CAZ	IPM	SAM	AMK	GEN	TOB	TET	CIP	LVX	
1	967/1308	BL	09/23/03	S	S	S	S	S	S	S	S	S	S	*qacE*, *uppP*, *folA*
2	2828/846	BL	03/28/06	R	R	R	I	R	R	R	R	R	I	*ampC*, *qacE*, *uppP*, *folA*
3	3340/847	BL	10/22/06	R	R	R	R	R	R	R	I	R	R	*ampC*, *qacE*, *uppP*, *folA*
4	3560/848	BL	12/14/06	R	R	S	S	R	R	R	R	R	R	*ampC*, *qacE*, *uppP*, *folA*
5	3638/849	STS	01/10/07	R	I	R	I	R	R	R	R	R	R	*ampC*, *qacE*, *uppP*, *folA*
6	3785/853	BL	03/18/07	R	R	S	R	R	R	S	R	R	R	*ampC*, *qacE*, *uppP*, *folA*
7	3806/854	STS	03/19/07	R	I	R	I	R	S	S	R	R	R	*ampC*, *qacE*, *uppP*, *folA*
9	3927/856	STS	05/18/07	I	R	S	R	R	R	R	I	R	S	*ampC*, *qacE*, *uppP*, *folA*
10	4025/858	W	06/24/07	R	R	S	R	S	R	S	R	R	R	*ampC*, *qacE*, *uppP*, *folA*
11	2046/859	W	06/26/07	R	R	S	R	S	R	S	R	R	R	*ampC*, *qacE*, *uppP*, *folA*
12	4027/860	W	06/26/07	R	R	S	R	S	R	S	R	R	R	*ampC*, *qacE*, *uppP*, *folA*
13	4052/863	WW	07/16/07	R	R	R	R	I	R	S	I	R	R	*ampC*, *qacE*, *uppP*, *folA*
14	4269/877	WW	10/16/07	R	R	R	R	R	R	S	I	R	R	*ampC*, *qacE*, *uppP*, *folA*
15	4448/899	WW	12/25/07	R	R	R	R	R	R	S	S	R	R	*ampC*, *qacE*, *uppP*, *folA*
16	4456/903	TA	12/30/07	R	R	I	S	R	R	R	R	R	R	*ampC*, *qacE*, *uppP*, *folA*
17	4496/906	WW	01/12/08	R	I	R	R	S	S	S	S	R	R	*ampC*, *qacE*, *uppP*, *folA*
18	4498/907	BL	01/13/08	R	R	S	R	S	R	S	R	R	R	*ampC*, *qacE*, *uppP*, *folA*
19	4795/930	STS BO	05/05/08	R	R	S	R	S	R	S	R	R	R	*ampC*, *qacE*, *uppP*, *folA*
20	4857/939	STS	05/28/08	R	R	I	S	R	R	R	R	R	R	*ampC*, *qacE*, *uppP*, *folA*
21	4878/941	WW	06/06/08	R	R	R	R	R	R	S	I	R	R	*ampC*, *qacE*, *uppP*, *folA*
22	4932/949	SP	07/04/08	R	R	R	R	R	R	S	R	R	R	*ampC*, *qacE*, *uppP*, *folA*
23	4957/951	STS BO	07/17/08	R	R	R	R	R	R	S	R	R	R	*ampC*, *qacE*, *uppP*, *folA*
24	4991/953	WW	08/03/08	R	R	R	I	S	R	S	R	R	R	*ampC*, *qacE*, *uppP*, *folA*
25	5001/954	BL	08/05/08	R	R	R	R	R	R	S	I	R	R	*ampC*, *qacE*, *uppP*, *folA*
26	5075/959	BO	09/01/08	R	R	R	R	R	R	R	S	R	R	*ampC*, *qacE*, *uppP*, *folA*
27	5197/960	STS	10/15/08	R	R	R	R	R	R	S	R	R	R	*qacE*, *uppP*, *folA*
28	5256/961	BL	11/11/08	R	R	S	S	R	R	R	R	R	R	*qacE*, *uppP*, *folA*
29	5674/963	BL	05/22/09	R	R	R	R	R	R	I	I	R	R	*ampC*, *qacE*, *uppP*, *folA*
30	5711/1310	BL	06/09/09	R	R	R	R	R	R	S	R	R	R	*ampC*, *qacE*, *uppP*, *folA*
31	866	STS	08/08/07	R	I	R	I	I	R	R	R	R	I	*ampC*, *qacE*, *uppP*, *folA*
32	875	STS	10/17/07	R	R	R	R	R	R	S	I	R	R	*ampC*, *qacE*, *uppP*, *folA*
33	892	U	12/24/07	R	R	R	R	R	R	S	I	R	R	*ampC*, *qacE*, *uppP*, *folA*
34	895	CSF	12/21/07	R	R	R	R	R	R	S	I	R	R	*ampC*, *qacE*, *uppP*, *folA*
35	901	SP	01/01/08	R	R	I	S	R	R	R	R	R	R	*ampC*, *qacE*, *uppP*, *folA*
36	908	STS	01/14/08	R	R	R	S	R	R	R	R	R	R	*ampC*, *qacE*, *uppP*, *folA*
37	920	W	03/26/08	R	R	I	S	R	R	R	R	R	R	*ampC*, *qacE*, *uppP*, *folA*
38	927	CSF	04/21/08	R	R	I	S	R	R	R	R	R	R	*ampC*, *qacE*, *uppP*, *folA*
39	955	STS	08/03/08	R	R	I	S	R	R	R	R	R	R	*ampC*, *qacE*, *uppP*, *folA*
40	1967	SP	10/22/06	R	R	R	R	R	R	R	I	R	R	*ampC*, *qacE*, *uppP*, *folA*
41	1969	STS CH	11/08/06	R	R	S	R	S	R	S	R	R	R	*ampC*, *qacE*, *uppP*, *folA*
42	1970	BAL	03/07/07	I	R	S	R	R	S	I	R	R	I	*ampC*, *qacE*, *uppP*, *folA*
43	1971	WW	03/19/07	R	R	S	S	R	R	I	S	R	R	*ampC*, *qacE*, *uppP*, *folA*
44	1972	STS	03/26/07	S	R	S	S	R	I	S	I	S	S	*ampC*, *qacE*, *uppP*, *folA*
45	1973	W	04/24/07	I	R	S	R	S	R	S	S	S	S	*ampC*, *qacE*, *uppP*, *folA*
46	1974	STS	05/24/07	R	R	S	S	R	R	R	R	R	R	*ampC*, *qacE*, *uppP*, *folA*
47	1975	BL	06/23/07	S	R	S	I	R	R	I	R	R	R	*ampC*, *qacE*, *uppP*, *folA*
48	1976	STS	06/30/07	R	R	S	R	S	R	S	R	R	R	*ampC*, *qacE*, *uppP*, *folA*
49	1977	BL	07/04/07	S	S	S	S	S	S	S	*S*	S	S	*ampC*, *qacE*, *folA*
50	1978	WW	07/13/07	S	I	S	S	S	R	I	S	R	I	*ampC*, *qacE*, *uppP*, *folA*
51	1979	STS AN	07/22/07	S	I	S	S	S	R	I	S	S	S	*qacE*, *uppP*
52	1980	BL	10/07/07	S	I	S	S	S	I	I	S	R	I	*qacE*, *uppP*, *folA*
53	1981	STS	10/22/07	S	S	S	S	S	S	S	S	S	S	*folA*
54	1982	WW	10/20/07	R	R	S	S	S	I	S	S	S	S	*ampC*, *qacE*, *uppP*, *folA*
55	1983	STS	10/27/07	I	R	S	I	I	R	I	R	R	R	*ampC*, *qacE*, *uppP*, *folA*
56	1984	STS	10/29/07	I	R	R	S	R	R	R	R	R	R	*ampC*, *qacE*, *uppP*, *folA*

(Continued on following page)

TABLE 1 (Continued)

Isolate no.	MRSN no.	Source tissue[a]	Date of isolation (mo/day/yr)	Resistance phenotype[b]										ARDM-positive controls
				FEP	CAZ	IPM	SAM	AMK	GEN	TOB	TET	CIP	LVX	
57	1985	WW	10/30/07	I	R	S	I	I	R	I	R	R	R	*ampC*, *qacE*, *uppP*, *folA*
58	1986	WW	12/11/07	I	R	R	I	R	R	R	R	R	R	*ampC*, *qacE*, *uppP*, *folA*
59	1987	STS	01/25/08	R	R	S	S	R	R	R	S	R	R	*ampC*, *qacE*, *uppP*, *folA*
60	1988	WW	02/05/08	R	R	S	S	S	R	I	R	R	R	*ampC*, *qacE*, *uppP*, *folA*
61	1989	U	02/12/08	S	S	S	S	S	S	S	S	S	S	*folA*
62	1990	SP	02/19/08	I	I	S	S	S	S	S	S	R	R	*ampC*, *qacE*, *uppP*, *folA*
63	1991	CSF	02/24/08	R	R	S	R	S	R	S	R	R	R	*ampC*, *qacE*, *uppP*, *folA*
64	1992	WW	03/09/08	R	R	S	S	S	R	R	R	R	R	*ampC*, *qacE*, *uppP*, *folA*
65	1993	WW	03/30/08	S	S	S	S	S	S	S	S	S	S	*ampC*, *folA*
66	1994	SP	05/01/08	S	S	S	S	S	S	S	S	S	S	*ampC*, *folA*
67	1995	SP	06/10/08	R	R	R	R	R	R	S	I	R	R	*ampC*, *qacE*, *uppP*, *folA*
68	1996	STS	06/28/08	R	R	S	R	S	R	S	R	R	R	*ampC*, *qacE*, *uppP*, *folA*
69	1997	WW	07/19/08	S	I	S	S	S	S	S	S	S	S	*folA*
70	1998	STS	08/05/08	R	R	I	S	R	R	R	R	R	R	*ampC*, *qacE*, *uppP*, *folA*
71	1999	BL	08/31/08	R	R	R	R	R	R	I	R	R	R	*ampC*, *qacE*, *uppP*, *folA*
72	2000	WW	08/31/08	R	R	R	R	R	R	I	R	R	R	*ampC*, *qacE*, *uppP*, *folA*
73	2001	STS	09/03/08	R	R	R	I	R	R	I	R	R	R	*ampC*, *qacE*, *uppP*, *folA*
75	2003	STS	09/12/08	R	R	R	I	R	R	I	R	R	R	*ampC*, *qacE*, *uppP*, *folA*
76	2004	U	09/11/08	S	S	S	S	S	S	S	S	S	S	*ampC*, *qacE*, *folA*
77	2005	W	10/08/08	R	R	R	R	R	R	S	I	R	R	*ampC*, *qacE*, *uppP*, *folA*
78	2006	AN	10/16/08	I	R	S	S	R	R	R	R	R	R	*ampC*, *qacE*, *uppP*, *folA*
79	2007	STS	10/25/08	R	R	R	R	R	R	S	R	R	R	*ampC*, *qacE*, *uppP*, *folA*
80	2008	U	10/29/08	I	I	S	R	S	S	S	S	R	R	*ampC*, *qacE*, *uppP*, *folA*
81	2009	WW	11/05/08	S	S	S	R	S	S	S	S	S	S	*ampC*, *qacE*, *uppP*, *folA*
82	2010	WW	11/16/08	R	R	I	S	R	R	R	R	R	R	*ampC*, *qacE*, *uppP*, *folA*
83	2011	WW	11/22/08	R	R	R	R	R	R	S	R	R	I	*ampC*, *qacE*, *uppP*, *folA*
84	2012	WW	12/03/08	R	R	R	R	S	R	S	R	R	I	*ampC*, *qacE*, *uppP*, *folA*
85	2013	STS	01/02/09	R	R	R	R	S	R	S	I	R	R	*ampC*, *qacE*, *uppP*, *folA*
86	2014	SP	01/16/09	R	R	R	I	I	S	S	S	R	I	*ampC*, *qacE*, *uppP*, *folA*
87	2015	STS	01/16/09	R	R	R	R	R	R	S	S	R	R	*ampC*, *qacE*, *uppP*, *folA*
88	2016	BL	01/17/09	R	R	I	S	I	S	S	S	R	I	*ampC*, *qacE*, *uppP*, *folA*
89	2017	MDR/G	01/18/09	R	R	R	I	R	R	R	R	R	R	*ampC*, *qacE*, *uppP*, *folA*
90	2018	STS	02/28/09	R	R	R	R	R	R	I	S	R	R	*ampC*, *qacE*, *uppP*, *folA*
91	2019	BL	05/11/09	S	I	S	S	S	S	S	S	S	S	*folA*
92	2020	C	06/01/09	R	R	R	R	R	R	S	I	R	R	*ampC*, *qacE*, *uppP*, *folA*
93	2021	MDR/G	06/16/09	R	R	S	S	R	R	I	S	R	R	*ampC*, *qacE*, *uppP*, *folA*
94	2022	AN	06/17/09	R	R	R	R	R	R	S	R	R	R	*ampC*, *qacE*, *uppP*, *folA*
95	2023	MDR/G	07/08/09	R	R	R	R	R	R	S	R	R	R	*ampC*, *qacE*, *uppP*, *folA*
96	2024	W	01/01/09	R	R	R	I	R	R	R	I	R	R	*ampC*, *qacE*, *uppP*, *folA*
97	2025	SL	01/01/09	S	S	S	S	S	S	S	S	S	S	*ampC*, *qacE*, *folA*
98	1966	BAL	07/31/03											*ampC*, *qacE*, *uppP*, *folA*
99	1968	U	11/01/06	R	R	R	R	R	R	R	I	R	R	*ampC*, *qacE*, *uppP*, *folA*

[a] AN, anaerobic; BAL, bronchoalveolar lavage; BL, blood; BO, bone; C, catheter; CH, chest; CSF, cerebrospinal fluid; MDR/G, groin swab; SL, scalp lesion; SP, sputum; STS, sterile tissue site; TA, tracheal aspirate; TI, tibia; U, urine; W, wound; WW, war wound.

[b] AMK, amikacin; SAM, ampicillin-sulbactam; FEP, cefepime; CAZ, ceftazidime; IPM, imipenem; GEN, gentamicin; TOB, tobramycin; TET, tetracycline; CIP, ciprofloxacin; LVX, levofloxacin; R, resistant; I, intermediate; S, sensitive.

lones has been reported in *A. baumannii* (30), *gyrA* and *parC* mutations are the most common mechanisms of fluoroquinolone resistance in *Acinetobacter* (31–33), and single nucleotide polymorphisms such as these are currently not detectable using the ARDM platform.

Four genome-sequenced strains (isolate no. 20, 26, 28, and 30) were among the isolates tested. Each of the 238 gene sequences represented in the ARDM was compared to the published sequences for these four isolates (28), and rates of true positives, true negatives, false positives, and false negatives were calculated based on >90% sequence identity with the reference sequence. Of the 238 genes on the microarray, no false positives and only 3 false negatives were observed among the four sequenced strains (see Table S3 in the supplemental material).

Determinants conferring resistance to β-lactams. A significant proportion of the ARDM v.2 content was devoted to genes

conferring resistance to β-lactam antibiotics due to their continued importance in clinical settings. Of the 46 β-lactamase genes or gene families represented in the ARDM, six were detected at various frequencies (Table 2). The *ampC* and $bla_{\text{OXA-51-like}}$ determinants were detected by the ARDM in 89% of the isolates tested. Targeted PCR analyses also demonstrated the presence of a $bla_{\text{OXA-51-like}}$ determinant in two additional samples where it had not been detected by microarray analysis; this apparent difference in sensitivity may potentially be due to nonoptimal probe design or poor amplification/labeling of the sample DNA. PCR amplification and DNA sequencing of each of the identified $bla_{\text{OXA-51-like}}$ genes revealed 18 variants, six of which had not been previously described and were assigned new OXA numbers (OXA-312 through OXA-317) (Table 3). Most of the isolates contained a $bla_{\text{OXA-51-like}}$ gene encoding one of three OXA-51-like variants: OXA-66 (and its derivative OXA-82; $n = 28$ isolates), OXA-69 ($n = 26$ isolates), and OXA-71 (and its derivatives OXA-64, OXA-113, OXA-121, OXA-312, OXA-313; $n = 26$ isolates). Previous studies have shown that these variants are associated with the highly successful *A. baumannii* clonal lineages WW2 (EU2), WW1 (EU1), and WW3 (EU3), respectively (34–36). The IS*Aba1* insertion sequence was found immediately upstream of the $bla_{\text{OXA-51-like}}$ gene in 15 isolates; in most cases, these isolates harbored OXA-71 (and its novel derivatives OXA-312 and OXA-313) and OXA-82. The presence of IS*Aba1* upstream of the $bla_{\text{OXA-51-like}}$ gene was highly correlated with phenotypic carbapenem resistance (or intermediate resistance) in the absence of the $bla_{\text{OXA-23}}$ carbapenemase gene (χ^2 test, $P < 0.005$).

ARDM analyses detected $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-58-like}}$ determinants in 40% and 3% of the isolates, respectively (Table 3). Specific PCR assays for these genes uncovered two additional isolates positive for $bla_{\text{OXA-23}}$ (isolate no. 34 and 35). The presence of the $bla_{\text{OXA-23}}$ determinant was always associated with phenotypic imipenem resistance. However, isolate no. 52 (in which $bla_{\text{OXA-58}}$ was the sole carbapenemase) was phenotypically susceptible to imipenem. While the presence of the $bla_{\text{OXA-58}}$ gene in *A. baumannii* is traditionally associated with carbapenem resistance, studies have also documented the carriage of $bla_{\text{OXA-58}}$ by carbapenem-susceptible *Acinetobacter* isolates (37, 38). Conversely, there was only one isolate that was phenotypically imipenem resistant (isolate no. 22) for which we were unable to detect a likely resistance determinant.

Genes encoding members of the TEM and GES families of β-lactamases were also detected in 14 and five isolates, respectively (Table 2). The bla_{GES}-positive samples were confirmed by PCR and DNA sequencing. All five genes were identified as $bla_{\text{GES-11}}$, a determinant encoding the extended-spectrum β-lactamase (ESBL) GES-11. In each case, a class 1 integron harboring cassettes containing $bla_{\text{GES-11}}$, *aac(6)-Ib*, *dfrA7 qacEΔ1*, and *sul1* was identified by DNA sequence analysis. Importantly, ARDM analyses had also identified the presence of these same genes.

Determinants conferring resistance to aminoglycosides. The ARDM v.2 probe content covers 43 different aminoglycoside (AG) resistance determinants, including those encoding AG-modifying enzymes (acetyltransferases [AACs], nucleotidyl transferases [ANTs], phosphotransferases [APHs]) and RNA methylases. Overall, 89% of the isolates contained at least one AG resistance determinant, while 80% of the isolates harbored multiple AG resistance determinants with a maximum of six unique determinants identified per strain (Table 2). ARDM-positive determinations correlated with phenotypic resistance to at least one of the AG compounds tested (χ^2 test, $P < 0.005$).

APH determinants were found to be the most prevalent and numerous, with 83% of the isolates harboring at least one APH gene and 41% harboring three or more. The most prevalent APH determinant, *aphA6*, was detected in 61% of the isolates, while *aphA1* was detected in 29% of the isolates. *aph(6)-Id* and *aph(3′′)* were detected at roughly equivalent frequencies and in most cases together.

ANT determinants were also prevalent, with *aadB* detected in 50% of the isolates, while the *aadA1* and *aadA2* family of AG 3′-adenylyltransferases was observed in 29% of the isolates. While some isolates hybridized well to all probes representing the *aadA1* and *aadA2* family, others barely met the minimal threshold for positive determination; these variations in hybridization efficiency may reflect the presence of alleles which differ in DNA sequence composition by as much as 10% (*aadA1*, *aadA1b*, *aadA2*). These differences could potentially be exploited to identify the specific allele present (versus detecting a member of this gene family) if a wider variety of probes for these related alleles is included in the ARDM content (26).

AAC determinants that encoded enzymes catalyzing acetylation at position 3 on AG compounds, such as *aac(3)-III* and *aacC1*, were detected in 3% and 31% of the isolates, respectively. Two AAC determinants encoding 6′-modifying enzymes were detected among the isolates. Six isolates were strongly positive for *aac(6)-Ib*, with all six confirmed by PCR, two by additional amplicon sequencing, and one by whole-genome sequencing (28). No *aac(6)-Ib-cr* variants conferring quinolone resistance were detected. A single isolate (no. 13) was positive for *aac(6′)-Ik*.

Thirteen RNA methylases are represented in ARDM v.2. The *armA* gene, which encodes a 16S RNA-modifying enzyme, was detected in a single isolate (no. 22) and was confirmed by PCR and DNA amplicon sequencing.

Determinants conferring resistance to macrolides and streptogramins. The only macrolide-lincosamide-streptogramin B (MLS) resistance determinants detected in this study belonged to the *msrA*, *msrB*, and *msrSA* gene family that encodes macrolide and streptogramin efflux pumps (Table 2). Observed in four isolates (no. 7, 13, 14, 21), these results were somewhat surprising given the minimal representation of *msr* genes among Gram-negative species (39). Interestingly, the DNA sequences of the hybridization-positive probes were identical to those of a published *msrA* gene found in *Pseudomonas* sp. 3U3-1 (40).

Determinants conferring resistance to tetracyclines. Two-thirds of the isolates tested were phenotypically either resistant or intermediate in sensitivity to tetracycline. Four determinants conferring tetracycline resistance were detected among the 97 isolates: *tet*(30) ($n = 4$), *tet*(39) ($n = 5$), *tet*(A) ($n = 20$), and *tet*(B) ($n = 20$). The *tet*(39), *tet*(A), and *tet*(B) genes were correlated with phenotypic resistance (χ^2 test, $P < 0.005$), although resistance was also observed in 23 samples that did not harbor any of these genes (Tables 1 and 2). The phenotypic tetracycline resistance of these latter samples also cannot be explained by Ade efflux pumps as recently reported (41, 42), and there must be another undefined resistance mechanism at play here.

Thirty of the isolates were tested for the presence or absence of the *tet*(39), *tet*(A), and *tet*(B) genes by gene-specific PCR, and the only discrepancy occurred in a single isolate that was PCR positive

TABLE 2 Resistance determinants detected by using ARDM v.2

Isolate no.	Resistance determinant(s) detected by the ARDM, sorted by antimicrobial class[a]							
	β-Lactam	Aminoglycoside	Macrolides-lincosamides-streptogramin B	Tetracycline	Chloramphenicol	Quaternary amines, streptothricin	Sulfonamide	Diaminopyrimidine
1	***bla*$_{\text{OXA-51-like}}$**							
2	***bla*$_{\text{OXA-23}}$**, ***bla*$_{\text{OXA-51-like}}$**	*aadB*, *aphAI*, *aph(3′′)*, *aph(6)-Id*, *aphA6*		***tet*(39)**			*sul2*	
3	***bla*$_{\text{OXA-23}}$**, ***bla*$_{\text{OXA-51-like}}$**, ***bla*$_{\text{OXA-58-like}}$**	*aadB*, *aphA6*					*sul1*	
4	***bla*$_{\text{OXA-51-like}}$**	*aadB*, *aphA6*		***tet*(A)**		*qacEΔ1*	*sul1*	
5	***bla*$_{\text{OXA-23}}$**, ***bla*$_{\text{OXA-51-like}}$**	*aadB*, *aphAI*, *aph(3′′)*, *aph(6)-Id*		***tet*(39)**			*sul2*	
6	*bla*$_{\text{TEM}}$, ***bla*$_{\text{OXA-51-like}}$**	*aph(3′′)*, *aph(6)-Id*		***tet*(B)**				
7	***bla*$_{\text{OXA-23}}$**, ***bla*$_{\text{OXA-51-like}}$**	*aphAI*, *aph(3′′)*, *aph(6)-Id*, *aphA6*	*msrA*, *msrB*	*tet*(30), ***tet*(39)**			*sul2*	
9	***bla*$_{\text{OXA-51-like}}$**	*aadB*, *aph(3′′)*, *aph(6)-Id*, *aphA6*					*sul2*	
10	*bla*$_{\text{TEM}}$, ***bla*$_{\text{OXA-51-like}}$**	*aacC1*, *aadA1* and *aadA2* family, *aphAI*, *aph(3′′)*, *aph(6)-Id*		***tet*(B)**		*qacEΔ1*	*sul1*	
11	*bla*$_{\text{TEM}}$, ***bla*$_{\text{OXA-51-like}}$**	*aacC1*, *aadA1* and *aadA2* family, *aphAI*, *aph(3′′)*, *aph(6)-Id*		***tet*(B)**		*qacEΔ1*	*sul1*	
12	*bla*$_{\text{TEM}}$, ***bla*$_{\text{OXA-51-like}}$**	*aacC1*, *aadA1* and *aadA2* family, *aphAI*, *aph(3′′)*, *aph(6)-Id*		*tet*(30), ***tet*(B)**		*qacEΔ1*	*sul1*	
13	***bla*$_{\text{OXA-23}}$**, ***bla*$_{\text{OXA-51-like}}$**	*aacC1*, *aph(3′′)*, *aph(6)-Id*, *aphA6*, *aac(6′)-Ik*	*msrA*, *msrB*	*tet*(30)			*sul2*	
14	***bla*$_{\text{OXA-23}}$**, ***bla*$_{\text{OXA-51-like}}$**	*aacC1*, *aph(3′′)*, *aph(6)-Id*, *aphA6*	*msrA*, *msrB*				*sul2*	
15	***bla*$_{\text{GES}}$**, ***bla*$_{\text{OXA-23}}$**, ***bla*$_{\text{OXA-51-like}}$**	***aac(6)-Ib* family,** *aadA1* and *aadA2 family*, *aadB*, *aph(3′′)*, *aph(6)-Id*, *aphA6*			*cmlA*	***qacEΔ1***	***sul2***	***dfrA7***
16	***bla*$_{\text{OXA-51-like}}$**	*aadB*, *aphA6*		***tet*(A)**		*qacEΔ1*	*sul1*	
17	***bla*$_{\text{OXA-23}}$**, ***bla*$_{\text{OXA-51-like}}$**						*sul1*	
18	***bla*$_{\text{TEM}}$**, ***bla*$_{\text{OXA-51-like}}$**	*aacC1*, *aadA1* and *aadA2* family, *aphAI*, *aph(3′′)*, *aph(6)-Id*		***tet*(B)**		*qacEΔ1*	*sul1*	
19	***bla*$_{\text{TEM}}$**, ***bla*$_{\text{OXA-51-like}}$**	*aacC1*, *aadA1* and *aadA2* family, *aphAI*, *aph(3′′)*, *aph(6)-Id*		***tet*(B)**		*qacEΔ1*	*sul1*	
20	***bla*$_{\text{OXA-51-like}}$**	*aadB*, *aphA6*		***tet*(A)**		*qacEΔ1*	*sul1*	
21	***bla*$_{\text{OXA-23}}$**, ***bla*$_{\text{OXA-51-like}}$**	*aacC1*, *aph(3′′)*, *aph(6)-Id*, *aphA6*	*msrA*, *msrB*	*tet*(30)			*sul2*	
22	*bla*$_{\text{TEM}}$, ***bla*$_{\text{OXA-51-like}}$**	*aac(6)-Ib* family, *aadA1* and *aadA2 family*, *aphAI*, *aph(3′′)*, *aph(6)-Id*, ***armA***				*qacEΔ1*	*sul1*, *sul2*	
23	***bla*$_{\text{OXA-23}}$**, ***bla*$_{\text{OXA-51-like}}$**	*aacC1*, *aph(3′′)*, *aph(6)-Id*, *aphA6*					*sul2*	
24	***bla*$_{\text{OXA-23}}$**, ***bla*$_{\text{OXA-51-like}}$**	*aacC1*, *aadA1* and *aadA2* family, *aphAI*		***tet*(A)**	*catA1* group	*qacEΔ1*	*sul1*	
25	***bla*$_{\text{OXA-23}}$**, ***bla*$_{\text{OXA-51-like}}$**	*aacC1*, *aph(3′′)*, *aph(6)-Id*, *aphA6*					*sul2*	
26	***bla*$_{\text{GES}}$**, ***bla*$_{\text{OXA-23}}$**, ***bla*$_{\text{OXA-51-like}}$**	***aac(6)-Ib* family,** *aadA1* and *aadA2* family, *aadB*, *aph(3′′)*, *aph(6)-Id*, *aphA6*			*cmlA*	***qacEΔ1***	***sul1***	***dfrA7***
27	***bla*$_{\text{OXA-23}}$**, ***bla*$_{\text{OXA-51-like}}$**	*aacC1*, *aadA1* and *aadA2* family, *aphAI*, *aph(6)-Id*, *aphA6*		***tet*(B)**		*sat2*	*sul1*, *sul2*	*dfrA1*
28	***bla*$_{\text{OXA-51-like}}$**	*aac*(3)*-III*, *aph(3′′)*, *aph(6)-Id*		***tet*(B)**			*sul2*	
29	***bla*$_{\text{GES}}$**, ***bla*$_{\text{OXA-23}}$**, ***bla*$_{\text{OXA-51-like}}$**	***aac(6)-Ib* family,** *aadA1* and *aadA2* family, *aadB*, *aph(3′′)*, *aph(6)-Id*, *aphA6*			*cmlA*	***qacEΔ1***	***sul1***	***dfrA7***
30	***bla*$_{\text{OXA-23}}$**, ***bla*$_{\text{OXA-51-like}}$**	*aph(3′′)*, *aph(6)-Id*, *aphA6*					*sul2*	
31	***bla*$_{\text{OXA-23}}$**, ***bla*$_{\text{OXA-51-like}}$**	*aadB*, *aphAI*, *aph(3′′)*, *aph(6)-Id*, *aphA6*		*tet*(39)			*sul2*	
32	***bla*$_{\text{OXA-23}}$**, ***bla*$_{\text{OXA-51-like}}$**	*aacC1*, *aph(3′′)*, *aph(6)-Id*, *aphA6*					*sul2*	
33	***bla*$_{\text{OXA-23}}$**, ***bla*$_{\text{OXA-51-like}}$**	*aacC1*, *aph(3′′)*, *aph(6)-Id*, *aphA6*					*sul2*	
34		*aacC1*, *aph(3′′)*, *aph(6)-Id*, *aphA6*					*sul2*	
35	***bla*$_{\text{OXA-51-like}}$**	*aadB*, *aphA6*		*tet*(A)		*qacEΔ1*	*sul1*	
36	***bla*$_{\text{OXA-51-like}}$**	*aadB*, *aphA6*		*tet*(A)		*qacEΔ1*	*sul1*	
37	***bla*$_{\text{OXA-51-like}}$**	*aadB*, *aphA6*		*tet*(A)		*qacEΔ1*	*sul1*	
38	***bla*$_{\text{OXA-51-like}}$**	*aadB*, *aphA6*		*tet*(A)		*qacEΔ1*	*sul1*	
39	***bla*$_{\text{OXA-51-like}}$**	*aadB*, *aphA6*		*tet*(A)		*qacEΔ1*	*sul1*	
40	***bla*$_{\text{OXA-23}}$**, ***bla*$_{\text{OXA-51-like}}$**, ***bla*$_{\text{OXA-58-like}}$**	*aadB*, *aphA6*					*sul1*	
41	*bla*$_{\text{TEM}}$, ***bla*$_{\text{OXA-51-like}}$**	*aacC1*, *aadA1* and *aadA2* family, *aphAI*, *aph(3′′)*, *aph(6)-Id*		*tet*(B)		*qacEΔ1*	*sul1*	
42	*bla*$_{\text{TEM}}$, ***bla*$_{\text{OXA-51-like}}$**	*aadA1* and *aadA2* family, *aphAI*, *aph(3′′)*, *aph(6)-Id*		*tet*(B)		*qacEΔ1*	*sul1*, *sul2*	
43	***bla*$_{\text{OXA-51-like}}$**	*aadB*, *aphA6*						
44	***bla*$_{\text{OXA-51-like}}$**	*aadB*					*sul2*	
45	*bla*$_{\text{TEM}}$, ***bla*$_{\text{OXA-51-like}}$**	*aacC1*, *aadA1* and *aadA2* family, *aphAI*				*qacEΔ1*	*sul1*	
46	***bla*$_{\text{OXA-51-like}}$**	*aadB*, *aphA6*		*tet*(A)		*qacEΔ1*	*sul1*	
47	***bla*$_{\text{OXA-51-like}}$**	*aadB*, *aphA6*		*tet*(A)		*qacEΔ1*	*sul1*	
48	*bla*$_{\text{TEM}}$, ***bla*$_{\text{OXA-51-like}}$**	*aacC1*, *aadA1* and *aadA2* family, *aphAI*, *aph(3′′)*, *aph(6)-Id*		*tet*(B)		*qacEΔ1*	*sul1*	
49								
50	***bla*$_{\text{OXA-51-like}}$**	*aadA1* and *aadA2* family, *aadB*, *aphA6*				*sat2*	*sul2*	*dfrA1*

51		*aadB*					
52	***bla*$_{\text{OXA-51-like}}$, *bla*$_{\text{OXA-58-like}}$**	*aadB*					
53							
54	***bla*$_{\text{OXA-51-like}}$**	*aac(3)-III*, *aph(3′′)*, *aphA6*					
55	***bla*$_{\text{OXA-51-like}}$**	*aadB*, *aphA6*	*tet*(A)		*qacEΔ1*	*sul1*	
56	***bla*$_{\text{OXA-51-like}}$**	*aadB*, *aphA6*	*tet*(A)		*qacEΔ1*	*sul1*	
57	***bla*$_{\text{OXA-51-like}}$**	*aadB*, *aphA6*	*tet*(A)		*qacEΔ1*	*sul1*	
58	***bla*$_{\text{OXA-51-like}}$**	*aadB*, *aphA6*	*tet*(A)		*qacEΔ1*	*sul1*	
59	***bla*$_{\text{OXA-51-like}}$**	*aadB*, *aphA6*				*sul2*	
60	***bla*$_{\text{OXA-51-like}}$**	*aadB*, *aphAI*	*tet*(B)			*sul2*	
61		*aadB*					
62	***bla*$_{\text{OXA-51-like}}$**	*aadB*			*qacEΔ1*	*sul1*	***dfrA19***
63	*bla*$_{\text{TEM}}$, ***bla*$_{\text{OXA-51-like}}$**	*aacC1*, *aadA1* and *aadA2* family, *aphAI*, *aph(3′′)*, *aph(6)-Id*	*tet*(B)		*qacEΔ1*	*sul1*	
64	***bla*$_{\text{OXA-51-like}}$**	*aadB*	*tet*(A)		*qacEΔ1*	*sul1*	***dfrA19***
65							
66							
67	***bla*$_{\text{OXA-23}}$**, ***bla*$_{\text{OXA-51-like}}$**	*aacC1*, *aph(3′′)*, *aph(6)-Id*, *aphA6*				*sul2*	
68	*bla*$_{\text{TEM}}$, ***bla*$_{\text{OXA-51-like}}$**	*aacC1*, *aadA1* and *aadA2* family, *aphAI*, *aph(3′′)*, *aph(6)-Id*	*tet*(B)		*qacEΔ1*	*sul1*	
69							
70	***bla*$_{\text{OXA-51-like}}$**	*aadB*, *aphA6*	*tet*(A)		*qacEΔ1*	*sul1*	
71	***bla*$_{\text{OXA-23}}$**, ***bla*$_{\text{OXA-51-like}}$**	*aadB*, *aphA6*				*sul2*	
72	***bla*$_{\text{OXA-23}}$**, ***bla*$_{\text{OXA-51-like}}$**	*aadB*, *aphA6*				*sul2*	
73	***bla*$_{\text{OXA-23}}$**, ***bla*$_{\text{OXA-51-like}}$**	*aadB*, *aphAI*, *aphA6*				*sul2*	
75	***bla*$_{\text{OXA-23}}$**, ***bla*$_{\text{OXA-51-like}}$**	*aadB*, *aphA6*				*sul2*	
76							
77	***bla*$_{\text{OXA-23}}$**, ***bla*$_{\text{OXA-51-like}}$**	*aacC1*, *aph(3′′)*, *aph(6)-Id*, *aphA6*				*sul2*	
78	***bla*$_{\text{OXA-51-like}}$**	*aac(3)-III*, *aph(3′′)*, *aph(6)-Id*	*tet*(B)			*sul2*	
79	***bla*$_{\text{OXA-23}}$**, ***bla*$_{\text{OXA-51-like}}$**	*aacC1*, *aadA1* and *aadA2* family, *aphAI*, *aph(6)-Id*, *aphA*	*tet*(B)		*qacEΔ1*, *sat2*	*sul1*, *sul2*	*dfrA1*
80	*bla*$_{\text{TEM}}$, ***bla*$_{\text{OXA-51-like}}$**	*aacC1*, *aadA1* and *aadA2* family, *aphAI*			*qacEΔ1*	*sul1*	
81	***bla*$_{\text{OXA-51-like}}$**						
82	***bla*$_{\text{OXA-51-like}}$**	*aadB*, *aphA6*	*tet*(A)		*qacEΔ1*	*sul1*	
83	***bla*$_{\text{OXA-23}}$**, ***bla*$_{\text{OXA-51-like}}$**	*aacC1*, *aadA1* and *aadA2* family, *aphAI*, *aph(6)-Id*, *aphA6*			*qacEΔ1*, *sat2*	*sul1*, *sul2*	*dfrA1*
84	***bla*$_{\text{OXA-23}}$**, ***bla*$_{\text{OXA-51-like}}$**	*aacC1*, *aadA1* and *aadA2* family, *aphAI*, *aph(6)-Id*			*qacEΔ1*, *sat2*	*sul1*, *sul2*	*dfrA1*
85	***bla*$_{\text{OXA-23}}$**, ***bla*$_{\text{OXA-51-like}}$**	*aacC1*, *aph(3′′)*, *aph(6)-Id*				*sul2*	
86	***bla*$_{\text{OXA-23}}$**, ***bla*$_{\text{OXA-51-like}}$**	*aadB*, *aphAI*, *aphA6*				*sul2*	
87	***bla*$_{\text{GES}}$**, ***bla*$_{\text{OXA-23}}$**, ***bla*$_{\text{OXA-51-like}}$**	*aac(6)-Ib* family, *aadA1* and *aadA2* family, *aadB*, *aph(3′′)*, *aph(6)-Id*, *aphA6*		*cmlA*	*qacEΔ1*	*sul1*	*dfrA7*
88	***bla*$_{\text{OXA-23}}$**, ***bla*$_{\text{OXA-51-like}}$**	*aadB*, *aphAI*, *aphA6*				*sul2*	
89	***bla*$_{\text{OXA-23}}$**, ***bla*$_{\text{OXA-51-like}}$**	*aadB*, *aph(3′′)*, *aph(6)-Id*, *aphA6*	*tet*(39)			*sul2*	
90	***bla*$_{\text{GES}}$**, ***bla*$_{\text{OXA-23}}$**, ***bla*$_{\text{OXA-51-like}}$**	***aac(6)-Ib* family,** *aadA1* and *aadA2* family, *aadB*, *aph(3′′)*, *aph(6)-Id*, *aphA6*		*cmlA*	***qacEΔ1***	***sul1***	***dfrA7***
91							
92	***bla*$_{\text{OXA-23}}$**, ***bla*$_{\text{OXA-51-like}}$**	*aacC1*, *aph(3′′)*, *aph(6)-Id*, *aphA6*				*sul2*	
93	***bla*$_{\text{OXA-51-like}}$**	*aadB*, *aphA6*		*floR*		*sul2*	
94	***bla*$_{\text{OXA-23}}$**, ***bla*$_{\text{OXA-51-like}}$**	*aacC1*, *aadA1* and *aadA2* family, *aphAI*, *aph(6)-Id*, *aphA6*	*tet*(B)		*qacEΔ1*, *sat2*	*sul1*, *sul2*	*dfrA1*
95	***bla*$_{\text{OXA-23}}$**, ***bla*$_{\text{OXA-51-like}}$**	*aacC1*, *aadA1* and *aadA2* family, *aphAI*, *aph(6)-Id*, *aphA6*	*tet*(B)		*qacEΔ1*, *sat2*	*sul1*, *sul2*	*dfrA1*
96	***bla*$_{\text{OXA-23}}$**, ***bla*$_{\text{OXA-51-like}}$**	*aadB*, *aphA6*				*sul2*	
97							
98	***bla*$_{\text{OXA-51-like}}$**	*aadB*, *aph(3′′)*, *aph(6)-Id*, *aphA6*	*tet*(A)		*qacEΔ1*	*sul1*, *sul2*	
99	***bla*$_{\text{OXA-51-like}}$**	*aadB*, *aphA6*	*tet*(A)		*qacEΔ1*	*sul1*	

[a] Bold text indicates determinants that were confirmed by PCR (with or without DNA sequencing).

TABLE 3 *Acinetobacter* oxacillinases

Isolate no.	ARDM-detected oxacillinases (and IS*Aba* promoter sequences) confirmed and identified by PCR and sequencing[a]							Phenotypic IPM susceptibility[b]
	bla$_{OXA-23}$		*bla*$_{OXA-51-like}$			*bla*$_{OXA-58-like}$		
	ARDM	PCR/seq	ARDM	PCR/seq	IS*Aba*[c]	ARDM	PCR/seq	
1	−	−	+	***bla*$_{OXA-314}$**	−	−	−	S
2	+	*bla*$_{OXA-23}$	+	*bla*$_{OXA-64}$	−	−	−	R
3	+	*bla*$_{OXA-23}$	+	*bla*$_{OXA-69}$	−	+	−	R
4	−	−	+	*bla*$_{OXA-71}$	−	−	−	S
5	+	*bla*$_{OXA-23}$	+	*bla*$_{OXA-64}$	−	−	−	R
6	−	−	+	*bla*$_{OXA-66}$	−	−	−	S
7	+	*bla*$_{OXA-23}$	+	*bla*$_{OXA-64}$	−	−	−	R
9	−	−	+	*bla*$_{OXA-69}$	−	−	−	S
10	−	−	+	*bla*$_{OXA-66}$	−	−	−	S
11	−	−	+	*bla*$_{OXA-66}$	−	−	−	S
12	−	−	+	*bla*$_{OXA-66}$	−	−	−	S
13	+	*bla*$_{OXA-23}$	+	*bla*$_{OXA-66}$	−	−	−	R
14	+	*bla*$_{OXA-23}$	+	*bla*$_{OXA-66}$	−	−	−	R
15	+	*bla*$_{OXA-23}$	+	*bla*$_{OXA-69}$	−	−	−	R
16	−	−	+	***bla*$_{OXA-312}$**	+	−	−	I
17	+	*bla*$_{OXA-23}$	+	*bla*$_{OXA-69}$	−	−	−	R
18	−	−	+	*bla*$_{OXA-66}$	−	−	−	S
19	−	−	+	*bla*$_{OXA-66}$	−	−	−	S
20	−	−	+	***bla*$_{OXA-312}$**	+	−	−	R
21	+	*bla*$_{OXA-23}$	+	*bla*$_{OXA-66}$	−	−	−	R
22	−	−	+	*bla*$_{OXA-66}$	−	−	−	R
23	+	*bla*$_{OXA-23}$	+	*bla*$_{OXA-66}$	−	−	−	R
24	+	*bla*$_{OXA-23}$	+	*bla*$_{OXA-69}$	−	−	−	R
25	+	*bla*$_{OXA-23}$	+	*bla*$_{OXA-66}$	−	−	−	R
26	+	*bla*$_{OXA-23}$	+	*bla*$_{OXA-69}$	−	−	−	R
27	+	*bla*$_{OXA-23}$	+	*bla*$_{OXA-69}$	−	−	−	R
28	−	−	+	*bla*$_{OXA-64}$	−	−	−	S
29	+	*bla*$_{OXA-23}$	+	*bla*$_{OXA-69}$	−	−	−	R
30	+	*bla*$_{OXA-23}$	+	*bla*$_{OXA-66}$	−	−	−	R
31	+	*bla*$_{OXA-23}$	+	*bla*$_{OXA-64}$	−	−	−	R
32	+	*bla*$_{OXA-23}$	+	*bla*$_{OXA-66}$	−	−	−	R
33	+	*bla*$_{OXA-23}$	+	*bla*$_{OXA-66}$	−	−	−	R
34	−	*bla*$_{OXA-23}$	−	*bla*$_{OXA-66}$	−	−	−	R
35	−	*bla*$_{OXA-23}$	+	***bla*$_{OXA-312}$**	+	−	−	I
36	−	−	+	***bla*$_{OXA-312}$**	+	−	−	R
37	−	−	+	***bla*$_{OXA-312}$**	+	−	−	I
38	−	−	+	***bla*$_{OXA-312}$**	+	−	−	I
39	−	−	+	***bla*$_{OXA-313}$**	+	−	−	I
40	+	*bla*$_{OXA-23}$	+	*bla*$_{OXA-69}$	−	+	−	R
41	−	−	+	*bla*$_{OXA-66}$	+	−	−	S
42	−	−	+	*bla*$_{OXA-66}$	−	−	−	S
43	−	−	+	*bla*$_{OXA-69}$	−	−	−	S
44	−	−	+	*bla*$_{OXA-100}$	−	−	−	S
45	−	−	+	*bla*$_{OXA-66/88}$	−	−	−	S
46	−	−	+	*bla*$_{OXA-71}$	−	−	−	S
47	−	−	+	*bla*$_{OXA-71}$	−	−	−	S
48	−	−	+	*bla*$_{OXA-66}$	−	−	−	S
49	−	−	−	−	−	−	−	S
50	−	−	+	***bla*$_{OXA-315}$**	−	−	−	S
51	−	−	+	*bla*$_{OXA-95}$	−	−	−	S
52	−	−	+	*bla*$_{OXA-100}$	−	+	*bla*$_{OXA-58}$	S
53	−	−	−	−	−	−	−	S

(Continued on following page)

TABLE 3 (Continued)

Isolate no.	ARDM-detected oxacillinases (and IS*Aba* promoter sequences) confirmed and identified by PCR and sequencing[a]							Phenotypic IPM susceptibility[b]
	bla$_{OXA-23}$		*bla*$_{OXA-51-like}$			*bla*$_{OXA-58-like}$		
	ARDM	PCR/seq	ARDM	PCR/seq	IS*Aba*[c]	ARDM	PCR/seq	
54	−	−	+	*bla*$_{OXA-100}$	−	−	−	S
55	−	−	+	*bla*$_{OXA-71}$	−	−	−	S
56	−	−	+	*bla*$_{OXA-113}$	+	−	−	R
57	−	−	+	*bla*$_{OXA-71}$	+	−	−	S
58	−	−	+	*bla*$_{OXA-113}$	+	−	−	R
59	−	−	+	*bla*$_{OXA-69}$	−	−	−	S
60	−	−	+	*bla*$_{OXA-69}$	−	−	−	S
61	−	−	−	*bla*$_{OXA-69}$	−	−	−	S
62	−	−	+	*bla*$_{OXA-82}$	+	−	−	S
63	−	−	+	*bla*$_{OXA-66}$	−	−	−	S
64	−	−	+	*bla*$_{OXA-82}$	+	−	−	S
65	−	−	−	−	−	−	−	S
66	−	−	−	−	−	−	−	S
67	+	*bla*$_{OXA-23}$	+	*bla*$_{OXA-66}$	−	−	−	R
68	−	−	+	*bla*$_{OXA-66}$	−	−	−	S
69	−	−	−	−	−	−	−	S
70	−	−	+	***bla*$_{OXA-313}$**	+	−	−	I
71	+	*bla*$_{OXA-23}$	+	*bla*$_{OXA-69}$	−	−	−	R
72	+	*bla*$_{OXA-23}$	+	*bla*$_{OXA-69}$	−	−	−	R
73	+	*bla*$_{OXA-23}$	+	*bla*$_{OXA-69}$	−	−	−	R
75	+	*bla*$_{OXA-23}$	+	*bla*$_{OXA-69}$	−	−	−	R
76	−	−	−	−	−	−	−	S
77	+	*bla*$_{OXA-23}$	+	*bla*$_{OXA-66}$	−	−	−	R
78	−	−	+	*bla*$_{OXA-64}$	−	−	−	S
79	+	*bla*$_{OXA-23}$	+	*bla*$_{OXA-69}$	−	−	−	R
80	−	−	+	***bla*$_{OXA-316}$**	−	−	−	S
81	−	−	+	*bla*$_{OXA-121}$	−	−	−	S
82	−	−	+	***bla*$_{OXA-312}$**	+	−	−	I
83	+	*bla*$_{OXA-23}$	+	*bla*$_{OXA-69}$	−	−	−	R
84	+	*bla*$_{OXA-23}$	+	*bla*$_{OXA-69}$	−	−	−	R
85	+	*bla*$_{OXA-23}$	+	*bla*$_{OXA-66}$	−	−	−	R
86	+	*bla*$_{OXA-23}$	+	*bla*$_{OXA-69}$	−	−	−	R
87	+	*bla*$_{OXA-23}$	+	*bla*$_{OXA-69}$	−	−	−	R
88	+	*bla*$_{OXA-23}$	+	*bla*$_{OXA-69}$	−	−	−	I
89	+	*bla*$_{OXA-23}$	+	***bla*$_{OXA-317}$**	−	−	−	R
90	+	*bla*$_{OXA-23}$	+	*bla*$_{OXA-69}$	−	−	−	R
91	−	−	−	−	−	−	−	S
92	+	*bla*$_{OXA-23}$	+	*bla*$_{OXA-66}$	−	−	−	R
93	−	−	+	*bla*$_{OXA-94}$	−	−	−	S
94	+	*bla*$_{OXA-23}$	+	*bla*$_{OXA-69}$	−	−	−	R
95	+	*bla*$_{OXA-23}$	+	*bla*$_{OXA-69}$	−	−	−	R
96	+	*bla*$_{OXA-23}$	+	*bla*$_{OXA-94}$	−	−	−	R
97	−	−	−	−	−	−	−	S
98	−	−	+	*bla*$_{OXA-71}$	−	−	−	?
99	−	−	+	*bla*$_{OXA-113}$	+	−	−	R
% ARDM sensitivity	95.1		97.8			100		
% ARDM specificity	100		100			97.9		
Positive predict value (%)	100		100			33		
Negative predict value (%)	81.8		100			00.7		

[a] Bold text indicates *bla*$_{OXA-51-like}$ genes that were determined to be new variants. +, positive by ARDM or PCR, as indicated; −, not detected by ARDM or PCR, as indicated.
[b] IPM, imipenem; S, sensitive; I, intermediate; R, resistant; ?, unknown.
[c] IS*Aba1* promoter sequence detected 5′ of *bla*$_{OXA-51-like}$ gene by PCR (see the supplemental material).

but ARDM negative for *tet*(B). Based on PCR verification of these 30 isolates, sensitivities for *tet*(39), *tet*(A), and *tet*(B) were 100%, 100%, and 89%, respectively, and specificity for all three genes was 100%. Repeated gene-specific PCR attempts were unable to confirm the presence of *tet*(30) in any of the four isolates that had tested positive via ARDM analysis. As all four ARDM identifications met only the minimal requirements for a positive determination, these findings may indicate the potential nonspecific nature of these probes or the presence of probe sequence motifs that are conserved among major facilitator superfamily (MFS) efflux pumps resulting in false-positive identifications. Interestingly, presumptive false-positive *tet*(30) results have been previously observed with other *A. baumannii* isolates (26).

Determinants conferring resistance to phenicol compounds. Determinants conferring resistance to chloramphenicol and phenicol compounds represented in ARDM v.2 encode both inactivating enzymes (five families of acetyltransferases) and specific efflux pumps (three phenicol-specific pumps). From these possibilities, the *floR* and *cmlA* genes, which encode MFS exporters, and the *catA1* group family of acetyltransferase genes were detected among the *A. baumannii* isolates analyzed (Table 2). The presence of the *cmlA* gene was PCR confirmed in all five isolates positive for *cmlA* by ARDM; amplicon sequencing identified the genes as *cmlA4*. Interestingly, all five of the *cmlA4*-positive samples possessed identical resistance determinant profiles: *ampC*, $bla_{\text{OXA-23}}$, $bla_{\text{OXA-51-like}}$, *aadA1* and *aadA2* family, *aadB*, *aph(3′′)*, *aph(6)-Id*, *aphA6*, and the five specific alleles present in the $bla_{\text{GES-11}}$-containing class 1 integron [$bla_{\text{GES-11}}$, *aac(6)-Ib*, *qacEΔ1*, *sul1*, and *dfrA7*].

Determinants conferring resistance to quaternary amines, sulfonamides, streptothricin, diaminopyrimidines, and detection of integrons. The ARDM v.2 content contains probes for two genes conferring resistance to quaternary amines: *qacEΔ1*, found in class 1 integrons, and *qacE* from *A. baumannii*, which exhibits no significant sequence homology to *qacEΔ1*. In our analyses, the detection of *qacE* was generally considered to be evidence that the hybridized DNA was derived from *A. baumannii*, as none of the other Gram-negative and Gram-positive species tested to date have been *qacE* positive (data not shown). There were, however, six isolates in this study that were *qacE* negative by ARDM analysis. Interestingly, all six were susceptible to at least nine of the 10 antimicrobials tested and harbored (at most) a single resistance determinant in addition to the positive control, *folA*.

Of the 97 isolates tested by the ARDM, 45% were shown to contain both *qacEΔ1* and *sul1* genes (Table 2). As both *qacEΔ1* and *sul1* are components of the 3′-conserved region of many, but not all, class 1 integrons, *intI1*-specific PCR confirmed the presence of the appropriate integrase gene in all 44 of these $qacE\Delta1^+$ and $sul1^+$ samples. However, an additional 14 samples tested positive for *intI1* by PCR but were ARDM negative for *qacEΔ1* (1 isolate) or both genes (13 isolates), potentially indicating the presence of class 1 integrons without these genes. In at least one sample—isolate no. 30, a genome-sequenced strain—the presence of a class 1 integron without *qacEΔ1* and *sul1* was confirmed (Table 2; see also Table S3 in the supplemental material).

Using PCR primers specific to the 5′- and 3′-conserved sequences of class 1 integrons and DNA sequencing of the resulting amplicons, we determined the gene cassette content of the class 1 integrons in 28 of the 44 $qacE\Delta1^+$ and $sul1^+$ (*intI1*-positive) samples. Most of the observed amplicons ($n = 20$) were <1 kb in size and presumptively contained just one gene cassette; the presence of *aadB* was confirmed in seven of these 20 samples. A second set of amplicons (~2.5 kb in size, isolate no. 41, 63, and 68) contained a four-cassette array: *aacC1*, *orfP*, *orfQ*, *aadA1*. Finally, using a modified 5′-conserved sequence primer, we were able to amplify an ~2.7-kb cassette array out of five of the 21 samples that were PCR negative when using both 5′ and 3′ conserved sequence primers. For these samples, DNA sequencing revealed the presence of the following three-cassette array: $bla_{\text{GES-11}}$, *aac(6′)-Ib*, *dfrA7*. We were unable to identify the content of the integron cassette arrays in the remaining 16 $qacE\Delta1^+$ and $sul1^+$ (*intI1*-positive) samples. The lack of amplification in these last samples could be the result of the cassette array being too large for PCR amplification or the consequence of the lack of a 3′-conserved sequence region (43, 44).

In addition to *sul1*, two additional genes conferring sulfonamide resistance are also present by ARDM v.2 (*sul2*, *sul3*). While the *sul2* gene was detected in 43% of the isolates, the *sul3* gene was not found.

ARDM v.2 also includes probes targeting 27 alternative dihydrofolate reductase genes (including the *A. baumannii*-specific *folA* control) that confer resistance to trimethoprim and other diaminopyrimidines. Besides the *folA* control, three *dfrA* genes were detected by the ARDM: *dfrA1*, *dfrA7*, and *dfrA19* (Table 2). *dfrA7* was detected in 5 isolates by ARDM analysis; these results were confirmed by PCR and sequence analysis, showing that the *dfrA7* gene was harbored within a class 1 integron, as described above. The *dfrA19* gene, previously detected only within members of the *Enterobacteriaceae*, was detected in two isolates (no. 62 and 64) and was subsequently confirmed by PCR and DNA sequencing.

Seven isolates contained the *dfrA1* gene. These same isolates also harbored the streptothricin resistance determinant *sat2* and an *aadA1* gene. The codetection of these genes—all commonly associated with class 2 integrons—suggested that these strains harbored class 2 integrons. The presence of class 2 integrons was confirmed via PCR amplification of the *intI2* gene in all seven of these $dfrA1^+$, $sat2^+$, and $aadA1^+$ isolates. However, we were unable to amplify the associated class 2 integron cassette arrays. PFGE profiles suggested that four of these isolates were closely related (isolate no. 27, 79, 83, and 84; data not shown) (45).

DISCUSSION

In this study, we determined the genetic assemblages that confer antimicrobial resistance in *A. baumannii* isolates from patients in military treatment facilities. For this purpose, the broad-spectrum screening capabilities of the ARDM was refined to include emerging, high-impact resistance determinants (e.g., $bla_{\text{NDM-1}}$), previously overlooked classes of determinants (e.g., 16S rRNA methylases, *cmlA*, *cmr*), and *Acinetobacter*-specific genes, including those for the four classes of class D carbapenemases most often responsible for carbapenem resistance in *Acinetobacter* spp. ($bla_{\text{OXA-51-like}}$, $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-58-like}}$) (46). Furthermore, inclusion of multiple *Acinetobacter*-specific positive controls allowed the quality of sample processing and hybridization to be assessed. Due to *A. baumannii*'s ability to acquire drug resistance determinants, it was not surprising that the vast majority of isolates tested harbored multiple resistance determinants; nine isolates had 13 detected resistance genes in addition to positive con-

TABLE 4 Cross-study comparison of the percentage of resistance determinants detected in military collections of *Acinetobacter* sp. isolates

Resistance determinant(s)	% of isolates				
	WRAMC (2003–2005)[a]	NNMC (2006)[b]	SAMMC (2006–2007)[c]	NNMC (2004–2005)[d]	This study (2003–2009)[e]
bla_{ADC}, *ampC*	99				90
$bla_{OXA\text{-}51\text{-}like}$, $bla_{OXA\text{-}58\text{-}like}$	97			100	90
$bla_{OXA\text{-}23}$	11	27		26	40[f]
$bla_{OXA\text{-}58\text{-}like}$	12			26	3[g]
bla_{TEM}	40				14
bla_{SHV}	1				0
$bla_{CTX\text{-}M\text{-}2}$	0				0
bla_{VEB}, bla_{PER}, bla_{IMP}, bla_{VIM}, bla_{GIM}	0				0
aacC1	56				31
aadA1	39				27
aadB	48				46
aphA6	71				60
qnrA, *qnrB*	0				0
tet(A)			19		20
tet(B)			17		20
tet(H), *tet*(L), *tet*(M), *tet*(41)			0		0
tet(39)			38		5

[a] No. of isolates tested = 75. WRAMC, Walter Reed Army Medical Center (19).
[b] No. of isolates tested = 102. NNMC, National Naval Medical Center (18).
[c] No. of isolates tested = 89. SAMMC, San Antonio Military Medical Center (21).
[d] No. of isolates tested = 65. NNMC, National Naval Medical Center (32).
[e] No. of isolates tested = 97.
[f] Forty-one percent were PCR confirmed.
[g] One percent were PCR confirmed.

trols. In fact, various isolates were found to contain up to four β-lactamase determinants and six AG resistance determinants; the prevalence of multiple AG resistance determinants in particular has been well documented (47–49).

While the ARDM v.2 detected $bla_{OXA\text{-}51\text{-}like}$ genes in 89% of the isolates tested, subsequent PCR and amplicon sequencing not only confirmed the microarray findings but also revealed six new variants of the OXA-51-like β-lactamases (now designated OXA-312, -313, -314, -315, -316, and -317). Based on the sequencing data, the prevalence of specific $bla_{OXA\text{-}51\text{-}like}$ variants in the analyzed collection suggested that most of them ($n = 80$) belonged to the WW1 (EU1/SG2), WW2 (EU2/SG1), and WW3 (EU3/SG3) clonal lineages.

Our own correlation between $bla_{OXA\text{-}23}$ detected by the ARDM and phenotypic carbapenem resistance (97%) is consistent with that of a recent study (21) which demonstrated that 87% of imipenem-resistant isolates from military personnel hospitalized from 2003 to 2008 harbored the $bla_{OXA\text{-}23}$ determinant. In both studies, imipenem resistance not attributable to OXA-23 could be correlated with IS*Aba1* sequences upstream of $bla_{OXA\text{-}51\text{-}like}$ genes; the role of IS*Aba* promoter sequences in the upregulation of $bla_{OXA\text{-}51\text{-}like}$ genes and phenotypic carbapenem resistance has been suggested (50–52). In addition, the correlation of carbapenem resistance with the presence of $bla_{OXA\text{-}58}$ may also be related to the presence of IS*Aba*-like promoter sequences upstream of the gene (24, 53, 54). However, as observed in another study of U.S. MTFs (24), there was no correlation of $bla_{OXA\text{-}58}$ with carbapenem resistance, but the presence of these IS*Aba*-like sequences upstream of $bla_{OXA\text{-}58}$ were not determined.

The high frequency of $bla_{OXA\text{-}23}$ genes detected by the ARDM (40% of the isolates) and phenotypic carbapenem resistance (45% of the isolates) were of particular concern. Earlier studies of MDR isolates from MTFs have documented a significantly lower prevalence of $bla_{OXA\text{-}23}$, ranging from ~11% in isolates collected between 2003 and 2005 (23, 24) to 15 to 27% in isolates collected between 2005 and 2008 (21, 22) (Table 4). However, caution should be used when comparing our own results with those of previous studies, given the diversity of our own isolates and unknown points of origin.

Interestingly, the present study shows an increase in the rate of $bla_{OXA\text{-}23}$ detection in isolates collected in the latter half of 2008 through 2009. PFGE profiles and optical mapping of $bla_{OXA\text{-}23}$-positive strains collected during this time period suggested close genetic relationships within, but not between, two pairs of isolates containing $bla_{OXA\text{-}66}$ (isolate no. 33 and 77 and isolate no. 23 and 25) and two sets of isolates containing $bla_{OXA\text{-}69}$ (isolate no. 27, 79, 83, and 84 and isolate no. 71, 72, 73, and 75) (data not shown). The presence of $bla_{OXA\text{-}23}$ among both related and unrelated isolates suggests that neither a single outbreak nor a single MTF is responsible for an increase in $bla_{OXA\text{-}23}$ carriage among the isolates collected during this time period. However, this apparent increase may simply be due to collection bias or over-/underrepresentation of different isolate sources or source facilities. As such, further study is needed to correlate these changes in gene distributions with individual MTFs, mode of transport and medical treatment facilities through which combat-injured personnel passed (in cases of war wounds), antimicrobial administration policies, and infection control measures (55–57).

The broad-spectrum screening capability used in this study enabled the detection of unexpected and emerging *A. baumannii* resistance determinants. For example, isolate no. 22 was found to harbor the *armA* gene encoding a 16S rRNA methylase that confers resistance to all 4,6-disubstituted deoxystreptamines (e.g., amikacin, gentamicin, tobramycin) (58). While this determinant

is most commonly found in the *Enterobacteriaceae*, its presence in *A. baumannii* has been documented, primarily in the Far East (59, 60), more recently in Europe and the Middle East (61–64), and only once in North America (65, 66). Similarly, the ARDM detected the presence of the *dfrA19* determinant in isolate no. 62 and 64, which was subsequently confirmed by PCR and amplicon sequencing. To our knowledge, this antibiotic resistance determinant has not been described in any species outside the *Enterobacteriaceae*. Although *A. baumannii* is naturally trimethoprim resistant, this antimicrobial compound is still commonly used as a first-line treatment for uncomplicated urinary tract infections and community-associated methicillin-resistant *Staphylococcus aureus* skin infections. As naturally occurring lateral transfer of genes from *Acinetobacter* to other species has been conjectured (67–69), *A. baumannii* may serve as a reservoir for resistance determinants such as *armA* and *dfrA19* for the subsequent transfer to other, unrelated bacterial species (70).

In many cases, identification of multiple classes of resistance determinants by ARDM v.2 suggested the possible presence of a number of genetic structures (i.e., integrons and resistance islands) often associated with antimicrobial resistance. For example, many class 1 integrons possess both *qacEΔ1* and *sul1* determinants. Indeed, *intI1*-specific PCR verified the presence of a class 1 integron in all 44 isolates where the ARDM detected both *qacEΔ1* and *sul1*. However, an additional 14 samples tested negative for one or both genes by ARDM analysis but were PCR positive for *intI1*. Whether these harbor class 1 integrons with alternative structures remains to be determined. In one of the genome-sequenced isolates, the presence of a class 1 integron that did not contain *qacEΔ1* or *sul1* genes was confirmed.

The ARDM also detected 4 additional determinants in common in the five bla_{GES}-containing isolates: *aac(6)-Ib*, *dfrA7*, *qacEΔ1*, and *sul1* determinants. Subsequent confirmatory PCR and DNA sequencing confirmed the presence of a class 1 integron harboring bla_{GES-11} and all four additional genes; this integron has previously been described in *A. baumannii* isolates from Belgium (71, 72). Interestingly, of the five isolates harboring this integron, only two (isolate no. 15 and 26) could be considered closely related, as determined by PFGE profiles and optical mapping (73) (data not shown).

Similarly, where ARDM analyses identified seven isolates harboring *dfrA1*, *sat2*, and *aadA1*, which are often associated with class 2 integrons, the presence of a class 2 integron was also confirmed by *intI2*-specific PCR. Five of these samples presented nearly identical profiles of the other determinants: bla_{OXA-23}, bla_{OXA-51}, *aacC1*, *aphA1*, *aph(6)-Id*, *tet*(B), *qacEΔ1*, *sul1*, and *sul2*. Based on the presence of both *qacEΔ1* and *sul1*, each of these isolates was also tested for *intI1*, and its presence confirmed that these five isolates harbored a class 1 integron as well. Interestingly, these five strains were isolated between October 2008 and July 2009 and most likely represent the WW1 clonal lineage based on the presence of bla_{OXA-69}. PFGE profiles indicated that four of these strains were closely related (isolate no. 27, 79, 83, and 84; data not shown).

The identified genetic assemblages also suggested that a number of isolates were harboring various resistance islands. Fourteen samples were found to harbor four determinants found in AbaR6 and AbaR7 (*aacC1*, *aadA1* and *aadA2* family, *aphA1*, and *sul1*). One of these isolates (no. 24) may harbor AbaR5, as it also contained the *tet*(A) determinant. This last isolate was also positive for *cmlA4* but was negative for bla_{TEM}, which are both carried by AbaR3; two strains isolated in 2004 from Walter Reed Army Medical Center (but not included in this study) were previously confirmed to contain AbaR3 (74, 75). Interestingly, of the 14 isolates potentially harboring AbaR6/AbaR7, 11 do carry bla_{TEM}. A detailed sequence analysis of the regions surrounding these determinants will determine whether these strains do indeed contain resistance islands or, alternatively, genomic islands and may further uncover uncharacterized genomic rearrangements within these gene clusters.

The present study has provided a broad-spectrum survey of the resistance determinants harbored by 97 *A. baumannii* isolates from U.S. MTFs. Not surprisingly, $bla_{OXA-51-like}$ and multiple aminoglycoside determinants were observed in the vast majority of isolates, and most of the other resistance determinants detected by the ARDM have been previously described and at similar levels in other studies of *A. baumannii* isolates from U.S. MTFs. While decreased rates of detection were noted for a few determinants [e.g., bla_{TEM}, bla_{OXA-58}, *aacC1*, *tet*(39)], we detected rare (*armA*) and unexpected (*dfrA19*) resistance genes as well. Furthermore, the apparent increases in the rates of detection for both the bla_{GES} and bla_{OXA-23} genes into both related and unrelated strains within the final 6 months of sample collection and the presumptive cointroduction and subsequent spread of class 1 and class 2 integrons in October 2008 suggest that the ARDM technology can complement other techniques (PFGE profiles, multilocus sequence typing, amplicon sequencing) in monitoring the evolution of MDR in *A. baumannii* over time. Overall, these findings suggest that the ARDM and similar tools can provide information helpful in tracking the movement of antimicrobial resistance determinants to and from *A. baumannii* in hospital environments, enable comparisons of MDR genetic assemblages between differing populations and countries (64), and potentially support more informed infection control strategies and chemotherapeutic treatment.

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