Sequential Outbreaks of Infections by Distinct *Acinetobacter baumannii* Strains in a Public Teaching Hospital in Houston, Texas[▽]

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Invasive disease due to *Acinetobacter baumannii* is an increasing problem in health care settings worldwide. Whether certain clones of *A. baumannii* are more likely to cause invasive disease in hospitalized patients is unknown. We studied all patients at a public teaching hospital in Houston, Texas, from whom the *Acinetobacter calcoaceticus*-*Acinetobacter baumannii* complex was isolated over a 14-month period in 2005 to 2006. One hundred seven unique patient isolates were identified, with 87 of the strains classified as being *A. baumannii*, the majority of which were multidrug resistant. The *A. baumannii* isolates were comprised of 18 unique pulsed-field types, with strains of clone A and clone B accounting for 66 of the 87 isolates. Epidemiologic analysis showed the predominance of the two *A. baumannii* clones at distinct time periods, with the remainder of the *A. baumannii* and non-*A. baumannii* strains being evenly distributed. Patients from whom clone A strains were isolated were more likely to be bacteremic than were patients with other *A. baumannii* isolates. Conversely, clone B strains were more likely to be isolated from patients with tertiary peritonitis. Patients from whom clone A was isolated had a significantly higher rate of mortality. Multilocus sequence typing demonstrated that clones A and B are related to each other and to *A. baumannii* strains previously isolated in Western Europe, sharing five of seven alleles. Taken together, we conclude that the outbreak of the *A. calcoaceticus*-*A. baumannii* complex in our institution was due to two distinct *A. baumannii* clones that were associated with significantly different patient outcomes.

Infections due to antimicrobial-resistant organisms are a major public health issue (10, 14, 35). *Acinetobacter baumannii* is a gram-negative bacterium that is increasingly being identified as a significant cause of serious antibiotic-resistant nosocomial infections, especially in the intensive care unit (ICU) setting (12, 15, 31, 34). Moreover, the presence of multidrug-resistant *A. baumannii* infections in personnel returning from the war in Iraq has drawn additional attention to this organism (4, 7, 17, 27, 30).

Our understanding of the clinical characteristics of *A. baumannii* infection and the molecular basis of its pathogenesis is hampered by several issues (2, 12). First, standard laboratory techniques do not reliably distinguish *A. baumannii* from other species of the *A. calcoaceticus*-*A. baumannii* complex (26). The *A. calcoaceticus*-*A. baumannii* complex consists of the phenotypically similar but genetically diverse *A. calcoaceticus* and *A. baumannii* strains, *Acinetobacter* genomic species 3, and *Acinetobacter* genomic species 13TU (33). Thus, studies that do not use molecular techniques to distinguish *A. baumannii* from related *Acinetobacter* species may generate inaccurate data regarding clinical epidemiology and disease outcomes. Second, *A. baumannii* often colonizes rather than infects patients,

thereby obscuring the true significance of organism isolation (25). Finally, given that patients are often critically ill at the time of infection with *A. baumannii*, whether *A. baumannii* infection actually increases patient mortality has been debated (11, 18, 20). These difficulties have left major gaps in our understanding of the epidemiology and clinical relevance of *A. baumannii* infections, which in turn limits the effectiveness of preventive and therapeutic strategies.

To date, the virulence of specific *A. baumannii* strains has been attributed mainly to the presence or absence of drug resistance elements (12, 20). However, factors other than drug resistance are likely to be involved in the pathogenesis of *A. baumannii* infection in a fashion similar to those of other bacteria (6). To this end, we investigated a recent *A. calcoaceticus*-*A. baumannii* complex outbreak at a public teaching hospital in Houston, Texas. We used a combination of epidemiologic and molecular techniques, including the newly described *A. baumannii* multilocus sequence typing (MLST), to investigate whether patient acquisition of a particular *A. calcoaceticus*-*A. baumannii* complex clone resulted in a difference in clinical outcome. The outbreak investigation showed that *A. baumannii* clone A strains, which are related to invasive strains previously isolated in Europe by MLST, caused high rates of bacteremia and were associated with increased patient mortality (1).

MATERIALS AND METHODS

Hospital description and infection control practices. The study was conducted at Ben Taub General Hospital, a 588-bed teaching facility that is the primary public hospital in Houston. There are three separate adult ICUs at Ben Taub

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General Hospital: (i) a 30-bed open unit and six-isolation-room surgical ICU, (ii) a 14-bed open unit and two-isolation-room neurosurgical ICU, and (iii) a 16-bed individual-room medical ICU located two floors above the surgical and neurosurgical ICUs. Before a perceived increase in serious *A. calcoaceticus*-*A. baumannii* complex infections in the winter of 2004 to 2005, standard infection control interventions included infection control audits and education, alcohol gel dispensers, and cohorting and contact isolation procedures for patients with multidrug-resistant isolates. Additional responses to the outbreak included increasing nursing and house staff education, intensive environmental cleaning, dedicated equipment for patients with multidrug-resistant isolates, and environmental sampling.

Sample and data collection. To gain insight into whether an outbreak was occurring, we compared the annual numbers of *A. calcoaceticus*-*A. baumannii* complex isolates from 1995 to 2004. Having determined that an outbreak was occurring, we sought to characterize the molecular epidemiology of the outbreak by saving all *A. calcoaceticus*-*A. baumannii* complex isolates beginning on 1 May 2005. This report details isolates collected between 1 May 2005 and 30 June 2006. The date of first isolation of an *A. calcoaceticus*-*A. baumannii* isolate and the isolate's antibiotic resistance profile were recorded for all patients. Subsequent reviews of the patients' medical records were utilized to extract epidemiologic data and clinical information including age, sex, hospital location, duration of ICU and hospital stay, date and type of antimicrobial therapy received, mechanical ventilation, renal replacement therapy, date of insertion and duration of central catheters, surgical intervention, and laboratory data. The severity of patient illness was calculated using the acute physiology and chronic health evaluation II (APACHE II) score at the time of admission (21). Data for calculating the Glasgow coma score were not consistently available retrospectively; therefore, the APACHE II score was modified to exclude the Glasgow coma score. A cluster of patients was defined as patients located within three beds of one another from whom an *A. calcoaceticus*-*A. baumannii* isolate with identical drug susceptibility patterns was isolated. The study was performed under a protocol approved by the Baylor College of Medicine Institutional Review Board.

Infection definitions. Infection was defined as the isolation of an *A. calcoaceticus*-*A. baumannii* complex isolate from a normally sterile site with signs and symptoms compatible with infection or as noted below. Criteria for defining catheter-related bloodstream infection were not consistently available (i.e., quantitative cultures from a catheter segment, simultaneous quantitative blood cultures from the catheter and peripheral blood, or differential period to positivity of catheter versus peripheral cultures), so we identified catheter-associated bloodstream infections according to Centers for Disease Control and Prevention guidelines: a primary bloodstream infection (i.e., the isolate was not related to an infection present at another site) that occurred in a patient with a central catheter that was in use in the 48 h prior to the development of the bacteremia (24). Ventilator-associated pneumonia was diagnosed using standard definitions, i.e., a patient on mechanical ventilation in whom a new or progressive pulmonary infiltrate developed on chest radiography, and when the organism was isolated from a lower respiratory tract sample or from either pleural fluid or blood (3). Tertiary peritonitis was defined as the isolation of an *A. calcoaceticus*-*A. baumannii* complex isolate from peritoneal fluid >48 h after treatment for either primary or secondary peritonitis (3). Colonization was defined as the isolation of an *A. calcoaceticus*-*A. baumannii* complex isolate from at least one clinical specimen in the absence of clinical symptoms consistent with infection.

Species-level identification and susceptibility testing. Organisms were identified as belonging to the *A. calcoaceticus*-*A. baumannii* complex by using standard microbiologic methods. Antimicrobial susceptibility was determined by broth automated microdilution testing and/or Kirby-Bauer disk diffusion testing. Organisms were defined as multidrug resistant if there was resistance to two or more of the following: expanded-spectrum cephalosporins or extended-spectrum β -lactams, quinolones, carbapenems, trimethoprim-sulfamethoxazole, or aminoglycosides. Species-level identification of organisms in the *A. calcoaceticus*-*A. baumannii* complex was performed using amplified ribosomal DNA restriction analysis (8).

Genotyping. Genotyping of all organisms identified as being *A. baumannii* isolates was performed using digestion with *Apal* followed by pulsed-field gel electrophoresis (PFGE) according to previously reported standardized techniques (28). Isolates were assigned to clonal groups according to the criteria described previously by Tenover et al. (29). For each of the major clonal types of *A. baumannii* identified by PFGE, five strains from unique patients that were not geographically or temporally related were selected for MLST, which was performed as previously described (1; <http://pubmlst.org/abaumannii/>).

Statistical analysis. Statistical analysis was performed using the NCSS statistical package, 2004 version (NCSS Statistical Software). A Student's *t* test was

used for analysis of continuous data with normal distribution (e.g., age), whereas the Wilcoxon rank sum test was used for analysis of nonparametric data (e.g., time from ICU admission to organism isolation). Categorical data were analyzed using the χ^2 test. Stepwise logistic regression was employed for multivariate analysis of variables found to have a *P* value of ≤ 0.20 by univariate analysis. All test results with a two-sided *P* value of < 0.05 were considered to be significant.

RESULTS

Outbreak description. From 1995 to 2003, the total number of *A. calcoaceticus*-*A. baumannii* isolates in the ICUs was, on average, 55 ± 12 . Without significant changes in ICU patient load or institution of new surveillance practices, the total number of *A. calcoaceticus*-*A. baumannii* complex isolates from the ICUs increased to 177 in 2004 and 193 in 2005. In response to a perceived increase in severe *A. calcoaceticus*-*A. baumannii* complex infections in the surgical ICU in the winter of 2004 to 2005, a hospital-wide prospective collection of *A. calcoaceticus*-*A. baumannii* complex isolates was begun on 1 May 2005. Over the 14 months of active surveillance, we identified 107 unique patients from whom *A. calcoaceticus*-*A. baumannii* complex isolates were isolated (Table 1). Ninety-nine patients (93%) met the definition of nosocomial acquisition. Ninety-five patients (89%) had an *A. calcoaceticus*-*A. baumannii* complex isolate initially isolated while in the ICU or within 48 h of leaving the ICU. The breakdown by ICU was as follows: 56 patients (52%) in the surgical ICU, 25 patients (23%) in the medical ICU, and 14 patients (13%) in the neurosurgical ICU. The number of isolates peaked during the summer of 2005, declined in the winter of 2005 to 2006, and then increased again as the summer of 2006 began (Fig. 1A). Increased environmental cleaning and patient cohorting began in spring 2005 and was reemphasized in the fall of 2005, with a subsequent decrease in *A. calcoaceticus*-*A. baumannii* complex isolation that was not sustained (Fig. 1A). Environmental sampling in the surgical ICU resulted in the detection of multidrug-resistant *A. calcoaceticus*-*A. baumannii* complex isolates on two separate occasions (Fig. 1A). Temporal and geographical clusterings were noted on 15 occasions, with 12 cases occurring in the open-ward surgical ICU. When clusterings were noted, intensive environmental cleaning and focused health care worker education were undertaken.

Cohort characteristics. Sixty-three patients (58%) met the definition for *A. calcoaceticus*-*A. baumannii* infection, with a breakdown as follows: 30 patients with catheter-associated bloodstream infection, 25 patients with ventilator-associated pneumonia, 7 patients with tertiary peritonitis, and one patient with brain abscess (Table 2). Overall, 43 patients (40.1%) were bacteremic due to either a catheter or another focus, usually pneumonia. The patients who were deemed to be colonized by *A. calcoaceticus*-*A. baumannii* had isolates obtained from non-sterile sites (e.g., sputum, urine, and surgical wounds) without objective evidence of infection. We note two unusual aspects of our cohort. First, more than 40% of our patients had bloodstream infection, which is higher than the typical 5 to 10% reported previously for other *A. calcoaceticus*-*A. baumannii* complex cohorts (25, 31). Moreover, the recovery of *A. calcoaceticus*-*A. baumannii* complex isolates from an intra-abdominal abscess, as occurred in seven of our patients, is distinctly unusual (16).

TABLE 1. Comparison of *A. calcoaceticus*-*A. baumannii* complex-colonized and -infected patients^a

Patient characteristic	Value for group:		
	Colonized (<i>n</i> = 44)	Infected (<i>n</i> = 63)	Total (<i>n</i> = 107)
Mean age (yr) (SD)	47.8 (18.3)	44.7 (15.2)	46.0 (16.8)
No. of males (%)	34 (77.3)	44 (69.8)	78 (75.7)
Race [no. (%)]			
African-American	17 (38.7)	25 (39.7)	42 (39.2)
White, non-Hispanic	9 (20.5)	16 (25.4)	25 (23.3)
Hispanic, any race	16 (30.4)	18 (28.6)	34 (31.7)
Asian	2 (4.5)	4 (6.3)	6 (5.6)
Hospital acquired [no. (%)]	37 (84.1)	62 (98.4)	99 (92.5) ^b
In ICU when organism was isolated [no. (%)]	33 (75.0)	62 (98.4)	95 (88.8) ^b
Mean time from ICU admission to isolate isolation (days) (SD)	15.3 (10.7)	18.7 (13.0)	17.4 (12.3)
Mechanically ventilated when organism isolated [no. (%)]	24 (54.5)	47 (74.6)	71 (66.4) ^b
Surgical procedure during hospitalization [no. (%)]	31 (70.5)	55 (87.3)	86 (80.4) ^b
Central bloodstream catheter present when organism isolated [no. (%)]	28 (63.6)	48 (76.2)	76 (71.0)
Renal replacement [no. (%)]	11 (25.0)	20 (31.7)	31 (28.9)
Received expanded-spectrum cephalosporin 30 days prior to initial organism isolation [no. (%)]	26 (59.1)	54 (85.7)	80 (74.8) ^b
Received quinolone 30 days prior to initial organism isolation [no. (%)]	9 (20.5)	25 (39.7)	34 (31.8) ^b
Mean modified APACHE II score (SD)	12.4 (7.7)	14.2 (8.5)	13.5 (8.2)
Isolation of multidrug-resistant organism [no. (%)]	25 (29.8)	59 (93.7)	84 (78.5) ^b
Died during hospitalization [no. (%)]	6 (13.6)	23 (36.5)	29 (25.2) ^b

^a Characteristics of infected versus colonized patients were compared with a Student's *t* test or Wilcoxon rank sum test for continuous variables and with χ^2 tests for categorical variables.

^b *P* < 0.05 for the colonized versus infected patients.

Comparison of infected versus colonized patients. Patients may or may not develop invasive disease following initial colonization with *A. calcoaceticus*-*A. baumannii* complex isolates, possibly depending on coexisting illnesses or perhaps due to characteristics of the *A. calcoaceticus*-*A. baumannii* complex isolate (25). We investigated the possibility that sicker patients were more likely to develop infection by comparing modified APACHE II scores at the time of hospital admission for colonized versus infected patients (Table 1). Interestingly, we found no statistical difference in the initial modified APACHE II scores among infected versus colonized patients (12.4 ± 7.7 for colonized patients versus 14.2 ± 8.5 for infected patients; *P* = 0.30). Although the baseline APACHE II scores were not significantly different, the mortality rate for patients infected with *A. calcoaceticus*-*A. baumannii* was significantly higher than that for colonized patients (36.5% versus 13.6%; *P* = 0.009). The initial modified APACHE II levels were significantly higher in patients who subsequently died than in patients who did not die, confirming that APACHE II scores in our cohort did provide prognostic information (17.2 ± 6.9

versus 12.1 ± 8.5 , respectively; *P* = 0.002). Given the similar clinical characteristics of the colonized versus infected patients, these data suggest that variability in the virulence of the *A. calcoaceticus*-*A. baumannii* complex isolates may have contributed to the development of invasive disease.

Characteristics of *A. calcoaceticus*-*A. baumannii* complex isolates. Standard automated microbiology techniques can assign organisms to the *A. calcoaceticus*-*A. baumannii* complex only, which comprises at least four distinct species (20). Using amplified ribosomal DNA restriction analysis, we found that 87 of the 107 *A. calcoaceticus*-*A. baumannii* complex isolates (81%) were *A. baumannii* (33). The remaining 20 isolates were either *Acinetobacter* genomic species 3 (15 isolates), *Acinetobacter* genomic species 13 (three isolates), or not identified (two isolates). Sixty-one of the 63 cases of infection were due to *A. baumannii* isolates, whereas 18 of the 44 colonizing isolates were non-*A. baumannii* strains (*P* < 0.001). Moreover, more than 90% of the *A. baumannii* isolates were multidrug resistant, compared to 15% of the non-*A. baumannii* isolates (Table 2) (*P* < 0.001). When the outbreak epidemiologic data were

FIG. 1. Temporal distribution of *Acinetobacter calcoaceticus*-*A. baumannii* complex isolates. (A) Number of *A. calcoaceticus*-*A. baumannii* isolates by month. Arrows refer to times of increased infection control initiatives as described in Materials and Methods. Stars refer to time of environmental sampling of the surgical ICU for *A. calcoaceticus*-*A. baumannii* isolates. (B) Number of *A. calcoaceticus*-*A. baumannii* isolates by month broken down by species identification as noted in the legend. (C) Number of *A. baumannii* isolates broken down by PFGE type as noted in the legend.

stratified by species, the variability in the rates of *A. calcoaceticus*-*A. baumannii* isolation was found to be due to *A. baumannii*; non-*A. baumannii* strain isolation rates remained steady (Fig. 1B). These data support previous observations that *A. baumannii* isolates cause the majority of serious infections among isolates of the *A. calcoaceticus*-*A. baumannii* complex and are more likely to be multidrug resistant than other *A. calcoaceticus*-*A. baumannii* complex species (2, 19, 22).

Genetic characterization of *A. baumannii* isolates. Next, we used PFGE to determine genetic relatednesses among the *A. baumannii* strains in our cohort (28). Of the 87 strains studied, there were 18 distinct pulsed-field types. We identified two major clonal types, with clone A comprising 44 strains and clone B consisting of 20 strains (Fig. 2). The remaining 23 strains belonged to one of 16 clonal types or were nonresolvable by PFGE (four strains). Over the 14 months of the study, the two major clones showed distinct temporal trends, with clone A predominating for several months before gradually being replaced by clone B (Fig. 1C). The other *A. baumannii*

TABLE 2. Antimicrobial susceptibility patterns of *Acinetobacter calcoaceticus*-*A. baumannii* complex isolates

Antibiotic	No. (%) of sensitive isolates	
	<i>A. baumannii</i> (n = 87)	Non- <i>A. baumannii</i> (n = 20)
Imipenem	71 (81.6)	20 (100)
Meropenem	16 (18.3)	18 (90.0)
Ampicillin-sulbactam	79 (90.1)	20 (100)
Ticarcillin-clavulanate	13 (14.9)	18 (90.0)
Ceftriaxone	12 (13.7)	17 (85.0)
Ceftazidime	15 (17.2)	18 (90.0)
Cefepime	23 (26.4)	19 (95.0)
Levofloxacin	11 (12.6)	18 (90.0)
Ciprofloxacin	10 (11.4)	18 (90.0)
Trimethoprim-sulfamethoxazole	8 (9.2)	17 (85.0)
Gentamicin	32 (42.5)	19 (95.0)
Tobramycin	56 (64.3)	19 (95.0)
Amikacin	24 (27.5)	18 (90.0)

isolates (not clone A or B) were evenly distributed over the 14-month period (Fig. 1C). Clone A was identified in all adult ICUs (25 isolates in the surgical ICU, 13 isolates in the medical ICU, and 6 isolates in the neurosurgical ICU), whereas clone B isolates were found predominantly in the surgical ICU (17 of 20 isolates). Of the 15 clusters of patients with *A. calcoaceticus*-*A. baumannii* isolates, 7 were due to clone A strains and 5 were due to clone B strains. The isolates in the remaining three clusters had no clear genetic relationship. Given that the majority of *A. baumannii* isolates were multidrug resistant, we could discern no statistically significant differences in the percentages of strains that were multidrug resistant for a particular clonal type. Although all clone A and clone B strains were resistant to amikacin, clone A strains were universally resistant to gentamicin, whereas 19 of 20 clone B strains were gentamicin susceptible, suggesting differing mechanisms of aminoglycoside resistance between the two major clones (23). We conclude that in our cohort, there were two major multidrug-resistant clones that made up the majority of the *A. baumannii* isolates.

Site specificity and virulence of the two major *A. baumannii* clones. In light of the large numbers of cases of bacteremia in

FIG. 2. Representative PFGE profiles of *A. baumannii* isolates digested with *Apa*I. Lanes 1 to 4 contain clone A strains. Lanes 5 and 6 contain clone B strains. Lanes 7 and 8 contain *A. baumannii* strains other than clone A or B.

TABLE 3. Comparison of patients with and those without *A. baumannii* clone A^a

Patient characteristic	Value for patients:		P value
	With clone A (n = 44)	Without clone A (n = 43)	
Mean age (yr) (SD)	43.0 (14.9)	48.9 (16.6)	0.087
No. of men (%)	30 (68.2)	31 (72.1)	0.691
Race [no. (%)]			
African-American	18 (40.9)	18 (41.9)	0.971
White, non-Hispanic	11 (25.0)	9 (20.9)	
Hispanic, any race	12 (27.3)	13 (30.2)	
Asian	3 (6.8)	3 (7.0)	
Hospital acquired [no. (%)]	39 (90.7)	44 (100)	0.052
In ICU when organism was isolated [no. (%)]	35 (81.4)	40 (90.9)	0.198
ICU specialty [no. (%)]			0.137
Surgical	25 (56.8)	28 (56.0)	
Medical	13 (29.5)	5 (11.6)	
Neurosurgical	6 (13.6)	3 (7.0)	
Mean time from ICU admission to isolate isolation (days) (SD)	17.8 (9.9)	14.7 (10.4)	0.114
Mechanically ventilated at time of initial organism isolation [no. (%)]	33 (62.8)	27 (75.0)	0.210
Central bloodstream catheter present at time of initial organism isolation [no. (%)]	35 (81.4)	32 (72.7)	0.337
Surgical procedure prior to organism isolation [no. (%)]	38 (86.4)	34 (79.1)	0.361
Received expanded-spectrum cephalosporin 30 days prior to initial organism isolation [no. (%)]	39 (88.6)	31 (72.1)	0.051
Mean modified APACHE II score (SD)	13.3 (7.0)	13.9 (9.1)	0.780
Bloodstream infection [no. (%)]	37 (84.1)	7 (15.9)	<0.001
Died during hospitalization [no. (%)]	20 (45.5)	8 (18.6)	0.007

^a Characteristics of infected versus colonized patients were compared with a Student's *t* test or Wilcoxon rank sum test for continuous variables and with χ^2 tests for categorical variables.

our cohort, we next investigated whether a particular *A. baumannii* clone was responsible for a disproportionate number of bacteremias. Indeed, 36 of the 43 cases (83.7%) of bacteremia were caused by clone A, although clone A accounted for only 41% of all *A. calcoaceticus*-*A. baumannii* isolates and 50% of *A. baumannii* isolates ($P < 0.001$ for clone A causing a disproportionate number of bacteremias). When looked at in a different manner, 36 of the 44 patients (81.8%) who had clone A isolates developed bacteremia, compared to only 7 patients (15.9%) who had other *A. baumannii* strains isolated. To evaluate whether clone A was found preferentially in patients at high risk of developing bacteremia, we compared the clinical characteristics of patients from whom clone A was isolated to those of patients from whom other *A. baumannii* strains were isolated (Table 3). No statistically significant differences in measured clinical criteria including modified APACHE II scores upon admission to the ICU and the presence or duration of use of an indwelling central venous catheter were observed between the two groups of patients (Table 3). However, the mortality rate for patients from whom clone A was isolated

was 45.5%, compared with 18.6% for patients from whom other *A. baumannii* strains were isolated ($P = 0.007$).

In addition to the large numbers cases of bloodstream infection, our cohort was also characterized by a number of intra-abdominal abscesses. Although clone A accounted for the vast majority of bloodstream infections in our cohort, there was only one case of intra-abdominal abscess involving this particular clone. The remaining intra-abdominal infections were all caused by clone B. Although clone B accounted for only 22% of the *A. baumannii* isolates, it caused 85% of the intra-abdominal abscesses in this cohort ($P < 0.001$). In contrast, clone B accounted for only 7% of the bacteremias due to *A. baumannii* strains and was not associated with increased mortality compared to other *A. baumannii* strains ($P = 0.910$). Taken together, we conclude that clone A caused a disproportionate number of bloodstream infections and was associated with increased mortality, whereas clone B caused a high percentage of intra-abdominal abscesses and was not associated with increased mortality. These data suggest both a site-specific predispo-

TABLE 4. Multilocus sequence typing of *A. baumannii* isolates^a

Strain	PFGE type	ST	Allele no. (closest match)						
			<i>gltA</i>	<i>gyrB</i>	<i>gdhB</i>	<i>recA</i>	<i>cpn60</i>	<i>gpi</i>	<i>rpoD</i>
SAB1	A	Novel	1	12	3	2	2	Novel (11)	3
SAB11	A	Novel	1	12	3	2	2	Novel (11)	3
SAB12	A	Novel	1	12	3	2	2	Novel (11)	3
SAB133	A	Novel	1	12	3	2	2	Novel (11)	3
SAB157	A	Novel	1	12	3	2	2	Novel (11)	3
SAB117	B	Novel	1	Novel (2)	3	2	2	Novel (4)	3
SAB125	B	Novel	1	Novel (2)	3	2	2	Novel (4)	3
SAB128	B	Novel	1	Novel (2)	3	2	2	Novel (4)	3
SAB172	B	Novel	1	Novel (2)	3	2	2	Novel (4)	3
SAB198	B	Novel	1	Novel (2)	3	2	2	Novel (4)	3
SAB49	F	Novel	1	Novel (4)	12	1	1	Novel (7)	3

^a Sequences of amplified genes were compared with sequences at the *A. baumannii* MLST website (<http://pubmlst.org/abaumannii/>). ST, sequence type.

sition for the two clonal lineages and a difference in virulence.

Multivariate analysis of mortality associated with clone A.

To determine whether the isolation of clone A was independently associated with increased mortality, we performed a multivariate analysis using logistic regression for all patients from whom *A. baumannii* strains were isolated. The variables entered into the model included all covariates associated with mortality at a *P* value of 0.20 or less in univariate analysis, which included isolation of clone A, modified APACHE II score, renal replacement therapy, and presence of a central bloodstream catheter. In the multivariate model, the odds ratio of death after acquisition of clone A was 3.31 (95% confidence interval, 1.27, 9.15; *P* = 0.024). Therefore, we conclude that isolation of clone A was associated with increased mortality independent of other risk factors, suggesting increased virulence compared with those of other *A. baumannii* strains isolated in this cohort.

MLST. We next determined whether strains identified as belonging to clone A by PFGE would have the same MLST profile by analyzing five clone A isolates from unique patients. Each of the five clone A strains had the same MLST profile (Table 4). We also performed MLST on five clone B isolates from unique patients and found that all five clone B isolates had the same MLST profile, which was distinct from that of clone A (Table 4). The clone A and clone B strains each shared five alleles (*gltA*, *gdhB*, *recA*, *cpn60*, and *rpoD*) but were distinct at the *gyrB* locus (4-bp difference) and the *gpi* locus (11-bp difference). In contrast, an *A. baumannii* PFGE type F strain shared *gltA* and *rpoD* alleles with clone A and clone B but had distinct *gyrB*, *gdhB*, *recA*, *cpn60*, and *gpi* alleles (Table 4). A comparison of clone A and clone B data to published MLST data found that each strain shares five alleles (*gltA*, *gdhB*, *recA*, *cpn60*, and *rpoD*) with sequence types 6, 10, and 19, which include invasive *A. baumannii* isolates from Spain and Germany (1). Therefore, we conclude that clone A and clone B are related to each other and to certain *A. baumannii* strains previously isolated in Europe. Finally, we performed MLST on *A. baumannii* strains isolated from environmental samples and found that clone A was isolated in December 2005, whereas clone B was isolated in May 2006 (Fig. 1A). These data demonstrate that the presence of the two *A. baumannii* clones in

the environmental samples was temporally associated with their isolation from patients.

DISCUSSION

In this cohort of all patients from whom the *A. calcoaceticus*-*A. baumannii* complex was isolated over a 14-month period, we found that the isolation of a specific *A. baumannii* clone (clone A) was associated with a high level of bacteremia and increased mortality compared to other strains of *A. baumannii* and to strains of the broader *A. calcoaceticus*-*A. baumannii* complex. We were unable to discern patient-related factors that explained these findings, suggesting that some intrinsic property of clone A itself may be responsible. Although significant efforts were made to control for confounding variables, it is important to note that there are limitations inherent in the retrospective design and single-center nature of our study. Our data have parallels with data from a recent report noting an increased rate of bacteremia for a particular methicillin-resistant *Staphylococcus aureus* clone and, as such, add to our understanding of serious nosocomial infections by multidrug-resistant bacteria (9).

At present, colonization with *A. baumannii* in a susceptible host is thought to be the major risk factor for the subsequent development of invasive disease (12, 20). However, there is tremendous genetic diversity among *A. baumannii* strains, and it seems reasonable to speculate that the development of invasive disease following colonization is not the same for all strains (13). Indeed, one of the key findings of our investigation was that clone A caused a disproportionate number of bacteremias compared to other clones of *A. baumannii*. Whereas clone A comprised about 50% of the *A. baumannii* isolates, it accounted for 84% of the bloodstream infections. Our data suggest that clone A may possess some inherent property, such as an ability to produce biofilm or to adhere to epithelial cells, that provides it with a capacity to produce bacteremia.

An unexpected finding of our study was the presence of *A. baumannii* strains in abdominal abscesses. Reviews of descriptive studies of *A. baumannii* colonization/infection show that the majority of isolates are recovered from sputum, urine, blood, wounds, and catheters (2, 20, 25). Most previously reported cases of peritonitis involving *A. baumannii* have involved patients receiving chronic ambulatory peritoneal dialysis (32). However, one study found that multidrug-resistant *A. baumannii* isolates could be recovered from the stool of patients in the ICU (5). As our patients with abdominal abscesses had gastrointestinal tract disruption secondary to various processes, it does not seem unexpected that *A. baumannii* might be recovered from patients with nosocomial peritonitis. Importantly, however, we found that all but one of the patients with an intra-abdominal abscess were infected with *A. baumannii* clone B. Although we cannot rule out the possibility that the finding of clone B in abdominal abscesses was related to its prevalence in the surgical ICU, we note that of the 36 patients in the surgical ICU that had *A. baumannii* strains other than clone B isolated, only 1 had an isolate from an abdominal abscess. This finding suggests that clone B may have a particular predilection for the gastrointestinal tract in a fashion similar to that of clone A, which seems predisposed to caused bloodstream infections.

Our data highlight the need for microbiology laboratories to provide clinicians and infection control practitioners with more discriminative information than reporting an isolate as belonging to the *A. calcoaceticus*-*A. baumannii* complex. Given the vastly different outcomes between patients infected with *A. baumannii* and those infected with non-*A. baumannii* strains, practitioners need to know what *A. calcoaceticus*-*A. baumannii* complex species has been isolated and perhaps be provided with molecular data regarding *A. baumannii* strains. Without molecular investigation, we would not have discovered that our two peaks of infection were actually caused by the dissemination of two genetically distinct *A. baumannii* strains (Fig. 1). MLST provides researchers with a tool for geographically diverse investigations and also allows the rapid molecular identification of strains in individual patients. Such information may help clinicians with therapeutic decisions and infection control departments with outbreak containment strategies.

In conclusion, we found that a particular clone of *A. baumannii* was significantly associated with the development of bacteremia and adverse patient outcomes compared to other *A. baumannii* strains and non-*A. baumannii* strains belonging to the *A. calcoaceticus*-*A. baumannii* complex. We present the first MLST data for *A. baumannii* strains isolated in North America and demonstrate that the dominant clones of *A. baumannii* at our facility are related to strains previously isolated in Western Europe. Our findings provide a framework for a more detailed investigation of *A. baumannii* pathogenesis and improved understanding of *A. baumannii* epidemiology.

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