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Original Article

Gene cassette arrays, antibiotic susceptibilities, and clinical characteristics of *Acinetobacter baumannii* bacteremic strains harboring class 1 integrons

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Background and purpose: *Acinetobacter baumannii* isolates containing class 1 integrons belong to different clones, but only a few strains are successful at causing infection. This study was conducted to compare the characteristics among these clones with different epidemicity.

Methods: Eighty eight bacteremic isolates of *A. baumannii* were collected in a medical center in Taiwan during a 3-year period. The gene cassettes and antibiotic susceptibilities of the bacterial isolates were delineated and the patients' characteristics were compared.

Results: Class 1 integrons were detected in 75 isolates (85.2%). Most of the isolates belonged to 2 major clones, but only 1 of the 2 clones caused outbreaks in several hospitals in Taiwan. Restriction analyses of variable regions of the integron revealed identical gene cassettes among isolates within the same clone. The cassette arrays of the 3 clones were *aacA4*, *catB8*, *aadA1* (clone I, epidemic clone); *dhfr XII*, unknown open reading frame (*orfF*), *aadA2* (clone II, endemic clone); and *aacC1*, 2 unknown open reading frames (*orfX*, *orfX'*), *aadA1a* (clone III). The epidemic and endemic strains were multidrug resistant, but the former presented a higher resistance rate to ampicillin-sulbactam. Infections with epidemic strains were significantly associated with prior use of cephalosporins, but didn't contribute to a higher mortality rate.

Conclusions: Judicious use of cephalosporins and rapid identification using the integron typing method might be helpful for the prevention of further spread of strains with epidemic potential.

Key words: *Acinetobacter baumannii*; Disease attributes; Disease outbreaks; Drug resistance, microbial; Integron integrase IntI1

Introduction

Acinetobacter baumannii has emerged as an important nosocomial pathogen. Most *A. baumannii* infections are caused by limited clones or strains with the propensity to cause outbreaks that often involve multiple facilities [1,2] or countries [3,4]. This suggests that certain clones of *A. baumannii* might have greater epidemic potential than the others [5]. To identify markers of epidemic potential in this bacterium, Koeleman et al compared several outbreak and sporadic strains by investigating various properties of the strains, including the presence of hemagglutination, capsules and fimbriae, binding to salivary mucins, resistance to drying, antibiogram typing, and genotyping [6]. Resistance to antibiotics was the only factor significantly associated with the epidemic behavior of *A. baumannii*.

A. baumannii tends to evolve toward multidrug resistance within a few decades [7]. The resistant genes are usually acquired through mobile elements, including

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plasmids, transposons, and integron-borne gene cassettes [8]. Integrons are genetic elements that have the ability to integrate and mobilize the gene cassettes, and most gene cassettes code for antibiotic resistance [9,10]. Six classes of integrons have been recognized on the basis of the sequence of the integrase genes [1], among which class 1 was the most prevalent detected in *Acinetobacter* spp. [4,11-15]. Previous studies have reported that integrons (especially class 1) were detected at a significantly higher frequency in the epidemic strains than in the sporadic strains, and the integron-borne strains were more resistant to antibiotics [1,2,5]. These results showed a strong relationship between integrons, antibiotic resistance, and epidemicity of *A. baumannii* [2]. Thus, the integrase gene has been regarded as a marker for screening strains of *A. baumannii* with epidemic potential [5]. However, integron-borne *A. baumannii* isolates are genetically heterogeneous [12,16], and most of the isolates belong to limited clones [1,2]. This suggests that not all integron-borne *A. baumannii* isolates are equal in epidemicity, but little is known about the differences among these diverse clones. This study was performed to identify clones of class 1 integron-borne *A. baumannii* in a medical center in Taiwan. Their gene cassettes and antibiotic susceptibilities were depicted, and patients' characteristics were compared.

Methods

Bacterial strains

Bacteremic isolates presumptively identified as *Acinetobacter calcoaceticus-A. baumannii* (*Acb*) complex were collected from Taipei Veterans General Hospital (TVGH), Taipei, Taiwan, from January 1999 to December 2001. The presumptive identification was carried out by the API ID 32 GN system (bioMérieux, Marcy l'Etoile, France) and the growth capability at 44°C in brain heart infusion (BHI) medium [17]. When multiple isolates were obtained from a single patient, only the first isolate was included in the studies. A reference strain of *A. baumannii* (American Type Culture Collection [ATCC] 19606^T) was used in every study as a control.

Genomic species identification of *Acinetobacter baumannii*

The partial intergenic spacer (ITS) regions were amplified by a forward primer P-Acb-ITS (5'-GTCAGAC CCACCATGACT-3') and a reverse primer P-23SU (5'-GGTACTTAGATGTTTCAGTTC-3'). The forward primer was designed based on a conserved region within the 5' ITS region of the 16S-23S rRNA gene of *Acinetobacter* genomic species 1 (*A. calcoaceticus*; GenBank accession number, U60278), genomic species 2 (*A. baumannii*; GenBank accession number, U60279), genomic species 3 (GenBank accession number, U60280) and genomic species 13TU (GenBank accession number, U60281) [18]. The reverse primer was designed based on a universal region within the 23S rRNA gene [19]. Polymerase chain reaction (PCR) was performed in a total reaction volume of 50 μL consisting of 10 mM trisaminomethane hydrochloride (Tris-HCL; pH 8.4), 50 mM potassium chloride, 1.0 mM magnesium chloride, 0.2 mM deoxynucleoside triphosphate, 50 pmol each of forward and reverse primers, and 1 U of Taq polymerase (New England BioLabs, Ipswich, MA, USA). Two μL of bacterial overnight cultures were directly used as templates. Amplification was performed in the GeneAmp™ PCR System 2700 (Applied Biosystems, Foster City, CA, USA) with a denatured temperature at 94°C for 5 min, followed by 30 cycles of 30 sec at 94°C, 30 sec at 55°C, and 1 min at 72°C. A final extension step of 10 min at 72°C was performed. The PCR products were resolved with 1% agarose gel electrophoresis in Tris-acetate ethylenediaminetetraacetic acid (TAE) buffer at 100 V and examined after ethidium bromide staining. All the isolates included in the study gave a band of approximately 700 bp. The amplification products were subjected to direct nucleotide sequencing (Mission Biotech, Taipei, Taiwan). The sequence analysis was taken using the Basic Local Alignment Search Tool (BLAST) available from the National Center for Biotechnological Information (NCBI) website to compare with NCBI nucleotide databases [20]. Nucleotide sequences of the 16S-23S rRNA ITS region of all available *Acinetobacter* genotypes were verified to be present in the databases, and they were used for the advanced sequence analysis using FASTA, PILEUP, and dendrogram analysis from GCG software suite (Genetics Computer Group, Madison, WI, USA).

Class 1 integron analysis

Detection of class 1 integrons was carried out by PCR amplification of an internal fragment of 160-bp within the class 1 integrase gene (*intI1*) using the primers intIF (5'-CAGTGGACATAAGCCTGTTC-3') and intIR (5'-CCCGAGGCATAGACT GTA-3'). To detect inserted gene cassettes, the variable regions of class 1 integrons

were amplified with primers 5'CS (5'-GGCATCC AAGCAGCAAG-3') and 3'CS (5'-AAGCAGACTTG ACCTGA-3'), as described previously [21]. Amplicon sizes and restriction patterns obtained using *Alu*I (New England BioLabs) were used to differentiate integron cassettes of isolates belonging to different clones. Restriction digestions were carried out at 37°C in 30 μL volumes containing integron cassette PCR product (26 μL), 3 μL of reaction buffer (final concentration 1 strength), and 1 μL of *Alu*I. The fragments after restriction digestion were resolved with 2% agarose gel electrophoresis in TAE buffer at 150 V, and examined after ethidium bromide staining. Amplicons of gene cassettes from representative isolates were purified, ligated to a TA cloning vector (Yeastern Biotech, Taipei, Taiwan), transformed into *Escherichia coli* DH5α plasmid DNA with the cloned gene cassettes, and extracted and sequenced (Mission Biotech). The gene cassettes were sequenced from either end, using the M13 forward and reverse primers. Additional primers were designed based on the sequences obtained to complete the whole sequence of the gene cassettes. Sequences analyses were carried out using the BLAST program.

Genetic relatedness determined by automated ribotyping system

The genetic relatedness of the integron-borne *A. baumannii* isolates were determined by using the automated Riboprinter™ Microbial Characterization System (Qualicon, Wilmington, DE, USA) following the manufacturer's instructions and protocols, as in Wu et al [22]. Colonies were picked and loaded into the Riboprinter™ Microbial Characterization Unit (MCU). Within the MCU, the total DNA was digested with *Eco*RI enzyme, and the DNA was separated by electrophoresis and transferred directly to nylon membranes. Ribopatterns were expressed by hybridization with a chemiluminescent DNA probe containing an rRNA operon (rrnB) from *E. coli*. The patterns were automatically imaged and stored in the MCU computer. The positions of standard markers were used to correct for both lane-to-lane and membrane-to-membrane variations in band position. The ribopattern for each isolate was compared with patterns in the Riboprinter™ database. Assignment to a particular ribotype was based upon differences in band numbers, band position, and signal intensity at a given banding position. Molecular Analyst Fingerprinting, Fingerprinting Plus, and Fingerprinting DST Software (Bio-Rad Laboratories, Richmond, CA, USA) were used to analyze ribotyping polymorphisms in each sample. Similarities between every pair of organisms were determined by calculating the Pearson correlation coefficient. Samples were grouped, and a dendogram was constructed from the matrix using the unweighted pair-group method with arithmetic averaging (UPGMA) clustering technique. Isolates with the same ribopatterns were allocated to a clone.

Antibiotic susceptibility test

Minimal inhibitory concentrations (MICs) of the isolates were determined by the agar dilution method with the use of the automated Sensititre Susceptibility Plate (TREK Diagnostic Systems Ltd, East Grinstead, UK) according to the recommendation of the Clinical Laboratory Standards Institute (CLSI) [23]. Two control strains of *E. coli* (ATCC 35218 and 25922) were included in the test.

Definitions of clinical data

The medical records of patients were retrospectively reviewed. Information on patients' demographic data, clinical presentations, microbiological studies, treatments, and outcomes were obtained for analysis. Immunocompromised patients were classified as those who met any of the following criteria: liver cirrhosis; solid organ or stem cell transplantation; leukopenia (white blood cell count $<4 \times 10^9$/L); receipt of cytotoxic agents within the previous 6 weeks, >2 doses of steroids, or other immunosuppressive agents within 2 weeks prior to bacteremia. Recent surgery was defined as surgery performed within 4 weeks prior to bacteremia. Prior antibiotic use was defined as use of antibiotics in the previous month, and recent stay in the intensive care unit (ICU) as admission to an ICU within the previous 2 weeks before the day on which the first blood culture positive for *A. baumannii* was obtained. Previous shock status was defined as a condition with a systolic blood pressure <90 mm Hg due to various causes, with inadequate tissue perfusion that occurred within 1 week before the onset of *A. baumannii* bacteremia. The source of bacteremia was determined if there was concomitant or previous isolation of the same organism from urine, wounds, aspirate from abscesses, or cerebrospinal fluid within 3 days. The source was considered to be from an intravascular catheter when the same organism was isolated from blood and the catheter tip, but not from any other body sites, and there were more than 15 colony

forming units in the semiquantitative culture of the tip [24], or when local inflammation over the insertion site of the catheter was evident and there was no other possible sources of bacteremia. Bacteremia was considered to be from the respiratory tract when clinical or radiological evidence of new-onset or progressing pneumonia were evident with *A. baumannii* isolated from blood concomitantly, or when isolates with the antibiotic susceptibility pattern identical to those isolated from blood were recovered from sputum, bronchial secretion, or other respiratory specimens. Polymicrobial bacteremia was defined as isolation of 1 or more microorganisms other than *A. baumannii* from blood during the same bacteremic episode. Antibiotic therapy was defined as appropriate if *A. baumannii* isolated from the blood was susceptible in vitro to at least 1 of the antibiotics used within 3 days. Death was attributed to bacteremia if the patient died within 7 days of the bacteremia episode and no other immediate cause of death was found.

Statistical analysis

To assess differences, the chi-squared test with Yate's correction or Fisher's exact test was used to compare the discrete variables; Student's *t* test or Mann Whitney rank sum test was used to analyze the continuous variables, as appropriate. Multivariate analysis with logistic regression was performed to identify prognostic factors independently associated with mortality from *A. baumannii* bacteremia caused by epidemic and endemic strains. All the analyses were processed by using the Statistical Package for the Social Sciences for Windows (Version 15.0; SPSS Inc., Chicago, IL, USA). A p value <0.05 was considered statistically significant.

Results

Eighty eight isolates were identified as genomic species 2, *A. baumannii*. *IntI1* was detected in 75 (85.2%) of the isolates analyzed. The isolates were distributed into 3 clones (Fig. 1). Most of the isolates belonged to clone

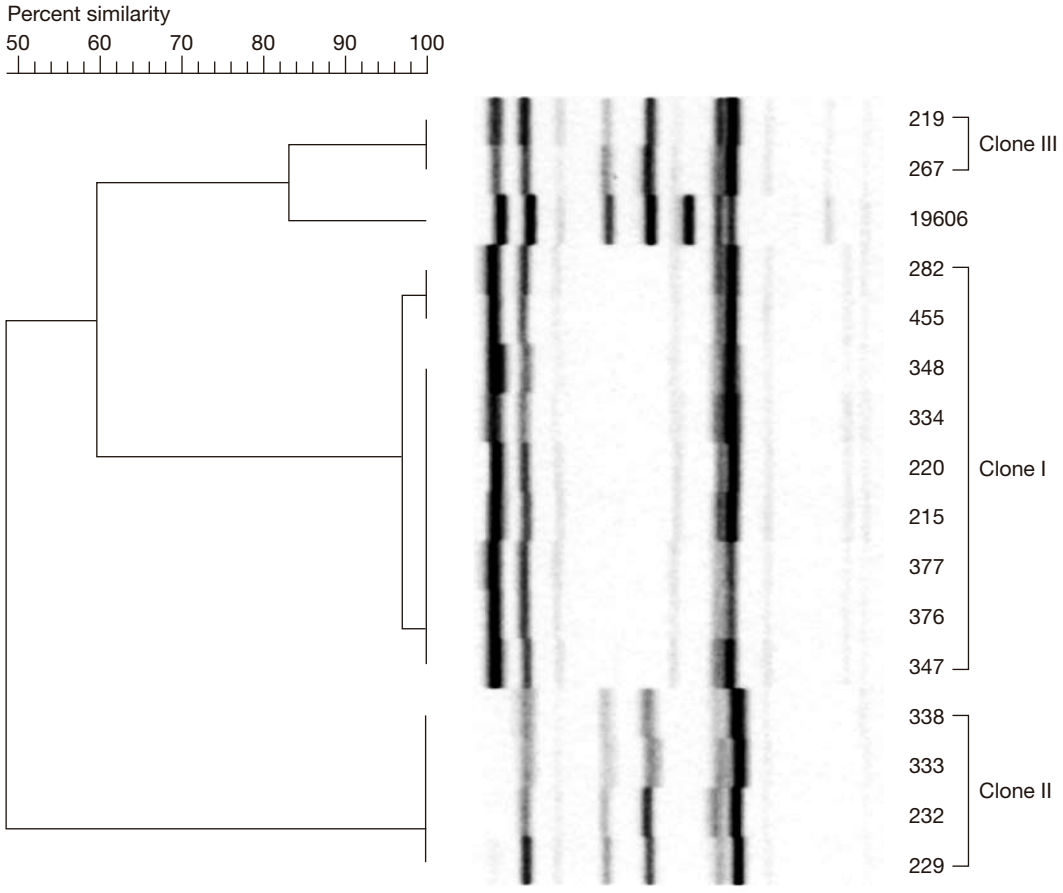

Fig. 1. Ribotypes of *Acinetobacter baumannii* isolates harboring class 1 integrons. Three clones were noted and several representative isolates are shown. The isolate designated 19606 was a prototype strain. Similarity was calculated by the unweighted-pair group method using arithmetic averages.

I (n = 51) and clone II (n = 22). Clone III comprised only 2 isolates. *A. baumannii* isolates belonging to clone I also caused disease outbreaks in 3 other medical centers in Taiwan (unpublished data), and was categorized as an epidemic clone. Isolates of clone II were found circulating in the TVGH but not in other hospitals; therefore clone II was defined as an endemic clone.

Detection of integron gene cassettes and sequences analyses

All the *intI1*-positive isolates gave a single PCR product by amplification of variable regions of integron. The amplicons were of 3 sizes (Fig. 2A). Isolates within the same clones had amplicons of identical size. The epidemic clone yielded an amplicon of approximately 2.4 kb. The amplicon sizes detected in clone II and clone III were approximately 1.9 kb and 2.6 kb, respectively. Restriction patterns of the amplicons following digestion with *Alu*I (Fig. 2B) were also identical among isolates belonging to the same clones, thus indicative of their sequence similarity. The arrangement of the gene cassette arrays of representative isolates from each clone are shown in Table 1.

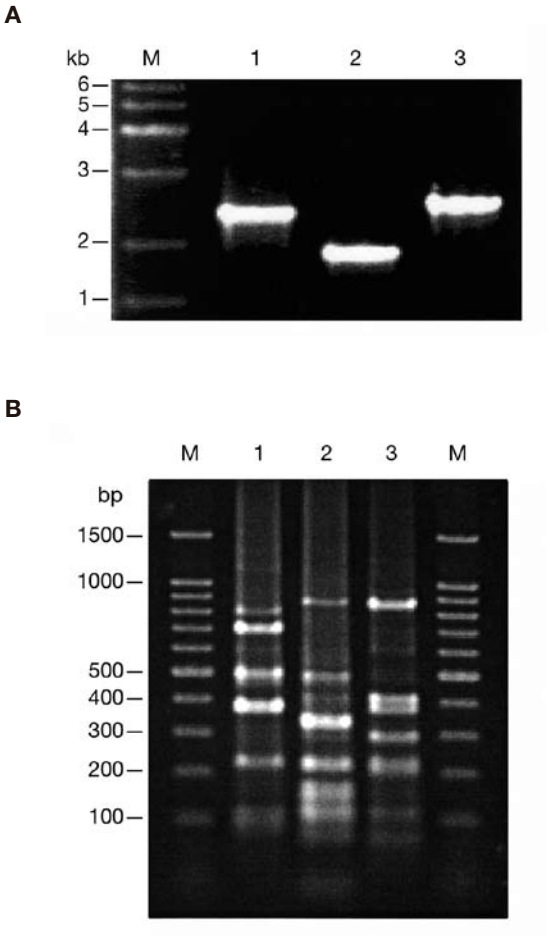

Fig. 2. (A) Amplicons obtained from the variable regions of class I integrons; and (B) the results of their restriction patterns following digestion with *Alu*I1. Size standards (1-kb and 100-bp ladder, respectively) were run in the lane labeled M. Lane 1, 286 (representative isolate of clone I); lane 2, 312 (clone II); lane 3, 267 (clone III).

Antimicrobial susceptibility

Isolates classified as clone III were more susceptible to extended-spectrum β-lactams and new fluoroquinolones than the epidemic and endemic clones (Table 2). Both the epidemic and endemic strains were multidrug resistant, and all were resistant to gentamicin, tobramycin, amikacin, piperacillin, ceftriaxone, aztreonam, ciprofloxacin, chloramphenicol, and trimethoprim-sulfamethoxazole. Resistance rates to ceftazidime, piperacillin-tazobactam, and moxifloxacin also exceeded 90% for isolates of both the epidemic and endemic clones. The epidemic strains showed a higher resistance rate to ampicillin-sulbactam than the endemic strains (88.2% vs 63.6%; $p = 0.022$), and tended to be more resistant to cefepime (90.2% vs 72.7%; $p = 0.077$). Although only 2 epidemic strains were resistant to imipenem, the MIC_{90} of imipenem for the epidemic strains was higher than that for the endemic strains. Forty eight epidemic strains had an MIC of imipenem of ≥2 μg/mL, but only 5 endemic strains reached such an MIC (94.1% vs 22.7%; $p = 0.009$). The levofloxacin resistance rate of the endemic strains was higher than that of the epidemic strains (95.5% vs 74.5%; $p = 0.051$).

Comparison of the clinical characteristics of infected patients

Two patients were infected with clone III isolates. One was a 74-year-old woman who was admitted to hospital for management of a large pressure sore. Empirical therapy with cefuroxime and netilmycin was initiated after debridement of the pressure sore. After 4 days, she developed nosocomial pneumonia, and died of sepsis 2 days later. The second patient was an 86-year-old man who attended the emergency department for a head wound after a fall. He developed bacteremia in the observation room, but fever was present for only 1 day and subsided spontaneously without antibiotic therapy.

For patients infected with epidemic strains and endemic strains, the medical records of 45 (88.2%) and 21 (95.5%) patients, respectively, were avail-

Table 1. Gene cassette arrays obtained from a representative isolate of 3 different clones of *Acinetobacter baumannii* harboring class 1 integrons.

Clone (no. of isolates)	Representative isolate	Amplicon size (bp)	Gene cassette array
I[a] (51)	286	2381	*aacA4, catB8, aadA1*
II[b] (22)	312	1823	*dhfr XII, orfF, aadA2*
III (2)	267	2545	*aacC1, orfX, orfX', aadA1a*

[a]Epidemic clone.
[b]Endemic clone.

Table 2. In vitro susceptibilities of integron-borne *Acinetobacter baumannii* isolates belonging to 3 different clones.

Antibiotic	Clone I (n = 51)			Clone II (n = 22)			Clone III (n = 2)		*p*
	MIC_{50} (μg/mL)	MIC_{90} (μg/mL)	Resistance rate No. (%)	MIC_{50} (μg/mL)	MIC_{90} (μg/mL)	Resistance rate No. (%)	MIC (μg/mL)	Resistance rate No. (%)	
Gentamicin	≥16	≥16	51 (100)	≥16	≥16	22 (100)	4, ≥16	1 (50.0)	<0.001
Tobramycin	≥16	≥16	51 (100)	≥16	≥16	22 (100)	0.5, 2	0 (0)	0.001
Amikacin	≥64	≥64	51 (100)	≥64	≥64	22 (100)	≥64	2 (100)	
Piperacillin	≥128	≥128	51 (100)	≥128	≥128	22 (100)	≥128	2 (100)	
Piperacillin-tazobactam	≥128/4	≥128/4	50 (98.0)	≥128/4	≥128/4	22 (100)	≥128/4	2 (100)	0.787
Ticarcillin-clavulanic acid	≥128/2	≥128/2	47 (92.2)	≥128/2	≥128/2	18 (81.8)	32/2, ≥128/2	1 (50.0)	0.112
Ampicillin-sulbactam	≥32/16	≥32/16	45 (88.2)	≥32/16	≥32/16	14 (63.6)	8/4, 16/8	0 (0)	0.001
Aztreonam	≥32	≥32	51 (100)	≥32	≥32	22 (100)	≥32	2 (100)	
Ceftriaxone	≥64	≥64	51 (100)	≥64	≥64	22 (100)	≥64	2 (100)	
Ceftazidime	≥64	≥64	51 (100)	32	≥64	20 (90.9)	16, ≥64	1 (50.0)	<0.001
Cefepime	≥32	≥32	46 (90.2)	≥32	≥32	16 (72.7)	8, ≥32	1 (50.0)	0.003
Imipenem	2	8	2	1	2	0 (0)	0.5, 1	0 (0)	0.616
Ciprofloxacin	≥4	≥4	51 (100)	≥4	≥4	22 (100)	≥4	2 (100)	
Levofloxacin	≥8	≥8	38 (74.5)	≥8	≥8	21 (95.5)	2	0 (0)	0.003
Moxifloxacin	≥4	≥4	51 (100)	≥4	≥4	21 (95.5)	1	0 (0)	<0.001
Chloramphenicol	≥32	≥32	51 (100)	≥32	≥32	22 (100)	≥32	2 (100)	
Trimethoprim-sulfamethoxazole	≥4/76	≥4/76	51 (100)	≥4/76	≥4/76	22 (100)	≥4/76	2 (100)	

Abbreviation: MIC = minimal inhibitory concentration.

able for comparison (Table 3). Previous receipt of antibiotic therapy was the only factor significantly associated with infection by epidemic strains (95% vs 76.2%; $p = 0.029$). Among antibiotic classes, only cephalosporins were prescribed at a higher frequency to patients infected with epidemic strains than to those with endemic strains (73.2% vs 42.9%; $p = 0.039$). No other factors, including ward distribution of the patients, was associated with bacteremia caused by epidemic strains (Table 3).

More patients infected with epidemic strains had been intubated than had those with endemic strains (84.4% vs 61.9%; $p = 0.059$). The source of bacteremia was considered to be the lung in 55.5% and 38.1% of patients infected with epidemic and endemic strains, respectively ($p = 0.585$). Due to the nature of multidrug resistance in both strains, only a few patients received appropriate antibiotic therapy (13.3% vs 28.6% for epidemic and endemic strains, respectively; $p = 0.175$). Infection with epidemic strains did not seem to be associated with a higher attributable mortality rate than that with endemic strains (40.0% vs 23.8%; $p = 0.313$). The prognosis for patients infected with either strain was related to immunocompromised status and impaired renal function (Table 4). Multivariate analysis showed that impaired renal function (odds ratio [OR], 7.98; 95% confidence interval [CI], 1.9-33.7; $p = 0.005$), malignancy (OR, 6.35; 95% CI, 1.4-29.3; $p = 0.018$), and immunodeficiency (OR, 4.52; 95% CI, 1.0-19.6; $p = 0.049$) were independent prognostic factors for mortality. Inappropriateness of antibiotic therapy was also associated with poor outcome.

Table 3. Clinical characteristics and underlying conditions of patients with bacteremia caused by epidemic (clone I) and endemic (clone II) strains of class 1 integron-borne *Acinetobacter baumannii* isolates.

Clinical characteristics and underlying conditions	Strain		*p*
	Clone I (n = 45) No. (%)	Clone II (n = 21) No. (%)	
Sex (male/female)	37/8	19/2	0.957
Age (years) [median ± SD]	67.28 ± 17.51	66.22 ± 16.43	0.54
Underlying condition			
Chronic obstructive pulmonary disease	19 (42.2)	10 (47.6)	0.885
Diabetes mellitus	10 (22.2)	4 (19.0)	1.000
Hypertension	17 (37.8)	11 (52.4)	0.395
Malignancy	15 (33.3)	7 (33.3)	1.000
Previous shock status	24 (53.3)	10 (47.6)	0.866
Recent intra-abdominal surgery	4 (8.9)	3 (14.3)	0.671
Recent stay in intensive care unit	28 (63.6)	11 (52.4)	0.551
Central catheter in situ	36 (80.0)	14 (66.7)	0.385
Ventilator dependent	38 (84.4)	13 (61.9)	0.059
Prior antibiotic therapy	43 (95.6)	16 (76.2)	0.029
Ward distribution			0.611
Intensive care unit	25 (55.6)	11 (52.4)	
Ordinary ward	19 (42.2)	7 (33.3)	
Emergency department	1 (2.2)	3 (14.3)	
Microbiologic data			
Hospital admission on day before bacteremia (median ± SD)	54.67 ± 80.9	18.48 ± 17.19	0.660
Polymicrobial	9 (20.0)	4 (19.0)	1.000
Source of bacteremia			0.133
Catheter	5 (11.1)	5 (23.8)	
Lung	25 (55.6)	8 (38.1)	
Urinary tract	0 (0)	1 (4.8)	
Gastrointestinal tract	0 (0)	1 (4.8)	
Wound	3 (6.7)	0 (0)	
Undetermined	12 (26.7)	6 (28.6)	

Table 4. Factors significantly associated with mortality in 66 patients with *Acinetobacter baumannii* bacteremia caused by epidemic or endemic strains harboring class 1 integrons.

Risk factor	Strain		*p*
	With risk factor No. of deaths/ patients (%)	Without risk factor No. of deaths/ patients (%)	
Immunodeficiency	16/25 (64.0)	7/41 (17.1)	<0.001
Malignancy	13/22 (59.1)	10/44 (22.7)	0.008
Previous shock status	11/16 (68.8)	12/50 (24.0)	0.003
Impaired renal function (serum creatinine >1.5 mg/dL)[a]	17/27 (63.0)	6/38 (15.8)	<0.001
Inappropriate antimicrobial therapy	23/54 (42.6)	0/12 (0)	0.005

[a]Data available for 65 patients.

Discussion

In this study, most *A. baumanni*i isolates (85.2%) harbored class 1 integrons, and were categorized into 3 clones. Two major clones were found, but only 1 clone caused outbreaks in several hospitals in Taiwan. This result suggested different epidemic potential among clones of *A. baumannii* harboring class 1 integrons. Therefore, the presence of the integrase gene is not sufficient to show the epidemicity of the isolates. To further delineate the clonality among different isolates, automated ribotyping [22] or pulsed-field

gel electrophoresis (PFGE) have been used [1]. However, these methods are either expensive (automated ribotyping) or time consuming (PFGE). The results of this study showed that restriction patterns of integron variable regions were identical among isolates within the same clone. Therefore, restriction analysis of such regions with gene cassettes is a useful method for discrimination among isolates, especially those from a hospital. Although it has been reported that isolates of the same genotype collected from a whole nation [1] or multiple countries [4] might have integrons with different cassette arrays, these reports also showed that genetically related isolates collected from a local area and within a short period (up to 2 years) often had the same cassette array [1,4,12,16]. These results implied that the acquisition and passage of integrons in *A. baumannii* are remarkably stable [12]. Only after selection in different areas or over long period of time might the gene cassette arrays vary.

There were 3 types of gene cassette arrays found in this study, and each belonged to a particular clone. Sequence analysis of the epidemic clone using the BLAST program showed that the same array had also been found in an *A. baumannii* isolate studied by another group in Taiwan (AY557339) [25] and in a major outbreak clone in the UK [1]. The cassette array of the integron associated with the endemic clone has been described in many bacteria, including *Klebsiella pneumoniae* (AY748453), *Citrobacter freundii* (AF550415), *Salmonella enterica* (DQ238105), *Serratia marcescens* (AF284063), *E. coli* (AB154407), and *Staphylococcus aureus* (AB191048), but was found to be present in another *A. baumannii* isolate only once before, which was reported from China (DQ141318) [26]. The situation may indicate that this cassette array was recently introduced into *A. baumannii* isolates from Asia.

The cassette array of clone III appears to be widely present among *A. baumannii* isolates in many European countries [1,4,12], but was only found in 2 isolates in the TVGH in 3 years. Although all 3 types of cassette arrays have been described previously in *A. baumannii* isolates [1,4,12,25,26], it seems that different gene cassettes gain success in outbreak strains from different areas. Interestingly, no β-lactamase gene was found within the gene cassettes in this study, despite the epidemic strains tending to exhibit a higher level of resistance to ampicillin-sulbactam, cefepime, and imipenem. Fournier et al found 3 integrons in a multidrug-resistant isolate from France, of which all 3 were located in a resistant island, 86 kb in size [8]. Genes encoding 2 β-lactamases ($bla_{\text{VEB-1}}$ and $bla_{\text{OXA-10}}$) were found in the largest integron with a size of approximately 9 kb, which might not have been amplified in this PCR protocol.

Most of the *A. baumannii* infections were caused by a few predominant clones. However, the determining factors of epidemicity have yet to be elucidated. The epidemic strains may be independently selected in different hospitals from a common ancestor, or they may have colonized in patients and spread across different hospitals through patient transfer. In this study, both the epidemic and endemic strains were multidrug resistant. However, the epidemic strains had a propensity to develop a higher level of resistance to cefepime, imipenem, and sulbactam than the endemic strains. The emergence of epidemic strains was associated with the use of cephalosporins. Long-term circulation of a strain with a propensity to develop antibiotic resistance and selection pressure posed by antibiotic overuse might ultimately select a superbug with a high level of resistance [22]. Indeed, isolates belonging to epidemic clones that were resistant to all available antibiotics (including imipenem) were discovered in 2004 in the TVGH ICU, where they caused an outbreak of nosocomial infections (unpublished data). Whether there are other properties that facilitate the spread of the epidemic strain of *A. baumannii* remains a challenge to ascertain. In this study, a higher percentage of patients infected with epidemic strains were intubated and 55% of bacteremic episodes were considered to originate from the lungs, indicating that a large proportion of epidemic strains might enter the bloodstream from the respiratory tract. It is worth determining whether epidemic strains have a greater ability to colonize the upper airway than other strains. Pharyngeal colonization might facilitate the spread of the bacteria across different hospitals by patient transfer. Lee et al reported that *A. baumannii* adheres to human bronchial epithelial cells in vitro as the initial step in the colonization process [27]. However, these researchers failed to prove that the adherence capacity of *A. baumannii* was associated with their epidemicity.

A. baumannii isolates with class 1 integrons found in a limited area within a short period belonged to 3 clones, among which their epidemicities were different. The epidemic strains had a higher resistance rate to ampicillin-sulbactam, and might be selected for by the use of cephalosporins.

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