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Clinical Outcomes of Hospital-Acquired Infection with *Acinetobacter nosocomialis* and *Acinetobacter pittii*

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The role of *Acinetobacter nosocomialis* and *Acinetobacter pittii*, which belong to the *A. calcoaceticus-A. baumannii* complex, in hospital-acquired infections is increasingly recognized. Here we describe a retrospective cohort study of hospital-acquired *A. calcoaceticus-A. baumannii* complex infections at a university hospital in Thailand. A total of 222 unique cases were identified between January 2010 and December 2011. The genomospecies of the *A. calcoaceticus-A. baumannii* complex isolates were classified as follows: *A. baumannii*, 197 (89%); *A. nosocomialis*, 18 (8%); and *A. pittii*, 7 (3%). All *A. nosocomialis* and *A. pittii* isolates were susceptible to imipenem and meropenem. The patients infected with *A. nosocomialis* and *A. pittii* had lower 30-day mortality than those infected with carbapenem-susceptible *A. baumannii* ($P = 0.025$) and carbapenem-resistant *A. baumannii* ($P = 0.013$). The factors influencing 30-day mortality were infection with non-*baumannii A. calcoaceticus-A. baumannii* complex (hazard ratio [HR], 0.12; 95% confidence interval [CI], 0.03 to 0.51; $P = 0.004$), infection with carbapenem-resistant *A. baumannii* (HR, 1.57; 95% CI, 0.89 to 2.79; $P = 0.105$), appropriate empirical antimicrobial therapy (HR, 0.38; 95% CI, 0.23 to 0.61; $P < 0.001$), and higher acute physiology and chronic health evaluation II (APACHE II) score (HR, 1.15; 95% CI, 1.10 to 1.19; $P < 0.001$). In *Galleria mellonella* assays, the survival rates were significantly higher for the larvae infected with *A. nosocomialis* or *A. pittii* than for those infected with either carbapenem-susceptible *A. baumannii* or carbapenem-resistant *A. baumannii*, but no differences in survival rates were observed between carbapenem-susceptible *A. baumannii* and carbapenem-resistant *A. baumannii*. These findings suggest intrinsic differences in virulence between non-*baumannii A. calcoaceticus-A. baumannii* complex species and *A. baumannii* but not between carbapenem-susceptible and resistant *A. baumannii*.

Acinetobacter species have emerged as one of the high-priority hospital-acquired pathogens causing substantial mortality and economic burdens (1). Among the *Acinetobacter* genomospecies described to date, those belonging to the *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex are the *Acinetobacter* genomospecies most commonly found in clinical specimens (2). The *A. calcoaceticus-A. baumannii* complex is comprised primarily of *A. baumannii* (genomospecies 2), *Acinetobacter nosocomialis* (genomospecies 13TU), *A. calcoaceticus* (genomospecies 1), and *Acinetobacter pittii* (genomospecies 3) (2, 3). Within the *A. calcoaceticus-A. baumannii* complex, *A. baumannii* is considered the species most clinically relevant and most frequently resistant to multiple classes of antimicrobial agents, whereas *A. nosocomialis* and *A. pittii* are also regarded as clinically relevant species with a geographically dependent incidence that is lower than that of *A. baumannii* but is likely increasing (4–6). However, the genomospecies within the *A. calcoaceticus-A. baumannii* complex cannot be identified by routine biochemical methods (7–9). Epidemiological and clinical studies of *Acinetobacter* spp. therefore often investigate the *A. calcoaceticus-A. baumannii* complex as a single entity, which is practical but limits the ability to differentiate the clinical features of infection due to *A. baumannii* and non-*baumannii A. calcoaceticus-A. baumannii* complex isolates (3). Nonetheless, recent reports are starting to reveal the distinct clinical characteristics and outcomes of patients infected with *A. baumannii* and non-*baumannii A. calcoaceticus-A. baumannii* complex species, primarily, *A. nosocomialis* and *A. pittii* (5, 6, 10, 11).

Carbapenems are widely used to treat infections caused by multidrug-resistant (MDR) *A. baumannii*, and several studies have reported significant associations between the carbapenem susceptibility of the infecting isolates and clinical outcomes, length of hospital stay, and hospital cost (12, 13). In general, non-*baumannii A. calcoaceticus-A. baumannii* complex isolates are more susceptible than *A. baumannii* isolates to carbapenems as well as to other classes of agents, including fluoroquinolones, aminoglycosides, and ampicillin-sulbactam (14). Carbapenem-resistant non-*baumannii A. calcoaceticus-A. baumannii* infections have been reported in intensive care units and tertiary care hospitals but appear to be relatively uncommon in comparison with carbapenem-resistant *A. baumannii* infections (5, 6, 10, 11).

Patients in hospitals in Thailand have suffered from a high incidence of MDR *A. baumannii* infections (15). However, data regarding the epidemiological and clinical role of non-*baumannii A. calcoaceticus-A. baumannii* complex species in this region of high endemicity have not been fully elucidated. To address this issue, we conducted a retrospective study to compare the clinical characteristics of non-*baumannii A. calcoaceticus-A. baumannii* complex infections and *A. baumannii* infections and to determine the impact of genomospecies on the clinical outcome with refer-

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ence to carbapenem susceptibility, empirical antimicrobial selection, and severity of infection. We then aimed to corroborate the clinical outcomes using a *Galleria mellonella* infection model of *A. calcoaceticus-A. baumannii* complex species.

MATERIALS AND METHODS

Ethics. This study was approved by the Institutional Review Board (IRB) of the Faculty of Medicine, Prince of Songkla University (EC: 54-080-14-1-2). The authorized researchers were granted the right to extract the data from the database with waiver of consent because of the retrospective nature of the study. The laboratory work was conducted under an IRB approval from the University of Pittsburgh (PRO12060302).

Patients. The study was conducted in Songklanagarind Hospital, an 800-bed university hospital located in southern Thailand. Adult (age ≥ 18 years) patients who were admitted between 1 January 2010 and 31 December 2011 and had an infection with an *A. calcoaceticus-A. baumannii* complex isolate were included in the study. The infection status was identified from the hospital microbiology database and confirmed to be hospital acquired in nature using the diagnostic criteria of the Centers for Disease Control and Prevention (CDC) (16). Pneumonia was diagnosed with the following criteria: new or progressive infiltration on chest radiographic examination and microbiological criteria (positive quantitative cultures of airway specimens yielding $\geq 10^5$ CFU). Those patients deemed to be colonized without infection were excluded. For urinary tract infection, at least two clinical parameters (fever ≥ 38°C, urgency or frequency of urination, dysuria, or suprapubic tenderness) had to be present, with urine culture yielding $\geq 10^5$ CFU/ml of *A. calcoaceticus-A. baumannii* complex species with no more than two species of microorganisms. Patients with skin- and soft-tissue infection, including surgical-site infection, were defined with clinical criteria (purulent drainages or abscess with at least one of the following symptoms: pain or tenderness, localized swelling, redness, or heat at the affected site) and positive culture from aseptically obtained fluid or tissue. Patients with intra-abdominal infection were defined by the presence of purulent drainage or abscess, or evidence of infection found at the time of surgical or radiological intervention, with *A. calcoaceticus-A. baumannii* complex species isolated from aseptically obtained culture or fluid from the affected organ or peritoneal space. Only the first episodes of infection were included in the analysis to avoid case duplication.

Data collection. Demographic and clinical data were directly retrieved from the surveillance database of the infection control unit of Songklanagarind Hospital. Additional clinical parameters were extracted from the electronic medical record as needed. The demographic variables included age, sex, indication for admission, comorbidities, previous antimicrobial therapy, and admitted wards. We defined admission from emergency rooms without appointment as an emergency indication for admission. The comorbidities included diabetes mellitus, cardiovascular diseases, cerebrovascular diseases, chronic kidney diseases, HIV infection, and other immunocompromised status such as immunosuppressive therapy and neutropenia. Immunosuppressive therapy was defined as receiving cytotoxic agents within 6 weeks or corticosteroids at a dosage equivalent to or higher than 10 mg of prednisolone daily for more than 5 days within 4 weeks prior to the onset of CRAB infection. Neutropenia was defined as an absolute neutrophil count $< 0.5 \times 10^9$ neutrophils/liter. The extracted clinical variables included site(s) of infection, severity of infection, and antimicrobial treatment given. We used the acute physiology and chronic health evaluation II (APACHE II) scores determined within 24 h of the onset of infection to determine the severity of illness. The treatment data collected included appropriateness of empirical antimicrobial selection prior to the report of antimicrobial susceptibility and nonantimicrobial interventions, including the use of mechanical ventilation, retention of intravascular/thoracic/intra-abdominal devices, and urinary catheterization. The outcomes included in-hospital mortality, 14-day and 30-day mortality, length of hospital stay, and hospital costs. Data regarding the durations from identification of infection to hospital disposition (discharged or expired) were also collected. The lengths of hospital stay were divided into the total lengths of stay and lengths of stay after identification of infection. Hospital costs were divided into antimicrobial pharmacy costs and the remaining costs.

Identification of *A. calcoaceticus-A. baumannii* complex species. *Acinetobacter* species were presumptively identified as Gram-negative, oxidase-negative, nonmotile, nonfermenting coccobacilli and were identified as *A. calcoaceticus-A. baumannii* complex on the basis of standard biochemical reactions. Following presumptive identification, the isolates were subjected to PCR for detection of $bla_{\text{OXA-51-like}}$ genes. PCR for detection of $bla_{\text{OXA-51-like}}$ genes was performed using primers F_oxa51_001 (5′-TAA TGC TTT GAT CGG CCT TG-3′) and R_oxa51_001 (5′-TGG ATT GCA CTT CAT CTT GG-3′) (17). The isolates with a positive result for $bla_{\text{OXA-51-like}}$ genes were assigned as *A. baumannii*. The isolates with a negative result for $bla_{\text{OXA-51-like}}$ genes underwent *rpoB* gene sequencing as reported previously (18), using primers rpoB-F (5′-TAY CGY AAA GAY TTG AAA GAA G-3′) and rpoB-R (5′-CMA CAC CYT TGT TMC CRT GA-3′). Nucleotide sequence homology searches of the *rpoB* gene sequences were performed using BLAST (http://www.ncbi.nlm.nih.gov/BLAST). Sequences were aligned and compared with the published *rpoB* sequences of the *Acinetobacter* type strains (2).

Antimicrobial susceptibility testing. MICs of 11 agents, including imipenem, meropenem, colistin, ciprofloxacin, amikacin, gentamicin, ceftriaxone, cefotaxime, ceftazidime, piperacillin-tazobactam, and ampicillin-sulbactam, were tested using the broth microdilution method and interpreted according to the CLSI guidelines (19). Cefoperazone-sulbactam susceptibility was determined with the disk diffusion method according to previously described criteria (20). Tigecycline susceptibility was determined with the disk diffusion method and interpreted using the U.S. Food and Drug Administration (FDA) breakpoints for *Enterobacteriaceae* (21).

***Galleria mellonella* survival assay.** The *G. mellonella* larvae infection model for *A. baumannii* was adapted from the method described by Peleg et al. (22). *A. calcoaceticus-A. baumannii* complex clinical isolates representing carbapenem-susceptible *A. nosocomialis*, carbapenem-susceptible *A. pittii*, carbapenem-susceptible *A. baumannii*, and carbapenem-nonsusceptible *A. baumannii* from patients with and without in-hospital mortality were included. Therefore, a total of 8 clinical isolates were used for this assay. The larvae were purchased from Grubco (Fairfield, OH). The bacterial suspension was prepared in 10 mM $MgSO_4$ at approximately 1.5×10^7 CFU/ml, and 10 μl of this suspension (approximately 1.5×10^5 CFU) was injected through the last proleg of each larva. The control larvae were injected with 10 μl of 10 mM $MgSO_4$ without bacteria. The larvae were incubated in petri dishes at 37°C for 6 days and observed for survival every 24 h. The larvae were considered dead when they were unresponsive to touch. Fifteen larvae were used for each experiment in triplicate for a total of 45 larvae per isolate tested.

Statistical analysis. Clinical characteristics and outcomes of non-*baumannii A. calcoaceticus-A. baumannii* complex and *A. baumannii* infection were compared by tabulation, followed by chi-square test or Fisher's exact test as appropriate for categorical variables and Student's *t* test for continuous variables. The differences of levels of variables were expressed with odds ratio (OR) and 95% confidence interval (CI). The OR for 30-day mortality was first identified by tabulation followed by chi-square test or Fisher's exact test as appropriate. The variables with *P* values < 0.2 were included in a multivariate logistic regression model. These models were fitted to assess the effect of each characteristic, expressed as adjusted ORs. All independent variables were included in the final model. The significance level was set at 0.05. The association of each variable with the outcome was expressed with adjusted OR and 95% CI.

Survival analysis with Cox proportional hazard regression was used to assess the differences in the durations of survival after developing non-*baumannii A. calcoaceticus-A. baumannii* complex and *A. baumannii* infection. The latter was further divided into carbapenem-susceptible and nonsusceptible groups. The time started was defined as the day infection

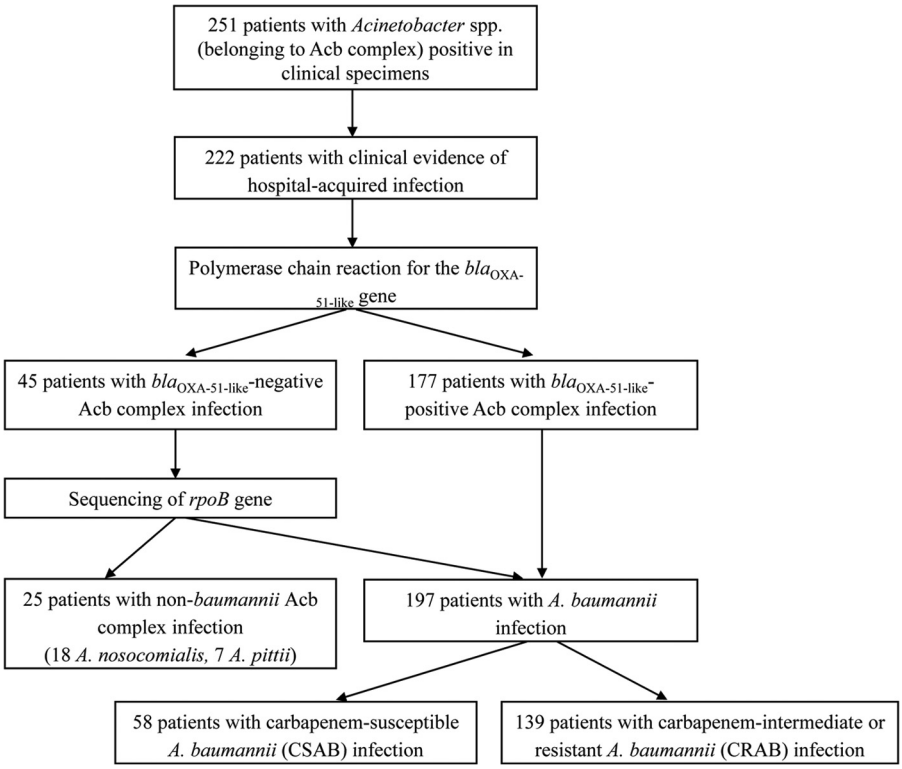

FIG 1 Flowchart of the study enrollment. A total of 25, 58, and 139 patients were included in the non-*baumannii Acinetobacter calcoaceticus-Acinetobacter baumannii* (Acb) complex group, carbapenem-susceptible *A. baumannii* (CSAB) group, and carbapenem-intermediate or -resistant *A. baumannii* (CRAB) group.

was identified. The time ended was defined as the date that the patient outcome was documented. The influences of *Acinetobacter* genomospecies, carbapenem susceptibility, APACHE II scores, and appropriate empirical antimicrobial therapy were expressed with hazard ratio (HR) and 95% CI. Student's *t* test was used to determine the differences in the number of days of survival of the larvae.

RESULTS

Genomospecies distribution. Between 1 January 2010 and 31 December 2011, 251 unique *A. calcoaceticus-A. baumannii* complex isolates were identified. Of these, 222 isolates were collected from the patients who met the criteria of hospital-acquired infection

TABLE 1 Comparisons of antimicrobial susceptibilities of non-*baumannii A. calcoaceticus-A. baumannii* complex and *A. baumannii*[c]

Antimicrobial agent(s)	No. (%) of susceptible isolates				
	Non-*baumannii A. calcoaceticus-A. baumannii* complex ($n = 25$)	Carbapenem-susceptible *A. baumannii* (CSAB; $n = 58$)	*P* value[a]	No. (%) of susceptible carbapenem-intermediate or -resistant *A. baumannii* (CRAB; $n = 139$) isolates	*P* value[b]
Imipenem	25 (100)	58 (100)	0.99	0 (0)	**<0.001**
Meropenem	25 (100)	58 (100)	0.99	0 (0)	**<0.001**
Ampicillin-sulbactam	19 (76)	19 (33)	**<0.001**	19 (13)	**<0.001**
Cefoperazone-sulbactam	23 (92)	43 (74)	0.12	16 (12)	**<0.001**
Piperacillin-tazobactam	20 (80)	35 (60)	0.14	9 (7)	**<0.001**
Gentamicin	20 (80)	32 (55)	0.06	9 (7)	**<0.001**
Amikacin	20 (80)	26 (45)	**0.007**	11 (8)	**<0.001**
Ciprofloxacin	21 (84)	31 (53)	**0.017**	5 (4)	**<0.001**
Trimethoprim-sulfamethoxazole	11 (44)	28 (48)	0.91	4 (3)	**<0.001**
Ceftriaxone	19 (76)	13 (22)	**<0.001**	3 (2)	**<0.001**
Cefotaxime	19 (76)	13 (22)	**<0.001**	3 (2)	**<0.001**
Ceftazidime	22 (88)	26 (45)	**<0.001**	5 (4)	**<0.001**
Colistin	25 (100)	58 (100)	0.68	139 (100)	0.40
Tigecycline	25 (100)	58 (100)	0.68	139 (100)	0.40

[a] Non-baumannii *A. calcoaceticus-A. baumannii* complex versus carbapenem-susceptible *A. baumannii* (CSAB).
[b] Non-baumannii *A. calcoaceticus-A. baumannii* complex versus carbapenem-intermediate or -resistant *A. baumannii* (CRAB).
[c] Boldface entries indicate values that reached the significance level set at 0.05.

TABLE 2 Comparisons of clinical features of the patients infected with non-*baumannii A. calcoaceticus-A. baumannii* complex and *A. baumannii*[e]

Parameter	Value(s) for patients infected with[a]: Non-*baumannii A. calcoaceticus-A. baumannii* complex ($n = 25$)	Carbapenem-susceptible *A. baumannii* (CSAB; $n = 58$)	*P* value[b]	Value(s) for patients infected with carbapenem-intermediate or -resistant *A. baumannii* (CRAB; $n = 139$)[a]	*P* value[c]
Demographics					
Age (yrs), median (IQR[d])	59 (46–73)	64 (48–74)	0.87	60 (45–74)	0.92
Male sex	17 (68)	42 (72)	0.87	78 (56)	0.37
Comorbidities	8 (32)	12 (23)	0.55	47 (34)	1
Clinical characteristics					
Emergency indication of admission	19 (76)	32 (55)	0.12	107 (77)	1
Initial admission (not mutually exclusive)					
Intensive care unit	8 (32)	16 (28)	0.87	54 (39)	0.67
Medical ward	10 (40)	30 (52)	0.34	64 (46)	0.81
Duration (days) from admission to infection, median (IQR)	12 (6–15)	4 (2–7)	**0.001**	15 (9–23)	**0.025**
Site(s) of infection			0.29		0.70
Bloodstream	1 (4)	2 (3)		15 (11)	
Respiratory tract	16 (64)	40 (69)		76 (55)	
Urinary tract	3 (12)	6 (10)		17 (12)	
Skin and soft tissue	2 (8)	9 (15)		20 (14)	
Intra-abdominal	2 (8)	0 (0)		5 (4)	
Two or more sites	1 (4)	1 (2)		6 (4)	
APACHE II score, median (IQR)	14 (12–17)	15 (12–20)	0.79	17 (12–22)	0.12
Invasive procedures	15 (60)	8 (15)	**<0.001**	107 (77)	0.12
Retention of medical devices	22 (88)	43 (81)	0.17	96 (69)	0.09
Previous antimicrobial therapy					
Penicillin(s)	4 (16)	5 (9)	0.42	21 (15)	1
Aminoglycoside(s)	8 (32)	19 (33)	1	49 (35)	0.93
Cephalosporin(s)	5 (20)	12 (21)	1	93 (67)	**<0.001**
Fluoroquinolone(s)	7 (28)	17 (29)	1	98 (71)	**<0.001**
Carbapenem(s)	2 (8)	2 (3)	0.58	83 (60)	**<0.001**
Appropriate empirical antimicrobial therapy	16 (64)	32 (56)	0.67	79 (57)	0.65

[a] Values represent number (percent) of patients unless otherwise indicated.
[b] Non-*baumannii A. calcoaceticus-A. baumannii* complex versus carbapenem-susceptible *A. baumannii* (CSAB).
[c] Non-*baumannii A. calcoaceticus-A. baumannii* complex versus carbapenem-intermediate or -resistant *A. baumannii* (CRAB).
[d] IQR, interquartile range.
[e] Boldface entries indicate values that reached the significance level set at 0.05.

described above. The genomospecies were as follows: *A. baumannii*, 197 (89%); *A. nosocomialis*, 18 (8%); and *A. pittii*, 7 (3%). Of 197 isolates of *A. baumannii*, 58 isolates were carbapenem-susceptible *A. baumannii* (CSAB), 10 isolates were carbapenem-intermediate *A. baumannii*, and 129 isolates were carbapenem-resistant *A. baumannii* (CRAB). As carbapenem-intermediate *A. baumannii* isolates are usually considered resistant to carbapenems in clinical practice, they are included in the CRAB group here. The non-*baumannii A. calcoaceticus-A. baumannii* complex group consisted exclusively of *A. nosocomialis* and *A. pittii*, and all of the isolates were susceptible to imipenem and meropenem. Figure 1 shows the patient enrollment and species identification data.

Antimicrobial susceptibility of non-*baumannii A. calcoaceticus-A. baumannii* complex and *A. baumannii* isolates. The antimicrobial susceptibility data for the three groups are shown in Table 1. All non-*baumannii A. calcoaceticus-A. baumannii* complex isolates were susceptible to imipenem and meropenem. They also had rates of susceptibility to all other agents tested that were significantly higher than those seen with the CRAB isolates and higher rates of susceptibility to amikacin, ciprofloxacin, ceftriaxone, cefotaxime, ceftazidime, and ampicillin-sulbactam than the CSAB isolates.

Clinical features of patients infected with non-*baumannii A. calcoaceticus-A. baumannii* complex and *A. baumannii*. Comparisons of the clinical features of patients infected with non-*baumannii A. calcoaceticus-A. baumannii* complex (*A. nosocomialis* or *A. pittii*) with those of patients infected with CSAB and CRAB are shown in Table 2. Previous therapy with fluoroquinolone, cephalosporin, and carbapenem was significantly less frequent among the patients infected with non-*baumannii A. calcoaceticus-A. baumannii* complex species than among those infected with CRAB, but no difference from those infected with CSAB was observed. In addition, the duration from admission to infection with non-*baumannii A. calcoaceticus-A. baumannii*

TABLE 3 Comparisons of outcomes for the patients infected with non-*baumannii A. calcoaceticus-A. baumannii* complex and *A. baumannii*[d]

Outcome	Values for patients infected with non-*baumannii A. calcoaceticus-A. baumannii* complex ($n = 25$)	Values for patients infected with carbapenem-susceptible *A. baumannii* (CSAB; $n = 58$)	*P* value[a]	Values for patients infected with carbapenem-intermediate or -resistant *A. baumannii* (CRAB; $n = 139$)	*P* value[b]
Mortality, no. (%) of patients					
In-hospital	3 (12)	20 (35)	0.067	79 (57)	**<0.001**
14 day	1 (4)	10 (17)	0.160	42 (30)	**<0.001**
30 day	2 (8)	20 (35)	**0.025**	78 (56)	**0.013**
Length of hospital stay after infection (days), median (IQR)	9 (3–14)	4 (1–9)	0.368	23 (12–52)	**<0.001**
Cost (baht[c]), median (IQR)					
Total hospital	88,443 (33,235-100,216)	38,845 (32,418-66,757)	0.055	123,552 (98,633-170,926)	**<0.001**
Antimicrobial	12,006 (7,902-23,902)	10,034 (9,000–12,987)	0.154	33,456 (22,997-43,757)	**<0.001**
Nonantimicrobial	45,456 (23,435-89,654)	32,334 (22,723-54,672)	0.104	89,312 (70,539-124,521)	**<0.001**

[a] Non-*baumannii A. calcoaceticus-A. baumannii* complex versus carbapenem-susceptible *A. baumannii* (CSAB).
[b] Non-*baumannii A. calcoaceticus-A. baumannii* complex versus carbapenem-intermediate or -resistant *A. baumannii* (CRAB).
[c] 1 U.S. dollar = 32.38 baht (as of 20 March 2014).
[d] Boldface entries indicate values that reached the significance level set at 0.05.

complex was significantly shorter than the duration to infection with CRAB whereas it was significantly longer than the duration to infection with CSAB. We also found that the proportion of patients who were exposed to invasive procedures among those infected with non-*baumannii A. calcoaceticus-A. baumannii* complex was higher than the proportion of those infected with CSAB whereas it was not different from the proportion of those infected with CRAB.

Clinical outcomes of the patients infected with non-*baumannii A. calcoaceticus-A. baumannii* complex and *A. baumannii*. Comparisons of the clinical outcomes are shown in Table 3. The patients infected with non-*baumannii A. calcoaceticus-A. baumannii* complex had more favorable outcomes, including mortality, hospital costs, and the length of stay after the onset of infection, than those infected with CRAB. Compared with CSAB-infected patients, those infected with non-*baumannii A. calcoaceticus-A. baumannii* complex species had nominally lower in-hospital and 14-day mortality and significantly lower 30-day mortality, though these differences were smaller than those seen with CARB-infected patients. The hospital costs were also nominally lower for non-*baumannii A. calcoaceticus-A. baumannii* complex infection cases than for CSAB infection cases, but the difference did not reach statistical significance.

Factors influencing 30-day mortality among patients infected with non-*baumannii A. calcoaceticus-A. baumannii* complex and *A. baumannii*. The demographic and clinical factors predictive of 30-day mortality are shown in Table 4. High APACHE II scores and infection with CRAB were significantly

TABLE 4 Factors influencing 30-day mortality among the 222 patients infected with *A. calcoaceticus-A. baumannii* complex

Parameter	Values[a]		Crude OR (95% CI)	Adjusted OR (95% CI)	*P* value[d]
	Survivor ($n = 122$)	Nonsurvivor ($n = 100$)			
Age (yrs), median (IQR)[b]	58 (45–71)	65 (48–76)	1.00 (0.98–1.00)	1.02 (0.96–1.04)	0.07
Male sex	82 (67.2)	55 (55.0)	0.64 (0.36–1.12)		
Comorbidities	31 (25.4)	36 (36.0)	1.47 (0.81–2.63)		
Emergency indication for admission	34 (27.9)	30 (30.0)	1.09 (0.59–2.00)		
APACHE II score, median (IQR)[b]	13 (11–15)	22 (17–25)	1.33 (1.23–1.43)	1.32 (1.20–1.43)	**<0.001**
Initial ICU[c] admission	40 (32.8)	38 (38.0)	1.25 (0.71–2.22)		
Retention of medical devices	90 (73.8)	71 (71.0)	0.84 (0.45–1.56)		
Bacteremia	13 (10.7)	13 (13.0)	1.61 (0.68–3.85)		
Pneumonia	77 (63.1)	63 (63.0)	1.03 (0.58–1.82)		
Appropriate empirical antimicrobial therapy	88 (72.1)	39 (39.0)	0.22 (0.12–0.39)	0.28 (0.13–0.63)	**0.002**
Infection with non-*baumannii A. calcoaceticus-A. baumannii* species	23 (18.9)	2 (2.0)	0.09 (0.02–0.41)	0.08 (0.01–0.63)	**0.005**
Infection with CRAB	61 (50.0)	78 (78.0)	3.57 (1.89–6.67)	2.50 (1.03–6.25)	**0.029**

[a] Values represent number (percent) of patients unless otherwise indicated.
[b] Continuous data.
[c] ICU, intensive care unit.
[d] Boldface entries indicate values that reached the significance level set at 0.05.

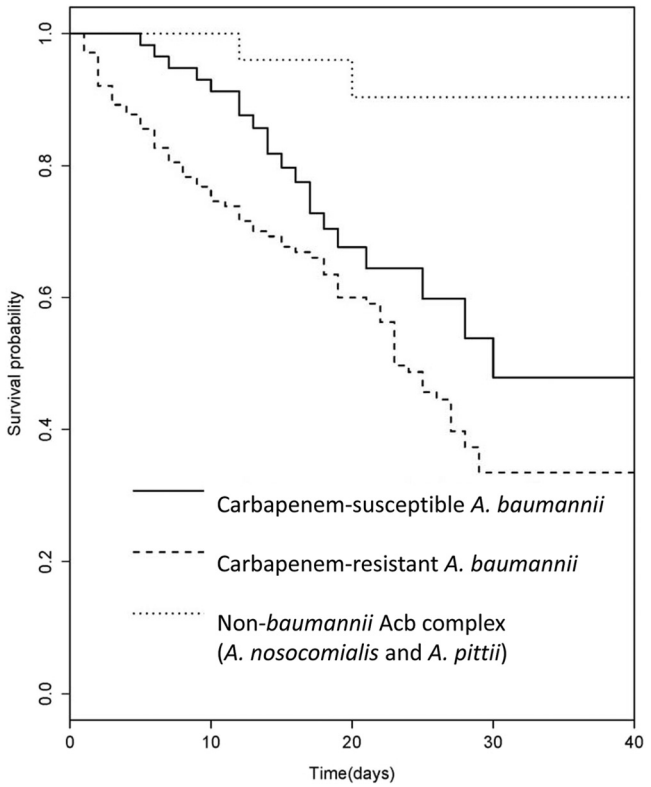

FIG 2 Kaplan-Meier survival curves of the patients in the non-*baumannii A. calcoaceticus-A. baumannii* complex group (*A. nosocomialis* or *A. pittii*), carbapenem-susceptible *A. baumannii* (CSAB) group, and carbapenem-intermediate or -resistant *A. baumannii* (CRAB) group. Levels of survival among these three groups were significantly different, with non-*baumannii A. calcoaceticus-A. baumannii* complex infection and CRAB infection associated with the lowest and highest mortality levels, respectively ($P < 0.001$, log-rank test).

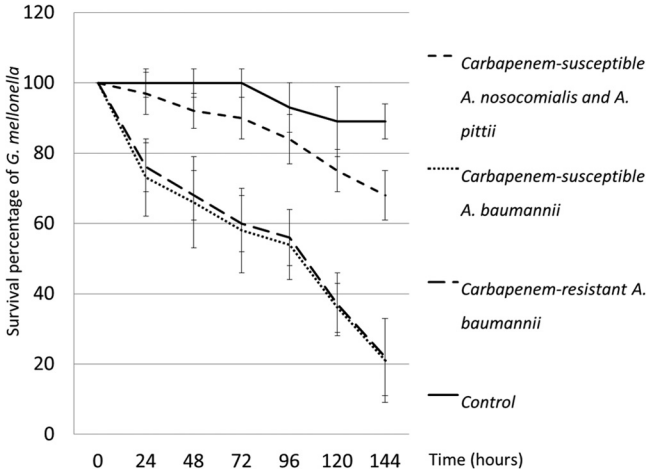

FIG 3 Survival of *G. mellonella*. The larvae were infected with 2 *A. nosocomialis*, 2 *A. pittii*, 2 carbapenem-susceptible *A. baumannii* (CSAB), and 2 carbapenem-resistant *A. baumannii* (CRAB) isolates and observed daily for 6 days. Survival rates were significantly higher for the larvae infected with non-*baumannii A. calcoaceticus-A. baumannii* complex species than for those infected with *A. baumannii*, either CSAB or CRAB ($P < 0.05$, Student's *t* test), whereas there was no significant difference in the levels of survival between the larvae infected with CSAB and those infected with CRAB.

associated with 30-day mortality, whereas infection with non-*baumannii A. calcoaceticus-A. baumannii* complex and appropriate empirical antimicrobial therapy were significant protective factors against 30-day mortality. Kaplan-Meier survival curves for the 30 days following infection caused by non-*baumannii A. calcoaceticus-A. baumannii* complex, CSAB, and CRAB are shown in Fig. 2. The survival rates of patients among these three groups were significantly different, with non-*baumannii A. calcoaceticus-A. baumannii* complex infection and CRAB infection associated with the lowest and highest mortality, respectively ($P < 0.001$, log-rank test). The survival analysis with the Cox proportional hazard model showed that the factors influencing 30-day mortality were infection with non-*baumannii A. calcoaceticus-A. baumannii* complex (HR, 0.12; 95% CI, 0.03 to 0.51; $P = 0.004$), appropriate empirical antimicrobial therapy (HR, 0.38; 95% CI, 0.23 to 0.61; $P < 0.001$), infection with carbapenem-resistant isolates (HR, 1.57; 95% CI, 0.89 to 2.79; $P = 0.105$), and higher APACHE II score (HR, 1.15; 95% CI, 1.10 to 1.19; $P < 0.001$).

***Galleria mellonella* assays.** To corroborate the findings from the clinical data discussed above, we used a *G. mellonella* infection model to determine survival of larvae infected with non-*baumannii A. calcoaceticus-A. baumannii* complex isolates, CSAB isolates, or CRAB isolates. *G. mellonella* survival data are shown in Fig. 3. Survival rates were significantly higher for the larvae infected with non-*baumannii A. calcoaceticus-A. baumannii* complex species than for those infected with *A. baumannii*, either CSAB or CRAB, in every observation between day 1 and day 6 ($P < 0.01$ by Student's *t* test). There was no significant difference in survival rates between the larvae injected with *A. nosocomialis* and those injected with *A. pittii* (data not shown). Furthermore, there was no significant difference in survival rates between the larvae infected with CSAB and those injected with CRAB with any of the observations (Fig. 3).

DISCUSSION

Non-*baumannii* species of the *A. calcoaceticus-A. baumannii* complex, in particular, *A. nosocomialis* and *A. pittii*, are increasingly recognized as significant causes of hospital-acquired infections (2, 5, 9). Observational clinical studies have demonstrated that the mortality rate for patients with infection due to non-*baumannii A. calcoaceticus-A. baumannii* complex species is lower than the mortality rate for those infected with *A. baumannii* (4, 5, 23, 24). However, *A. baumannii* is consistently less susceptible to various antimicrobials than the non-*baumannii A. calcoaceticus-A. baumannii* complex species; thus, the mortality data are often affected by confounding influences from the differences in the appropriateness of therapy, especially for the empirical phase of treatment. In practice, resistance to carbapenem is the most significant factor affecting the appropriateness of therapy, because it is the class of choice when hospital-acquired infection from *Acinetobacter* spp. is suspected, and the vast majority of carbapenem-resistant isolates across studies have been *A. baumannii* (4, 5, 23, 24). We therefore aimed to address this limitation by (i) comparing the clinical characteristics and outcomes of non-*baumannii A. calcoaceticus-A. baumannii* complex infection with those of carbapenem-susceptible and carbapenem-resistant *A. baumannii* infections separately and (ii) comparing rates of survival of infections by these three groups using a *G. mellonella* waxworm model, where no therapy is given and, thus, appropriateness of therapy does not affect the outcome.

Our study yielded several interesting observations. First, severity of illness, infection with CRAB, and inappropriate empirical therapy were independently associated with 30-day mortality, whereas infection with non-*baumannii A. calcoaceticus-A. baumannii* complex species was protective against mortality, an observation which was comparable with those of previous reports (4, 5, 23, 24). Accordingly, in-hospital, 14-day, and 30-day mortality rates were significantly higher for *A. baumannii* infection than for non-*baumannii A. calcoaceticus-A. baumannii* complex infection. However, when only the CSAB cases were included for the comparison, the overall mortality and 14-day mortality were no longer significantly higher for *A. baumannii* infections than for non-*baumannii A. calcoaceticus-A. baumannii* complex infections. This finding suggested that at least part of the excess mortality from *A. baumannii* infection was caused by antimicrobial resistance and the resulting inappropriateness of therapy. This would likely be prominent in the empirical-therapy setting, but, given the somewhat uncertain clinical efficacy of regimens (e.g., colistin and tigecycline) used for carbapenem-resistant cases, it may also negatively affect the therapeutic benefit from definitive, or pathogen-specific, therapy, thus contributing to higher mortality.

Second, survival of *G. mellonella* larvae was significantly better when they were infected with non-*baumannii A. calcoaceticus-A. baumannii* complex species (*A. nosocomialis* or *A. pittii*) than when they were infected with *A. baumannii*. On the other hand, there was no difference in the survival rates of the larvae that were infected with CSAB or CRAB isolates. While this is a relatively crude *in vivo* model of infection, the findings appear to complement the findings from the clinical study and support the notions that (i) *A. baumannii* is intrinsically more virulent and causes higher mortality than the non-*baumannii A. calcoaceticus-A. baumannii* complex species and (ii) the difference in the mortality rates observed clinically between patients infected with CSAB and CRAB may at least partly be accounted for by the difference in the appropriateness and efficacy of the antimicrobial therapies given.

Although the proportions of patients who received appropriate empirical therapy upon infection with non-*baumannii A. calcoaceticus-A. baumannii* complex species were not different from the proportions of those infected with CSAB and CRAB, the 30-day morality rates among these 3 groups were significantly different. This finding also suggests the impact of genomospecies for clinical outcomes. However, there are several potential caveats as we interpret these data. Appropriateness of therapy is defined based on *in vitro* activity data. In addition, agents considered appropriate for CRAB include colistin and tigecycline, which may have their own limitations such as low serum and epithelial lining fluid concentrations (21, 25).

There were several limitations in this study that must be acknowledged. First, the retrospective study design did not allow us to investigate factors influencing the physicians in selecting antimicrobial as well as overall treatment approaches. Second, this study was conducted in a single tertiary teaching hospital; thus, the findings may not be generalizable to other settings. Third, because of the relatively small number of patients who were infected with non-*baumannii A. calcoaceticus-A. baumannii* complex species, we could not analyze the characteristics and outcomes of patients infected with *A. nosocomialis* and *A. pittii* separately. Fourth, the screening test for *A. baumannii* with $bla_{\text{OXA-51-like}}$ genes may be affected by the emergence of carbapenem-resistant non-*baumannii A. calcoaceticus-A. baumannii* complex species harboring this gene, which appears to be a rare event (26). Finally, the observations in *G. mellonella* assays would require confirmation in vertebrate animal models.

In conclusion, the patients infected with *non-baumannii A. calcoaceticus-A. baumannii* complex species had more favorable outcomes than those infected with either carbapenem-susceptible or carbapenem-resistant *A. baumannii*. This difference appeared to stem primarily from the intrinsic virulence of the organism in addition to the appropriateness of therapy given. Our findings call for further investigation of virulence and pathogenesis among different genomospecies within the *A. calcoaceticus-A. baumannii* complex.

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