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Phenotypic and Molecular Characterization of *Acinetobacter* Clinical Isolates Obtained from Inmates of California Correctional Facilities[▽]†

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***Acinetobacter* spp. increasingly have been wreaking havoc in hospitals and communities worldwide. Although much has been reported regarding *Acinetobacter* isolates responsible for nosocomial infections, little is known about these organisms in correctional facilities. In this study, we performed species identification, examined the antibiotic resistance profiles, and determined the mechanisms of resistance and clonal relationships of 123 *Acinetobacter* isolates obtained from inmates of 20 California correctional facilities (CCFs). We found that 57.7% of the isolates belong to *A. baumannii*, followed by isolates of *Acinetobacter* genomic species 3 (gen. sp. 3; 23.6%) and of *Acinetobacter* gen. sp. 13TU (10.6%). Multidrug-resistant (MDR) CCF isolates were found in only six CCFs. Additionally, DNA sequences of *gyrA* and *parC* genes were consistent with fluoroquinolone (FQ) susceptibility phenotypes. Furthermore, the presence of class 1 integrons was detected in 15 CCF isolates, all of which are MDR. Integron-associated gene cassettes encode several aminoglycoside modification enzymes, which correlate with most of the aminoglycoside-resistant phenotypes. Antimicrobial susceptibility testing in the presence of Phe-Arg-β-naphthylamide dihydrochloride and 1-(1-naphthylmethyl)-piperazine indicated the involvement of efflux pumps in the FQ resistance of only a few CCF isolates. Finally, genetic profiling showed that there was no evidence of *A. baumannii* outbreaks in CCFs. Instead, our analyses revealed only limited clonal dissemination of mostly non-MDR *A. baumannii* strains in a few facilities. This study represents the first report to characterize phenotypic and molecular features of *Acinetobacter* isolates in correctional facilities, which provides a baseline for monitoring the antimicrobial resistance changes and dissemination patterns of these organisms in such specialized institutions.**

The role of Gram-negative *Acinetobacter* spp. as a cause of nosocomial infections worldwide has been recognized as a growing concern (30, 32, 52, 64, 67, 69). Chief among them, *A. baumannii* is responsible for a significant proportion of nosocomial infections, including urinary tract infections, endocarditis, surgical-site infections, meningitis, septicemia, and ventilator-associated pneumonia among intensive care unit patients in hospitals (3, 5, 17, 29, 36, 38, 44, 58, 70, 74). In particular, multidrug-resistant (MDR) clinical isolates of this bacterium have been reported as infectious agents in many soldiers wounded in Afghanistan and Iraq (2, 24, 27, 78). In addition, *A. baumannii* is recognized as an increasingly important cause of community-acquired pneumonia and other infections (8, 16, 46, 47). Besides *A. baumannii*, other *Acinetobacter* spp., including genomic species 3 (gen. sp. 3), gen. sp. 13TU, *A. lwoffii*/gen. sp. 9, *A. ursingii*, *A. johnsonii*, and *A. parvus*, have been found to be clinically relevant (28, 30, 64). The report of Turton and coworkers on 690 nonduplicate clinical *Acinetobacter* isolates revealed the significant presence of other species, including *A. lwoffii*/gen. sp. 9 (8.8%), *A. ursingii* (4%), *A. johnsonii* (1.7%), and *A. parvus* (1.3%), besides the expected *A. baumannii* isolates (78%) (64). Most of these non-*A. baumannii* isolates were from blood and were associated with bacteremia or septicemia (64). Especially noteworthy was the implication of some *A. johnsonii*, gen. sp. 13, and *A. beijerinckii* isolates in endocarditis (64). In contrast, Karah and colleagues recently reported that among 113 consecutive blood culture isolates of *Acinetobacter* species collected between 2005 and 2007 in Norway, the most prevalent species were gen. sp. 13TU (46.9%) and gen. sp. 3 (19.5%), followed by *A. baumannii* (8%) and *A. lwoffii*/gen. sp. 9 (7.1%) (28). These results demonstrated the increasingly recognized clinical role of some non-*A. baumannii* species of the *Acinetobacter* genus.

The growing importance of *Acinetobacter* spp. in hospitals has sparked concerns about their potential effect on other closed-environment institutions (26, 58). A few years ago, we became aware of *Acinetobacter* spp. being frequently isolated from inmates in California state prisons (California correctional facilities, or CCFs). Although a few published reports describing prison outbreaks exist, such reports focused primarily on methicillin-resistant *Staphylococcus aureus* (MRSA) and *Mycobacterium tuberculosis* clinical isolates obtained from correctional facilities in the United States (4, 12, 41). Currently, there is a lack of published studies focusing on the prevalence of multidrug resistance, dissemination patterns, and mechanisms of antibiotic resistance of *Acinetobacter* clinical isolates from correctional facilities. The availability of 123 *Acinetobacter* clinical isolates from inmates of 20 CCFs provided us a rare opportunity to determine the prevalence of multidrug-resistant

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isolates of this genus and whether clonal disseminations or even outbreaks of *A. baumannii* or other species occurred within or among these facilities. In this study, we identified the CCF isolates to the species level using molecular techniques, determined their antimicrobial susceptibility profiles, characterized molecular resistance determinants, and studied their genomic fingerprints and clonal relationships.

MATERIALS AND METHODS

Bacterial strains and isolates. All CCF *Acinetobacter* clinical isolates, obtained from a clinical laboratory, are listed in Table 1 along with facility designation, age and sex of inmate patients, and date of isolation. These bacterial isolates were identified initially as *A. baumannii* at the clinical laboratory using the Vitek 2 system with the Gram-negative (GN) colorimetric reagent identification cards (bioMérieux, Inc., Durham, NC) by following the instructions and protocols of the manufacturer. For comparison purposes, a number of genetically distinct clinical isolates obtained from nosocomial outbreaks in Los Angeles County also were used (67). Quality control strains used in antimicrobial susceptibility testing (*Escherichia coli* [ATCC 25922], *Pseudomonas aeruginosa* [ATCC 27853], *Enterococcus faecalis* [ATCC 29212], and *Staphylococcus aureus* [ATCC 29213]) were purchased from the American Type Culture Collection (ATCC; Manassas, VA). All strains were stored in LB broth and 40% glycerol at −80°C.

Species-level identification using molecular methods. Species of these CCF isolates were determined further based on the sequence analysis of 16S-23S rRNA intergenic spacer (ITS) regions of the CCF isolates using modified methods based on those of Chang and coworkers (7). Briefly, genomic DNA preparations were isolated from each of the CCF *Acinetobacter* isolates using a Wizard genomic DNA purification kit (Promega, Madison, WI). The amplification of the ITS region and sequence analysis were performed based on the procedures described by Chang and coworkers (7), but with a different PCR program consisting of an initial denaturation at 95°C for 5 min, followed by 36 cycles of denaturation at 95°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 1 min, with a final extension step at 72°C for 10 min. Also, *Taq* polymerase MasterMix from 5 Prime (Gaithersburg, MD) was used, and approximately 100 ng of genomic DNA was used as the template. PCR amplification was carried out in the GeneAmp 9700 system (Applied Biosystems, Foster City, CA) without a mineral oil overlay. ITS sequence analysis was performed by NCBI's BLAST program using each ITS sequence bordered by the 5′ and 3′ ends defined by Chang et al. (7). The isolate was given the name of the species whose ITS sequences have the greatest overall similarity (greater than 90% identity) across the entire ITS region to that of the query sequence.

Additionally, trilocus multiplex PCR (63) was performed using genomic DNA from these CCF isolates to determine clonal relationships of some *A. baumannii* isolates (European/International clone I, II, or III). Briefly, six pairs of PCR primers designed by Turton and colleagues (63) were used to perform two sets of multiplex PCR amplifications of *ompA*, *csuE*, and $bla_{\text{OXA-51-like}}$ gene fragments in the CCF isolates also using 5 Prime *Taq* polymerase MasterMix and a GeneAmp 9700 system. PCR parameters consisted of an initial denaturation at 94°C for 3 min, 30 cycles of denaturation at 94°C for 45 s, annealing at 57°C for 45 s, extension at 72°C for 1 min, and a final extension at 72°C for 5 min. CCF isolates were designated *A. baumannii* European clones I, II, and III based on criteria established for group 2, group 1, and group 3 strains, respectively (63).

Susceptibility testing. The antimicrobial susceptibility of the clinical isolates or strains were determined using the broth microdilution protocols of the Clinical and Laboratory Standards Institute (CLSI) against a total of 18 known antibiotics according to methods described previously (67).

Sequencing of QRDRs of *gyrA* and *parC* genes. Sequencing and analysis of the quinolone resistance determinant regions (QRDRs) of the *A. baumannii* genes *gyrA* and *parC* was performed according to the methods described previously (67), except a new forward PCR/sequencing primer for the *gyrA* QRDR was used: 5′-GTGTGCTTTATGCCATGCAC-3′. DNA sequences obtained were aligned with known sequences using the BLASTX option (on the NCBI website) to generate amino acid alignment within the QRDRs. Sequence comparisons were made against amino acid sequences of wild-type *A. baumannii* GyrA (accession no. X82165) and ParC (accession no. X95819) QRDRs (72, 73).

Detection of class 1 integrons. The presence or absence of class 1 integrons in all 123 of the CCF clinical isolates was determined by PCR using previously reported primers specific for the *intI1* genes: *int*I1L primer, 5′-ACATGTGAT GGCGACGCACGA-3′; and *int*I1R primer, 5′-ATTTCTGTCCTGGCTGGCG A-3′ (53). Amplification was performed using genomic DNA as the template or by colony PCR as described previously for the QRDR of the *gyrA* and *parC* genes (67), with the following parameters: an initial template denaturation at 94°C for 2 min; 36 cycles of 1 min of denaturation at 95°C, 30 s of annealing at 60°C, and 2 min of extension at 72°C; and a final extension at 72°C for 10 min. Similarly, primers specific to the 5′ and 3′ conserved segments (CS) of class 1 integrons (5′ CS primer, 5′-GGCATCCAAGCAGCAAG-3′; 3′ CS primer, 5′-AAGCAGAC TTGACCTGA-3′) also were chosen to amplify the variable regions, as previously described by Lévesque and coworkers (35). The PCR products were separated on 1% agarose gels, observed, and recorded under UV light after being stained with ethidium bromide. Successful amplicons were sequenced as previously described for the QRDR of *gyrA* and *parC* genes.

Effects of efflux pump inhibitors. Efflux pump inhibitors were used to evaluate whether certain efflux pumps contribute to the resistance of some antibiotics based on methods described previously (67). Briefly, susceptibility to the antibiotics in the presence and absence of 100 μg/ml of the efflux pump inhibitor PAβN (phe-arg-β-naphthylamide dihydrochloride) or NMP [1-(1-naphthylmethyl)-piperazine] was tested as described previously (67). Following the addition of an antibiotic and the bacterial cell inocula, 2 μl of the 5-mg/ml stock of either PAβN or NMP was added to the microplate wells (total volume, 100 μl). The rest of the procedures were carried out as described previously (67).

PFGE. Bacterial genomic DNA plugs were made from CCF *Acinetobacter* clinical isolates according to the PulseNet standardized laboratory protocol (http://www.cdc.gov/pulsenet/protocols.htm) for the molecular subtyping of *Escherichia coli* O157:H7, nontyphoidal *Salmonella* serotypes, and *Shigella sonnei* with the following modifications. Genomic DNAs (in agarose plugs) released from lysed cells of all isolates were digested with ApaI (40 U/μl; New England BioLabs, Ipswich, MA) for 2 h at 30°C. Samples were run on 1% SeaKem Gold pulsed-field agarose gels in 0.5 × Tris-borate-EDTA using a Bio-Rad contour-clamped homogeneous electric field DR III electrophoresis system (Bio-Rad Laboratories, Hercules, CA) for 14 h with an initial pulse at 5 s and a final pulse at 35 s. To compare the results of each clinical isolate on different gels, a genomic lambda ladder (New England BioLabs) was used as the DNA size standard. The pulsed-field gel electrophoresis (PFGE) results were interpreted by categorizing gels visually according to guidelines described by Tenover as used in our previous study (61, 67). The genomic DNA banding patterns of the various CCF clinical isolates generated by PFGE were analyzed for clonal relationships using BioNumerics software, version 5.1 (Applied Maths, Austin, TX).

RESULTS

Demographics of inmate patients from whom clinical isolates were obtained. One hundred twenty-three *Acinetobacter* clinical isolates were kindly provided by a clinical laboratory, which obtained the isolates from 20 different institutions (Institutions A to S in Table 1), representing the majority (19) of the 33 adult institutions in California, during a nearly 1-year period. As seen in Table 1, we obtained the highest number of *Acinetobacter* isolates from Institution A (with 18) and the lowest number of isolates from Institution P (only 1). By the end of 2008, the adult correctional facilities in California had a combined inmate population of 158,931, with 93% males and 7% females (6). The majority (78%) of the CCF clinical isolates were from male inmates, and only 22% of all isolates were from female inmates.

Species-level identification of CCF isolates using molecular methods. These CCF isolates were identified initially by the clinical laboratory as *A. baumannii* based on phenotypic methods (Vitek 2 system). Since the phenotypic identification of *Acinetobacter* isolates to the species level is not reliable (7, 79), we subsequently performed ITS sequencing and trilocus multiplex PCR to more accurately identify each isolate's species and, if it was *A. baumannii*, whether or not it belongs to one of the three European clones (I, II, and III) (Table 1). The ITS sequencing determined that 71 (57.7%) of the isolates were *A. baumannii*. Another 29 (23.6%) isolates were determined to be *Acinetobacter* gen. sp. 3, and another 13 (10.6%) were deter-

TABLE 1. CCF *Acinetobacter* isolates, source information, and species typing

Isolate code[a]	Facility	Age of inmate	Sex[d]	Date of collection (mo/day/yr)	Species typing[b]	PFGE type[c]
CCF-52A	A	45	M	2/28/2008	*A. baumannii*	PT1
CCF-124A	A	48	M	8/14/2008	*A. baumannii*	PT27
CCF-55A	A	48	M	2/22/2008	*A. baumannii*	PT29
CCF-104A	A	45	M	6/27/2008	*A. baumannii*	PT40a
CCF-43A	A	48	M	1/25/2008	*A. baumannii*	PT40a
CCF-92A	A	23	M	6/14/2008	*A. baumannii*	PT40a
CCF-40A	A	47	M	1/16/2008	*A. baumannii* (clone I)	PT30a
CCF-56A	A	47	M	2/22/2008	*A. baumannii* (clone I)	PT30b
CCF-63A	A	47	M	3/1/2008	*A. baumannii* (clone I)	PT30c
CCF-34A	A	36	M	12/14/2007	*A. baumannii* (clone II)	PT13
CCF-11A	A	30	M	9/22/2007	*A. baumannii* (clone II)	PT70
CCF-45A	A	49	M	1/22/2008	*A. baumannii* (clone II)	PT8a
CCF-46A	A	49	M	1/22/2008	*A. baumannii* (clone II)	PT8a
CCF-7A	A	42	M	9/15/2007	gen. sp. 13TU	PT21
CCF-120A	A	32	M	7/28/2008	gen. sp. 14BJ	PT55
CCF-101A	A	29	M	7/15/2008	gen. sp. 3	PT46
CCF-1A	A	46	M	9/7/2007	gen. sp. 3	PT49
CCF-96A	A	48	M	6/2/2008	gen. sp. 3	PT67
CCF-135B	B	44	M	8/3/2008	*A. baumannii*	PT24
CCF-28B	B	22	M	12/6/2007	Closest to gen. sp. 14BJ	PT56
CCF-6B	B	46	M	9/11/2007	gen. sp. 13TU	PT16
CCF-42B	B	51	M	1/19/2008	gen. sp. 13TU	PT17
CCF-74B	B	21	M	5/1/2008	gen. sp. 13TU	PT19
CCF-32B	B	31	M	11/17/2007	gen. sp. 13TU	PT32
CCF-2B	B	42	M	9/10/2007	gen. sp. 13TU	PT66
CCF-73B	B	31	M	4/28/2008	gen. sp. 13TU	PT9
CCF-17B	B	22	M	10/18/2007	gen. sp. 14TU	PT47
CCF-91B	B	72	M	6/6/2008	gen. sp. 3	PT2
CCF-72B	B	29	M	4/22/2008	gen. sp. 3	PT71
CCF-134C	C	40	M	8/8/2008	*A. baumannii*	PT65
CCF-117C	C	42	M	8/22/2008	*A. baumannii*	PT75
CCF-54C	C	35	M	2/26/2008	*A. baumannii* (clone II)	PT10a
CCF-8C	C	34	M	9/21/2007	*A. baumannii* (clone II)	PT10a
CCF-94C	C	35	M	6/2/2008	*A. baumannii* (clone II)	PT10a
CCF-9C	C	34	M	9/18/2007	*A. baumannii* (clone II)	PT10a
CCF-61C	C	35	M	2/7/2008	*A. baumannii* (clone II)	PT10b
CCF-13C	C	41	M	9/27/2007	*A. baumannii* (clone II)	PT48
CCF-22C	C	34	M	11/3/2007	gen. sp. 3	PT52
CCF-116D	D	40	M	8/11/2008	*A. baumannii*	PT31
CCF-5D	D	28	M	9/13/2007	*A. baylyi*	PT34
CCF-12D	D	26	M	9/22/2007	gen. sp. 3	PT11
CCF-4D	D	65	M	9/15/2007	gen. sp. 3	PT15
CCF-44E	E	59	F	1/25/2008	*A. baumannii*	PT61a
CCF-109E	E	41	F	6/17/2008	*A. baumannii*	PT64
CCF-60E	E	45	F	2/7/2008	*A. baumannii*	PT68
CCF-62E	E	34	F	3/1/2008	gen. sp. 13TU	PT4
CCF-112E	E	46	F	7/26/2008	gen. sp. 3	PT22
CCF-16E	E	45	F	10/18/2007	gen. sp. 3	PT25c
CCF-50E	E	50	F	1/31/2008	gen. sp. 3	PT25d
CCF-26E	E	36	F	12/11/2007	gen. sp. 3	PT39
CCF-58E	E	39	F	2/16/2008	gen. sp. 3	PT42
CCF-10E	E	35	F	9/21/2007	gen. sp. 3	PT57
CCF-99F	F	26	F	7/12/2008	*A. baumannii*	PT23
CCF-77F	F	52	M	5/5/2008	*A. baumannii*	PT24
CCF-38F	F	28	M	12/22/2007	*A. baumannii*	PT74b
CCF-130F	F	38	M	8/7/2008	gen. sp. 3	PFGE not available
CCF-14F	F	55	M	10/1/2007	gen. sp. 3	PT38
CCF-65G	G	27	F	3/10/2008	*A. baumannii*	PT20
CCF-75G	G	47	F	5/2/2008	*A. baumannii*	PT61b
CCF-47G	G	24	F	1/21/2008	*A. baumannii*	PT63a
CCF-98G	G	26	F	7/12/2008	*A. baumannii* (clone II)	PT77
CCF-131G	G	33	F	7/31/2008	gen. sp. 13TU	PT44
CCF-132G	G	33	F	7/31/2008	gen. sp. 13TU	PT44
CCF-64G	G	31	F	3/4/2008	gen. sp. 13TU	PT59
CCF-115G	G	43	F	7/21/2008	gen. sp. 3	PT25a
CCF-128G	G	42	F	8/11/2008	gen. sp. 3	PT26
CCF-18G	G	30	F	10/22/2007	gen. sp. 3	PT28

Continued on following page

TABLE 1—*Continued*

Isolate code[a]	Facility	Age of inmate	Sex[d]	Date of collection (mo/day/yr)	Species typing[b]	PFGE type[c]
CCF-21G	G	21	F	10/6/2007	gen. sp. 3	PT36
CCF-81G	G	26	F	6/2/2008	gen. sp. 3	PT43
CCF-111G	G	30	F	7/8/2008	gen. sp. 3	PT76
CCF-19H	H	71	M	11/2/2007	*A. baumannii*	PT69
CCF-27H	H	72	M	12/11/2007	*A. baumannii*	PT69
CCF-102I	I	28	M	7/7/2008	*A. baumannii*	PT24
CCF-83I	I	29	M	5/27/2008	*A. baumannii*	PT6
CCF-84I	I	29	M	5/27/2008	*A. baumannii*	PT6
CCF-37I	I	45	M	12/21/2007	*A. baumannii* (clone II)	PT7
CCF-23I	I	19	M	10/5/2007	gen. sp. 13TU	PT18
CCF-85I	I	45	M	5/23/2008	gen. sp. 3	PT51
CCF-78J	J	47	M	5/8/2008	*A. baumannii*	PFGE not available
CCF-87J	J	66	M	5/19/2008	*A. baumannii*	PT3
CCF-106J	J	29	M	6/24/2008	gen. sp. 14BJ	PT33
CCF-20J	J	34	M	11/3/2007	gen. sp. 14BJ	PT33
CCF-69K	K	36	M	3/25/2008	*A. baumannii*	PT24
CCF-76K	K	33	M	5/9/2008	*A. baumannii*	PT60
CCF-49K	K	40	M	1/19/2008	*A. baumannii*	PT63b
CCF-24K	K	44	M	11/8/2007	gen. sp. 3	PT25b
CCF-57K	K	32	M	2/19/2008	gen. sp. 3	PT45
CCF-119K	K	57	M	8/1/2008	Sequencing failed	PT25a
CCF-90L	L	38	M	5/17/2008	*A. baumannii*	PT24
CCF-79L	L	48	M	5/1/2008	*A. baumannii*	PT53a
CCF-80L	L	48	M	5/1/2008	*A. baumannii*	PT53a
CCF-136L	L	58	M	8/2/2008	*A. baumannii*	PT53b
CCF-125L	L	52	M	8/14/2008	*A. baumannii*	PT62
CCF-123L	L	49	M	8/14/2008	*A. baumannii*	PT73
CCF-103L	L	52	M	7/25/2008	gen. sp. 14BJ	PT35
CCF-95L	L	32	M	6/6/2008	gen. sp. 3	PT37
CCF-89L	L	48	M	5/17/2008	Sequencing failed	PFGE not available
CCF-82M	M	39	M	5/27/2008	*A. baumannii*	PT40a
CCF-108M	M	64	M	6/23/2008	*A. baumannii*	PT40b
CCF-121M	M	31	M	8/23/2008	*A. baumannii*	PT50
CCF-86M	M	42	M	5/20/2008	*A. baumannii*	PT78
CCF-59M	M	48	M	2/12/2008	*A. baumannii*	PT8b
CCF-70M	M	35	M	3/29/2008	*A. baumannii* (clone II)	PT5
CCF-88M	M	41	M	5/17/2008	*A. baumannii* (clone II)	PT72
CCF-93M	M	49	M	6/9/2008	*A. baumannii* (clone III)	PT79
CCF-126M	M	45	M	8/12/2008	gen. sp. 13TU	PT58
CCF-66M	M	40	M	3/18/2008	gen. sp. 3	PT14
CCF-29M	M	37	M	11/28/2007	gen. sp. 3	PT67
CCF-31M	M	45	M	11/19/2007	gen. sp. 3	PT67
CCF-30N	N	55	M	11/20/2007	*A. baumannii*	PT64
CCF-67N	N	48	M	3/21/2008	*A. baumannii*	PT74a
CCF-39O	O	55	M	12/22/2007	*A. baumannii*	PFGE not available
CCF-36O	O	47	M	12/22/2007	*A. baumannii*	PT41
CCF-53P	P	40	M	2/28/2008	*A. calcoaceticus*	PFGE not available
CCF-33Q	Q	21	M	12/14/2007	*A. baumannii*	PT24
CCF-129Q	Q	31	M	8/8/2008	*A. baumannii*	PT78
CCF-68Q	Q	45	M	3/22/2008	*A. baumannii*	PT78
CCF-97Q	Q	31	M	7/18/2008	*A. baumannii*	PT78
CCF-127R	R	23	F	8/12/2008	*A. baumannii*	PT54
CCF-71R	R	32	F	4/7/2008	*A. baumannii*	PT61a
CCF-25R	R	39	F	10/18/2007	*A. baumannii* (clone II)	PT12
CCF-118S	S	77	M	8/21/2008	*A. baumannii*	PFGE not available
CCF-114S	S	46	M	7/24/2008	*A. baumannii*	PT24

[a] For ease of tracking, each isolate code ends with a letter corresponding to the one-letter designation of the facility from which it was isolated.
[b] Species of the *Acinetobacter* isolates were identified by ITS sequencing (gen. sp. 3, genomic species 3; gen. sp. 13TU, genomic species 13TU; gen. sp. 14TU, genomic species 14TU; gen. sp. 14BJ, genomic species 14BJ) (7). European clone designations (in parentheses) were determined by trilocus multiplex PCR (63).
[c] PFGE types (PTs) were assigned to isolates with at least a six-band difference according to the rules of Tenover and colleagues (61), with subtypes being assigned to isolates of similar profiles.
[d] M, male; F, female.

mined to be *Acinetobacter* gen. sp. 13TU (Table 1). One was determined to be *A. calcoaceticus*, and one sample was determined to be *A. baylyi*. Five samples were determined most likely to be either gen. sp. 14TU or 14BJ. These particular five assignments are tentative, because only one representative reference sequence is available in GenBank. One additional isolate was found to be related to gen. sp. 14BJ with 83% identity. Two strains could not be assigned to a species due to repeated

failures to obtain ITS sequences. Trilocus multiplex PCR analysis showed that the majority of the *A. baumannii* isolates could not be assigned to one of the three European clonal groups. While only 3 isolates and 1 isolate of the 71 *A. baumannii* isolates were identified as strains of European clones I and III, respectively, 15 *A. baumannii* isolates were found to belong to European clone II (Table 1).

Antimicrobial susceptibility testing. To determine the antimicrobial susceptibility of these *Acinetobacter* CCF clinical isolates, MICs were determined for a panel of 18 antibiotics against 123 clinical isolates obtained from 20 CCFs (data not shown). Based on the available breakpoint values of the antibiotics from the CLSI (except for tigecycline, for which the U.S. FDA tigecycline susceptibility breakpoints listed for *Enterobacteriaceae* were applied to *Acinetobacter* spp.) (77), susceptibility rankings for these 18 antibiotics were assigned to the 123 CCF clinical isolates according to the MICs against each of these isolates (see Fig. S1 in the supplemental material). Our results indicated that the five least effective antibiotic drugs (i.e., yielding the largest number of isolates with nonsusceptibility) are ceftriaxone (71%), cefotaxime (64%), piperacillin (48%), tetracycline (36%), and ceftazidime (36%) (see Fig. S1). In contrast, antibiotics still maintaining high potency against the majority of the CCF isolates (i.e., isolates for which the MICs were within susceptible breakpoint ranges) are polymyxin B (100%), tigecycline (94%), minocycline (93%), amikacin (90%), and meropenem (89%) (see Fig. S1). Because the CCF clinical isolates are arranged according to the institutional codes (A to S), it is apparent that the majority of multidrug-resistant (with resistant phenotypes to three or more classes of antibiotics) phenotypes were exhibited by clinical isolates obtained from four correctional facilities (A, C, M, and Q) (see Fig. S1). Additionally, MDR clinical isolates accounted for only 20% of the CCF isolates and were found in only 6 of the 20 institutions (see Fig. S1 in the supplemental material). Among these six institutions, for four institutions more than 40% of their clinical isolates were multidrug resistant (see Fig. S1): institutions A (61%), C (56%), M (42%), and Q (75%). These MDR isolates are 100% resistant to ciprofloxacin, and they all belong to *A. baumannii* species (Table 1; also see Fig. S1). Furthermore, there were 27 isolates (22%) that were susceptible to all 18 antibiotics tested (see Fig. S1). It is interesting that several tetracycline analogs, such as doxycycline, minocycline, and tigecycline (one of the newest antibiotics on the market), still possess potent activity against these CCF clinical isolates to some extent (see Fig. S1). In particular, tigecycline was active against 94% of the CCF isolates, minocycline was active against 92% of the isolates, and doxycycline was active against 85% of the isolates. Finally, our results show that between 74 and 79% of the CCF clinical isolates were susceptible to the fluoroquinolones (FQs) (see Fig. S1).

Mechanism of FQ resistance. It has been well accepted that the primary mechanism of resistance to FQs was due to point mutations in genes encoding the GyrA and ParC subunits of DNA gyrase and DNA topoisomerase IV, respectively. To confirm if this is the case for the CCF isolates, the QRDRs of the *gyrA* and *parC* genes of all 123 CCF isolates were amplified via PCR and sequenced. The PCR-amplified DNA products for the QRDRs of the *gyrA* and *parC* genes were shown to be consistent with the respective lengths of the amplicons (data not shown). Sequencing summary results indicated that while the majority of FQ-susceptible CCF isolates contained no mutations in the QRDR regions (data not shown), the CCF isolates exhibiting resistant phenotypes to all three FQs (24/24) possess point mutations that result in amino acid substitutions in both GyrA and ParC polypeptides (Table 2). These 24 FQ-resistant isolates all had common serine-to-leucine conversions (GyrA, Ser-83 to Leu-83; and ParC, Ser-80 to Leu-80) except for one isolate, CCF-55A, in which ParC Ser-80 was converted to a phenylalanine (Table 2). These results are consistent with the findings that mutations in both GyrA and ParC are required for high-level FQ resistance phenotypes (72). In addition, among the few CCF isolates that were resistant to ciprofloxacin but not to gatifloxacin or levofloxacin, only three isolates had substitutions in GyrA but not in ParC: CCF-75G, possessing an uncommon point mutation that converted the glycine at position 81 (Gly-81) in GyrA to a cysteine, and CCF-19H and CCF-27H, with *gyrA* mutations resulting in Ser-83-to-Leu-83 conversions (Table 2). No additional mutations on other hot spots of *gyrA* and *parC* QRDRs were observed (data not shown).

Detection of class 1 integrons. To examine the role of class 1 integrons and their associated antibiotic resistance gene cassettes in antibiotic resistance phenotypes in CCF *Acinetobacter* isolates, we screened the 123 CCF isolates for the presence of class 1 integrons by the amplification of the integrase gene using PCR. Our results indicated that the class 1 integrase gene was detected in only 15 out of the 123 CCF isolates (12%) (Table 2). Subsequent conserved segment PCR amplification and DNA sequencing identified antibiotic resistance gene cassettes for 14 of the 15 integrase-positive isolates (Table 2). Specifically, the class 1 integrase gene and/or gene cassettes were identified only from multidrug-resistant CCF isolates obtained from four institutions (A, C, M, and Q) (Table 2; also see Fig. S1 in the supplemental material). Interestingly, the presence of at least two aminoglycoside resistance genes [*aacA4*, *aac*(*6′*)*-Im*, *aadA1*, *aadA2*, and/or *aadB*, encoding a variety of aminoglycoside modification enzymes] within the integron gene cassettes was associated with resistance phenotypes against two or more drugs (amikacin, gentamicin, and tobramycin) of this class (Table 2). Gene *aacA4* encodes aminoglycoside 6′-*N*-acetyltransferase, which confers resistance to amikacin and tobramycin, among others (55, 59). The presence of this gene is tightly associated with amikacin resistance (Table 2). On the other hand, aminoglycoside 2′-*O*-adenylyltransferase, encoded by *aadB*, confers gentamicin and tobramycin resistance but not resistance to amikacin (55). All five CCF isolates containing this gene (CCF-86M, CCF-93M, CCF-68Q, CCF-97Q, and CCF-129Q) were found to be resistant to gentamicin but not to amikacin, possibly due to the absence of the *aacA4* gene (Table 2). The presence of aminoglycoside modification enzyme genes at least partially explained the resistance phenotypes of the 14 CCF isolates to amikacin, tobramycin, and/or gentamicin (Table 2). Interestingly, CCF-55A was found to contain an *aadA1* gene disrupted by an insertion sequence (IS26).

Effects of efflux pump inhibitors. One of the mechanisms of antibiotic resistance is via the activities of efflux pumps located on the cell envelope that remove antibiotics from the cytoplasm. With the aid of efflux pump inhibitors, the contribution

TABLE 2. GyrA and ParC amino acid substitutions and detection of class 1 integrons in select *Acinetobacter* isolates obtained from correctional facilities[a]

Isolate code	Species typing	FQ breakpoint			Change in GyrA amino acid:			Change in ParC amino acid:			Aminoglycoside breakpoint			Class 1 integron detection	
		CIP	GAT	LVX	Gly-81 (GGT)	Ser-83 (TCA)	Ala-84 (GCT)	Glu-87 (GAA)	Ser-80 (TCG)	Glu-84 (GAA)	AMK	GEN	TOB	Presence of integrase	Cassette organization
CCF-34A	*A. baumannii* (clone II)	R	R	R	–	Leu (TTA)	–	–	Leu (TTG)	–	S	S	S	–	
CCF-40A	*A. baumannii* (clone I)	R	R	R	–	Leu (TTA)	–	–	Leu (TTG)	–	R	R	R	+	*aacA4-catB8-aadA1*
CCF-43A	*A. baumannii*	R	R	R	–	Leu (TTA)	–	–	Leu (TTG)	–	S	S	S	+	
CCF-45A	*A. baumannii* (clone II)	R	R	R	–	Leu (TTA)	–	–	Leu (TTG)	–	R	R	R	+	*aacA4-catB8-aadA1*
CCF-46A	*A. baumannii* (clone II)	R	R	R	–	Leu (TTA)	–	–	Leu (TTG)	–	R	R	R	+	*aacA4-catB8-aadA1*
CCF-55A	*A. baumannii*	R	R	R	–	Leu (TTA)	–	–	Phe (TTT)	–	I	S	R	+	*aac(6′)-Im-(aadA1/IS26/aadA1)*
CCF-56A	*A. baumannii* (clone I)	R	R	R	–	Leu (TTA)	–	–	Leu (TTG)	–	R	R	R	–	
CCF-63A	*A. baumannii* (clone I)	R	R	R	–	Leu (TTA)	–	–	Leu (TTG)	–	R	R	R	+	*aacA4-catB8-aadA1*
CCF-92A	*A. baumannii*	R	R	R	–	Leu (TTA)	–	–	Leu (TTG)	–	S	S	S	–	
CCF-96A	gen. sp. 3	R	I	I	–	–	–	–	–	–	S	S	S	–	
CCF-104A	*A. baumannii*	R	R	R	–	Leu (TTA)	–	–	Leu (TTG)	–	S	S	S	–	
CCF-120A	gen. sp. 14BJ	S	S	S	–	–	–	–	Leu (TTG)	–	S	S	S	–	
CCF-08C	*A. baumannii* (clone II)	R	R	R	–	Leu (TTA)	–	–	Leu (TTG)	–	R	R	R	+	*aacA4-catB8-aadA1*
CCF-09C	*A. baumannii* (clone II)	R	R	R	–	Leu (TTA)	–	–	Leu (TTG)	–	R	R	R	+	*aacA4-catB8-aadA1*
CCF-54C	*A. baumannii* (clone II)	R	R	R	–	Leu (TTA)	–	–	Leu (TTG)	–	R	R	R	+	*aacA4-catB8-aadA1*
CCF-61C	*A. baumannii* (clone II)	R	R	R	–	Leu (TTA)	–	–	Leu (TTG)	–	R	R	R	+	*aacA4-catB8-aadA1*
CCF-94C	*A. baumannii* (clone II)	R	R	R	–	Leu (TTA)	–	–	Leu (TTG)	–	R	R	R	–	
CCF-75G	*A. baumannii*	R	S	S	Cys	–	–	–	–	–	S	S	S	–	
CCF-19H	*A. baumannii*	R	S	S	–	Leu (TTA)	–	–	–	–	S	I	I	–	
CCF-27H	*A. baumannii*	R	I	S	–	Leu (TTA)	–	–	–	–	S	S	S	–	
CCF-37I	*A. baumannii* (clone II)	R	R	R	–	Leu (TTA)	–	–	Leu (TTG)	–	S	S	S	–	
CCF-102I	*A. baumannii*	I	S	S	–	–	–	–	–	–	S	S	S	–	
CCF-20J	gen. sp. 14BJ	S	S	S	–	–	–	–	Leu (TTG)	–	S	S	S	–	
CCF-59M	*A. baumannii*	R	R	R	–	Leu (TTA)	–	–	Leu (TTG)	–	R	R	R	–	
CCF-70M	*A. baumannii* (clone II)	R	R	R	–	Leu (TTA)	–	–	Leu (TTG)	–	S	R	S	–	
CCF-82M	*A. baumannii*	R	R	R	–	Leu (TTA)	–	–	Leu (TTG)	–	S	S	S	–	
CCF-86M	*A. baumannii*	R	R	R	–	Leu (TTA)	–	–	Leu (TTG)	–	S	R	R	+	*aadB-aadA2*
CCF-93M	*A. baumannii* (clone II)	R	R	R	–	Leu (TTA)	–	–	Leu (TTG)	–	S	R	R	+	*aadB-aadA2*
CCF-108M	*A. baumannii*	R	R	R	–	Leu (TTA)	–	–	Leu (TTG)	–	S	S	S	–	
CCF-121M	*A. baumannii*	R	S	S	–	–	–	–	–	–	S	R	I	–	
CCF-68Q	*A. baumannii*	R	R	R	–	Leu (TTA)	–	–	Leu (TTG)	–	S	R	R	+	*aadB-aadA2*
CCF-97Q	*A. baumannii*	R	I	I	–	Leu (TTA)	–	–	Leu (TTG)	–	S	R	R	+	*aadB-aadA2*
CCF-129Q	*A. baumannii*	R	R	R	–	Leu (TTA)	–	–	Leu (TTG)	–	S	R	R	+	*aadB-aadA2*

[a] Shown here are only isolates intermediate or resistant to relevant antibiotics, mutant genotype in the *gyrA* or *parC* QRDR, or positive with class 1 integrase PCR; all other isolates had identical susceptible phenotypes, were wild type in the *gyrA* or *parC* QRDR, and were negative in class 1 integrase PCR. –, No change from the wild type. Wild-type amino acids and codons are from published sequences (GenBank accession no. X82165 for *gyrA* and GenBank accession no. X95819 for *parC*). Isolates were designated susceptible (S), intermediate (I), or resistant (R) according to antibiotic breakpoint guidelines of the CLSI (10) for *Acinetobacter* spp. Antibiotic abbreviations: AMK, amikacin; CIP, ciprofloxacin; GAT, gatifloxacin; GEN, gentamicin; LVX, levofloxacin; and TOB, tobramycin.

TABLE 3. Effects of efflux pump inhibitors on susceptibility of FQs and tigecycline

Isolate	MIC (μg/ml)[a]											
	CIP	CIP-NMP	CIP-PAβN	GAT	GAT-NMP	GAT-PAβN	LVX	LVX-NMP	LVX-PAβN	TIG	TIG-NMP	TIG-PAβN
CCF-34A	>256	128 (>2×)	64 (>4×)	16	16	8 (2×)	32	16 (2×)	8 (4×)			
CCF-40A	>256	128 (>2×)	64 (>4×)	256	16 (16×)	8 (32×)	32	16 (2×)	8 (4×)			
CCF-43A	128	32 (4×)	32 (4×)	16	8 (2×)	8 (2×)	16	4 (4×)	4 (4×)			
CCF-45A	256	128 (2×)	64 (4×)	16	16	16	16	16	8 (2×)			
CCF-46A	128	64 (2×)	64 (2×)	16	8 (2×)	4 (4×)	16	8 (2×)	8 (2×)			
CCF-55A	64	4 (16×)	8 (8×)	32	2 (16×)	2 (16×)	64	4 (16×)	2 (32×)			
CCF-56A	>256	64 (>4×)	64 (>4×)	>256	16 (>16×)	8 (>32×)	>256	16 (>16×)	8 (>32×)	8	4 (2×)	4 (2×)
CCF-63A	>256	64 (>4×)	64 (>4×)	128	16 (8×)	16 (8×)	64	16 (4×)	8 (8×)	8	4 (2×)	4 (2×)
CCF-104A	128	16 (8×)	32 (4×)	16	4 (4×)	8 (2×)	8	4 (2×)	8			
CCF-8C	>256	128 (>2×)	128 (>2×)	128	32 (4×)	16 (8×)	128	32 (4×)	16 (8×)	16	8 (2×)	4 (4×)
CCF-9C	>256	128 (>2×)	128 (>2×)	128	32 (4×)	16 (8×)	128	32 (4×)	16 (8×)	16	8 (2×)	8 (2×)
CCF-54C	>256	256	128 (>2×)	256	64 (4×)	16 (16×)	128	64 (2×)	16 (8×)	16	16	8 (2×)
CCF-59M	128	128	64 (2×)	32	16 (2×)	8 (4×)	32	16 (2×)	8 (4×)			
CCF-70M	256	64 (4×)	32 (8×)	>256	16 (>16×)	8 (>32×)	>256	16 (>16×)	4 (>64×)			
CCF-93M	128	64 (2×)	64 (2×)	8	8	8	16	16	8 (2×)			
CCF-108M	>256	64 (>4×)	128 (>2×)	16	8 (2×)	16	16	8 (2×)	8 (2×)			

[a] Values in parentheses are reductions (*n*-fold) in the MIC after the addition of the indicated efflux pump inhibitors. Antibiotic abbreviations: CIP, ciprofloxacin; GAT, gatifloxacin; TIG, tigecycline; LVX, levofloxacin; NMP, 1-(1-naphthylmethyl)-piperazine; PAβN, phe-arg-β-naphthylamide dihydrochloride.

of efflux pumps to the antibiotic resistance phenotypes can be studied. Here, two efflux pump inhibitors, PAβN and NMP, were added to the susceptibility testing of three FQs and tigecycline to compare MICs in the presence and absence of either inhibitor. Our results with select multidrug-resistant CCF isolates suggested that efflux pumps influenced by both inhibitors contributed to the FQ resistance of CCF-55A, CCF-56A, and CCF-70M (up to a 64× reduction in MIC in the presence of the efflux pump inhibitor), while an efflux pump(s) sensitive to PAβN slightly increased the resistance of CCF-63A, CCF-8C, and CCF-9C to gatifloxacin and levofloxacin (with moderate fold changes) (Table 3). With only five CCF isolates exhibiting phenotypes of low resistance to tigecycline (MIC, 8 or 16 μg/ml), the contribution of efflux pumps that can be inhibited by these two efflux pump inhibitors was not evident (Table 3).

Genetic profiling of genomic DNA. The dendrogram generated from PFGE profiles of 123 CCF isolates indicated that there was a large number of *Acinetobacter* isolates with diverse genetic profiles (see Fig. S2 in the supplemental material). This was expected based on the large number of correctional facilities (19) from which these isolates were obtained. A closer analysis revealed several groups of CCF isolates with identical or closely related PFGE profiles. For ease of description and discussion, based on the rules of Tenover and colleagues (61) CCF isolates with more than six different bands were assigned distinct (major) PFGE types (PTs), and isolates with similar PFGE profiles (fewer than six different bands) were subtypes of a major PT. Consequently, a total of 79 major PTs were assigned, and among these 79 PTs, 17 contain more than 1 isolate of either identical or similar PFGE profiles (see Fig. S2). For example, CCF-40A, CCF-56A, and CCF-63A of institution A appeared to be closely related (with two to three bands being different), thus these isolates were classified as subtypes (PT30a, PT30b, and PT30c) of PT30 (see Fig. S2). Since these three isolates all belong to European clone I of *A. baumannii* and were obtained from 47-year-old inmates, these three strains most likely were isolated from the same individual about a couple of months apart (Table 1). Additionally, there were five isolates from institution C (CCF-8C, CCF-9C, CCF-54C, CCF-61C, and CCF-94C) sharing identical or closely related PFGE profiles and were assigned subtypes of PT10 (see Fig. S2). Interestingly, CCF-8C and CCF-9C most likely were obtained from an inmate of 34 years of age on two separate days in September 2007, while the other three (CCF-54C, CCF-61C, and CCF-94C) most probably originated from an inmate of 35 years of age on three separate days in 2008 (7 February, 26 February, and 2 June 2008, respectively) (Table 1). Because these five isolates belong to European clone II of *A. baumannii* (Table 1), it is quite possible (but impossible to confirm without access to the clinical records) that they were obtained from the same individual who turned 35 after September 2007. Further evidence supporting this claim came from identical or closely related susceptibility profiles of these five isolates (see Fig. S1 in the supplemental material) as well as most of the *gyrA* and *parC* gene mutation and class 1 integron results (Table 2). Similarly, several additional pairs of isolates with identical PFGE profiles (within each pair) obtained from the same institutions appeared to come from single individuals: CCF-131G and CCF-132G (PT44), CCF-83I and CCF-84I (PT6), CCF-79L and CCF-80L (PT53), CCF-97Q and CCF-129Q (PT78), and CCF-19H and CCF-27H (PT69) (Table 1; also see Fig. S2 in the supplemental material). These pairs of isolates also were typed to the same species by molecular typing methods (Table 1).

What has been the most worrisome problem with *Acinetobacter* spp. in a nosocomial setting is their clonal dissemination (or worse, clonal outbreak). Although the multidrug resistance prevalence is lower in CCFs than in hospitals in southern California (67) or elsewhere (14, 23, 25, 66), our results did reveal a few incidents of clonal dissemination within correctional institutions. For example, CCF-43A and CCF-92A share identical PFGE profiles (see Fig. S2 in the supplemental material) but were obtained from inmates of apparently different ages in institution A (Table 1). In addition, intrainstitutional clonal dissemination may have occurred in institutions J (CCF-106J and CCF-20J), L (CCF-79L and CCF-136L), K (CCF-

24K and CCF-119K), Q (CCF-68Q and CCF-97Q), and M (CCF-29M and CCF-31M; CCF-82M and CCF-108M) (see Fig. S2). Except for CCF-43A, CCF-92A, CCF-68Q, and CCF-97Q, all of the others involved in clonal dissemination were not multidrug resistant (see Fig. S1 in the supplemental material). Unexpectedly, our results also have revealed that isolates with identical PFGE profiles were obtained from different institutions: CCF-96A and CCF-29M (and CCF-31M) (gen. sp. 3), CCF-44E and CCF-71R (both *A. baumannii*), CCF-82M and CCF-43A (both *A. baumannii*), and CCF-97Q and CCF-86M (both *A. baumannii*) (see Fig. S2 in the supplemental material). The biggest surprise, which is difficult to explain, was that seven isolates of *A. baumannii* species (CCF-102I, CCF-33Q, CCF-69K, CCF-77F, CCF-114S, CCF-135B, and CCF-90L) from seven different correctional facilities were found to share identical PFGE profiles (PT24) (see Fig. S2 in the supplemental material) as well as nearly identical susceptibility profiles (mostly susceptible but some intermediate) (see Fig. S1 in the supplemental material). All of these seven CCF isolates cannot be assigned to any of the three prominent *A. baumannii* European clonal groups (clone I, II, or III) (Table 1).

DISCUSSION

There have been a number of reports characterizing antimicrobial resistance and molecular features of bacterial pathogens in correction facilities. The limited number of reports published, however, focused primarily on MRSA, followed by *Mycobacteria tuberculosis* and *Chlamydia* (4, 12, 13, 40–42, 62). In this report, the antimicrobial susceptibility of 123 CCF *Acinetobacter* isolates against 18 antibiotics was determined, followed by a series of experiments to elucidate the mechanisms of resistance in FQs, aminoglycosides, and tigecycline in these CCF isolates. Additionally, PFGE analysis and trilocus multiplex PCR were performed to determine clonal relationships of the isolates. To our knowledge, this study is the first report of the phenotypic and molecular characterization of *Acinetobacter* isolates from any correctional facility. In a case-control study to investigate an MRSA outbreak in a Missouri correctional facility, it was found that the improper care of infected skin lesions by inmates, poor personal hygiene by inmates, and close contact in confined spaces are the main risk factors for MRSA infection in this facility (62). Although there was no evidence of an *A. baumannii* outbreak in CCFs in our study, clonal dissemination among inmates was observed in a number of facilities, probably as a result of poor personal hygiene among inmates and close contact in confined spaces. Lowy and colleagues conducted a survey to determine the prevalence of nasal colonization with *S. aureus* in two New York state prisons and compared the colonized strains to clinical isolates obtained from the same correctional facilities (40). Their results indicated that the colonization rate was 25.5%, with 10.5% of the colonized isolates being MRSA. In contrast, clinical isolates from inmates had an MRSA prevalence rate of 48.3% (40). In our study, however, the MDR prevalence rate for *Acinetobacter* clinical isolates from all CCFs combined was 21%, and all MDR isolates belong to *A. baumannii*. Although this rate is lower than that of MRSA described by Lowy et al. (40) and of MDR *A. baumannii* in hospitals in general (14, 25), the MDR prevalence rates among isolates in four facilities where most of the MDR strains were obtained were much higher, ranging from 42 to 75%. This should be a cause for alarm: if the MDR *A. baumannii* isolates are spread more widely in correctional facilities, they could cause outbreaks due to the nature of these institutions, which are crowded and confined and in which inmates are prone to bodily injuries because of fighting among inmates. Furthermore, we find it intriguing and difficult to interpret that seven CCF *A. baumannii* isolates not related to the three European clonal groups (I, II, and II) from seven different CCFs share identical PFGE profiles as well as nearly identical susceptibility patterns. Perhaps this clonal strain colonized many inmates in a facility, and isolates of the clone were disseminated to other facilities via inmate transfers. Alternatively, this clone could have been disseminated to the CCFs when inmate patients receive medical care from local hospitals.

Although no report has been published that describes *Acinetobacter* spp. in a prison, there have been increased reports of nursing home-associated or community-acquired *Acinetobacter* infections (16, 34, 46, 47, 58). For instance, Sengstock and coworkers identified MDR *A. baumannii* as an emerging pathogen among older adults in nursing homes (58), institutions that are distinct from hospitals. During the 6-year study period, it was found that 560 community-dwelling and 280 nursing home-dwelling older adult patients admitted to the community hospitals had *Acinetobacter* isolated within 2 days of admission (58). More alarmingly, during the 6-year period *Acinetobacter* prevalence increased 25%, and their resistance to imipenem and ampicillin-sulbactam increased from 1.8 to 13.6% (58). Additionally, Ong et al. found that eight patients in Singapore were diagnosed with community-acquired pneumonia due to infections by *A. baumannii* (46).

We found that most (92.7%) of the CCF *Acinetobacter* isolates belong to the three most clinically relevant species: *A. baumannii*, *Acinetobacter* gen. sp. 3, and *Acinetobacter* gen. sp. 13TU, with 58.5% being *A. baumannii*. These three species of *Acinetobacter* also have been isolated from patients in hospitals around the world, where the dominant species also was *A. baumannii* (9, 30, 64, 69, 75). For example, Chuang et al. showed that 64.4, 26.7, and 8.9% of the *Acinetobacter* spp. were *A. baumannii*, *Acinetobacter* gen. sp. 3, and *Acinetobacter* gen. sp. 13TU, respectively, and patients with *A. baumannii* bacteremia were more likely to have pneumonia than were patients with bacteremia due to gen. sp. 13TU (9). In another study, a gen. sp. 13TU isolate was identified to have caused an outbreak in a hospital in The Netherlands (68). On the other hand, a recent study (28) by Karah et al. of *Acinetobacter* spp. from Norway indicated an unusual prevalence of the top three *Acinetobacter* spp. These researchers found that both gen. sp. 13TU (46.9%) and gen. sp. 3 (19.5%) were more prevalent than *A. baumannii* (8.8%) (28). They also found that none of the *A. baumannii* isolates belonged to European clone I, II, or III (28).

Of the 71 *Acinetobacter baumannii* isolates studied, only 19 isolates (26.8%) were found to belong to European clone I, II, or III. The majority (15/19) of these isolates belonged to European clone II, but this still represented only 21.1% of the total number of *A. baumannii* isolates. This differs from other international studies of *A. baumannii* isolates (15, 21). For example, among their sample of 492 *A. baumannii* clinical isolates from hospitals of various countries (including the

United States), Higgins and coworkers found that isolates belonging to the three European clones represented 62.6% of the total number of *A. baumannii* isolates analyzed (21), which is much higher than the 26.8% found in this study. Similarly, Di Popolo and coworkers reported recently that 57.1% of the 35 representative MDR *A. baumannii* isolates obtained from 28 outbreaks across Mediterranean hospitals were found to belong to one of the three European clones (15). The noted difference in prevalence of European clones between hospital-derived *A. baumannii* isolates and correctional facility-derived isolates (in this study) reflects the different types of institutions involved. This is consistent with the limited clonal dissemination and low percentage of MDR *A. baumannii* isolates in most of the CCFs. On the individual clone level, Higgins and coworkers found that European clone II was the largest (49.0%) and the most widespread epidemiological group (found in the United States and across Europe, Israel, Asia, Australia, and South Africa) (21). Additionally, these investigators also found five other epidemiological groups of *A. baumannii* (outside European clones I, II, and III), with the top two (WW4 and WW5) representing 16.3 and 5.5% of the total isolate population (21). These two groups of *A. baumannii* isolates also were widespread. Isolates of WW4 came from Asia, Europe, and South America, while those belonging to WW5 were obtained from North and South America and Europe (21). Furthermore, clusters of *A. baumannii* isolates other than European clones I, II, and III also were identified from Mediterranean countries by Di Popolo and colleagues (15). These studies further revealed the complex population genetics of nosocomial *A. baumannii* isolates that have been involved in hospital infections and outbreaks (15). Additional investigations are needed to determine if the *A. baumannii* CCF isolates not belonging to European clones I, II, and III are genetically linked to those new epidemiological groups found in hospitals.

Resistance to FQs in Gram-negative bacteria primarily results from point mutations in *gyrA* and/or *parC* gene sequences that render significantly lower drug affinity for DNA gyrase (containing mutated GyrA) and DNA topoisomerase (composed of mutated ParC) (1, 57, 65). In *A. baumannii*, the most common amino acid substitutions occur at position 83 of GyrA and position 80 of ParC (31, 72, 73, 76). Additionally, while changes in GyrA sequence are necessary for moderate levels of FQ resistance, additional changes in ParC sequence are required to confer high levels of resistance to FQs (67, 72). As is consistent with the previous observations, our results showed that most of the FQ-susceptible CCF clinical isolates do not harbor mutations in the *gyrA* and *parC* genes, and high-level FQ-resistant CCF clinical isolates (those resistant to all three FQs tested) all possess double mutations in both *gyrA* and *parC* genes. While nosocomial clinical isolates obtained from Los Angeles County hospitals were found to have a variety of ParC amino acid substitutions at positions 80 and 84 (Ser-80 to Leu-80, Ser-80 to Phe-80, or Glu-84 to Lys-84), the FQ-resistant CCF clinical isolates predominately had Ser-80-to-Leu-80 conversions in ParC, with a single case of Ser-80 to Phe-80 among a total of 24 cases. Interestingly, a rare GyrA amino acid substitution, Gly-81 to Cys-81, was found in one CCF clinical isolate (CCF-75G), which was observed previously in FQ-resistant *A. baumannii* (19).

Integrons contribute significantly to the emergence and dissemination of antibiotic resistance genes in Gram-negative bacteria due to their ability to integrate/excise gene cassettes into or out of themselves via site-specific recombination using self-coded integrases (50, 54, 56). In particular, class 1 integrons have been found in clinical isolates of *A. baumannii* all over the world (1, 18, 33, 37, 39, 43, 60). We have detected the presence of class 1 integrons in 15 CCF clinical isolates, all of which are multidrug resistant. From 14 out of these 15 isolates, we were able to amplify associated resistance gene cassettes via PCR and sequenced the genes involved. The organizations of class 1 integron-associated resistance gene cassettes include primarily two types: *aacA4-catB8-aadA1* and *aadB-aadA2*. The first type has been widely distributed among *A. baumannii* clinical isolates worldwide; it was found, for example, in 26 clinical isolates of MDR *A. baumannii* obtained in central Ohio (60), in 35 clinical isolates of MDR *A. baumannii* from Taiwan (37), and in 29 clinical isolates of *A. baumannii* from China (18). The second type was extremely rare in the literature (60). Both types of gene cassettes contain either the *aadA1* or *aadA2* gene, which encodes variants of adenylyl transferases, conferring resistance to spectinomycin and streptomycin (55), older aminoglycoside antibiotics not included in our study. However, we predict that these isolates are resistant to spectinomycin and streptomycin. One CCF isolate (CCF-55A) contained an IS26-disrupted, apparently nonfunctional *aadA1* gene cassette downstream of the *aac(6′)-Im* gene. DNA sequence analysis revealed, surprisingly, that these gene cassettes (including the IS26 sequence) shares 99% sequence homology with an atypical integron harbored by 19 out of 26 imipenem-resistant outbreak strains of *A. baumannii* in Korea (20).

Efflux pumps have been recognized as a mechanism of antibiotic resistance in Gram-negative bacteria (45, 48, 71). Recently, efflux pump inhibitors have been employed to probe the contribution of efflux pumps to *A. baumannii* isolates' resistance to FQs (11, 49, 67) and tigecycline (51). For example, Coban and coworkers examined the effect of NMP on antibiotic drug susceptibility in 42 clinical isolates of *A. baumannii* (11). The presence of NMP restored ciprofloxacin susceptibility in 15 intermediate and 2 resistant isolates (11). We evaluated the contribution of efflux pumps that can be inhibited by the two efflux pump inhibitors (NMP and PAβN) to the susceptibility of FQs in a select group of CCF clinical isolates. Especially noteworthy was the observation that an efflux pump(s) inhibited by both NMP and PAβN contribute partially to the resistance to all three FQs tested in three particular *A. baumannii* isolates (CCF-55A, CCF-56A, and CCF-70M). The presence of the efflux pump inhibitors restored only one isolate's (CCF-55A) susceptibility to two FQs (gatifloxacin and levofloxacin). This probably was due to the high levels of FQ resistance exhibited by these CCF isolates for which *gyrA* and *parC* are still the major resistance determinants. In contrast, the influence of efflux pumps (at least those inhibited by NMP and PAβN) was not evident (2- to 4-fold change in MIC) in five CCF isolates found to be moderately resistant to tigecycline (MIC, 8 or 16 μg/ml). The ranges of tigecycline MICs for five CCF tigecycline-resistant isolates are similar to those for two clinical isolates described by Hornsey and colleagues recently (22). Hornsey et al. determined that the tigecycline-resistant phenotype (MIC, 8 and 16 μg/ml) in two posttherapy clinical isolates was correlated with the elevated expression of

adeABC (22). Since *adeABC* genes encode an RND (resistance-nodulation-division) type of efflux pump on which at least PAβN was shown to be a potent inhibitor (49), it is difficult to explain the failure of both inhibitors to restore CCF isolates' tigecycline sensitivity to the susceptible range in our study. Perhaps additional genetic determinants (such as other efflux pumps) are involved.

In conclusion, in the first report examining antimicrobial susceptibility and molecular characteristics of *Acinetobacter* clinical isolates from prisons in the United States, we found, with much relief, only a limited clonal dissemination of mostly *A. baumannii* isolates in just 6 of 20 correctional facilities. Most of these isolates (except for two each from institutions A and Q) are not multidrug resistant. Although the number of isolates studied in each institution was limited, we did find high rates of MDR isolates in a few facilities. Due to the possibility that these MDR isolates will spread out of control and cause outbreaks in prisons, our findings did raise alarm. Additionally, our molecular analyses of antibiotic resistance determinants in the CCF isolates correlate well with their phenotypic characteristics. Taken together, the findings of this report provide baseline knowledge of the current antimicrobial susceptibility, species composition and diversity, dissemination patterns, and antibiotic resistance mechanisms of a population of *A. baumannii* clinical isolates in correctional facilities, a unique type of institution that is unlike either hospitals or free-moving communities.

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