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ORIGINAL ARTICLE

Outbreak of imipenem-resistant *Acinetobacter calcoaceticus—Acinetobacter baumannii* complex harboring different carbapenemase gene-associated genetic structures in an intensive care unit

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Background and Purpose: To investigate the clinical and molecular epidemiology of the imipenem-resistant *Acinetobacter calcoaceticus—Acinetobacter baumannii* (IRAcb) complex during an outbreak in an intensive care unit (ICU).
Methods: Forty-six clinical and 11 environmental isolates of the IRAcb complex were collected from the ICU of Taipei Veterans General Hospital, Taiwan between December 2003 and March 2004. These isolates were genotyped using pulsed-field gel electrophoresis (PFGE). Carbapenemase genes and their associated genetic structures were analyzed using PCR. Clinical data obtained from the patients were also reviewed and analyzed.
Results: The isolates were identified at the genomic species level as *A. baumannii* (42 clinical and five environmental isolates) and *Acinetobacter* genomic species 13TU (four clinical and six environmental isolates). Both species were comprised of two pulsotypes, but those of *A. baumannii* were closely related (83% similar). IS*1008*-ΔIS*Aba3*-$bla_{\text{OXA-58-like}}$ and IS*Aba1*-$bla_{\text{OXA-51-like}}$ were identified in 22 and 21 clinical isolates of *A. baumannii*, respectively (one isolate contained both). The IS*Aba3*-bracketed $bla_{\text{OXA-58-like}}$ gene was detected in all isolates of *Acinetobacter* genomic species 13TU. Patient transfers between different sections of the ICU were important factors that contributed to the spread of the two pulsotypes of *A. baumannii*. However, among the *A. baumannii* isolates identified, only those carrying IS*1008*-ΔIS*Aba3*-$bla_{\text{OXA-58-like}}$ could be

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found in the environment, indicating an additional route of transmission. The prior use of carbapenem or cefepime was associated with the subsequent infection with *A. baumannii* carrying the IS*Aba1*-$bla_{\text{OXA-51-like}}$ gene, while prior piperacillin/tazobactam use was associated with the subsequent infection with *A. baumannii* carrying the IS*1008*-ΔIS*Aba3*-$bla_{\text{OXA-58-like}}$ gene.
Conclusion: *A. baumannii* isolates carrying different carbapenemase genes and their associated genetic structures might be transmitted or selected in different ways.

Introduction

Members of the genus *Acinetobacter*, particularly *Acinetobacter baumannii*, *Acinetobacter* genomic species 13TU, and *Acinetobacter* genomic species 3, are important opportunistic pathogens that are responsible for a variety of nosocomial infections, especially among critically ill patients in intensive care units (ICUs).[1,2] However, these species cannot be differentiated by commercial identification methods and are, therefore, grouped into the so-called *Acinetobacter calcoaceticus—A. baumannii* (Acb) complex.[1] Most outbreaks are caused by *A. baumannii*, although *A.* genomic species 13TU and *A.* genomic species 3 have also been responsible for several outbreaks.[3,4] A more complicated situation is that, at times, more than one species can be found within the same period of time,[5,6] and different clones belonging to a single species can be responsible for the same outbreak.[7,8] Therefore, the identification of the genomic species and the application of molecular epidemiological methods are warranted in order to delineate the actual situation of an outbreak caused by the Acb complex.

The recently observed increase in carbapenem-resistant *Acinetobacter* strains has been almost exclusively associated with hospital outbreaks.[9] Among *A. baumannii* isolates, the overproduction of carbapenem hydrolyzing class D beta-lactamases (CHDLs), including those belonging to the OXA-23, -24, -51, and -58 families, are the most prevalent mechanisms for carbapenem resistance.[10,11] The overexpression of these CHDL genes are frequently driven by a strong promoter provided by an upstream insertion sequences.[12–15] For isolates of non-*baumannii Acinetobacter* spp., the production of metallo-beta-lactamases, and sometimes CHDLs, are usually responsible for carbapenem resistance.[2,16,17]

An increase in the number of cases with the imipenem-resistant Acb (IRAcb) complex was observed between December 2003 and March 2004 in the ICU of Taipei Veterans General Hospital. The aim of this study is to characterize the epidemiology of this outbreak in detail by determining the genomic species of the outbreak strains, their clonalities, the presence of any carbapenemase gene-associated genetic structures, and by investigating the patients' clinical features and epidemiological data.

Methods

Hospital setting and bacterial strains

Taipei Veterans General Hospital is a 2900-bed tertiary-care teaching hospital located in Taipei, Taiwan. This hospital has its own medical-surgical ICU. The ICU is divided into three sections—A, B and C—which are located side-by-side and connected by two corridors. Each section has 14 beds and is run by its own independent nursing staff. Beds in sections A and B include private rooms that are reserved for medical and surgical patients, respectively. All of the beds in section C are located in a single large room, providing care for both medical and surgical patients. As a consequence, the physicians and surgeons move between sections A/C and section B/C, respectively. Respiratory therapists are shuttled between all three sections. During the study period, patients were transferred to section C from section A or B when their conditions stabilized, and transferred from section C to B or A for the purpose of isolation if culturing detected imipenem-resistant, gram-negative bacilli or vancomycin-resistant enterococci.

Once it was determined that an outbreak was occurring, we sought to characterize the molecular epidemiology of the outbreak by screening all of the patients in the ICU for IRAcb colonization upon admission and twice weekly thereafter. The screened specimens included sputum, intravascular catheter tips, surgical wounds, urine, and other significant clinical samples; these samples were obtained from each patient in the ICU from December 29, 2003 through March 22, 2004. We also obtained environmental samples after the first three case patients were identified by swabbing the objects in the environment and equipment adjacent to the patients in sections A, B, and C (including the headboard, bed rails, door handles, and external surfaces of the intravenous pump machines, ventilators, and monitors), as well as by culturing samples from the hands of 70 hospital care workers (doctors, nurses, and respiratory therapists) over the first three consecutive days after the first three case patients were identified, as previously described.[18] The presumptive identification of any isolates was obtained using the API ID 32 GN system (bioMérieux, Marcy l'Etoile, France).

Definitions

Nosocomial infection was defined based on the definition of nosocomial infection provided by the Centers for Disease Control and Prevention.[19] Colonization was defined as the isolation of an Acb complex isolate from at least one clinical specimen in the absence of the clinical symptoms consistent with infection. Case patients were defined as those in the ICU with infection or those identified as carrying the IRAcb complex during the study period. Acute renal failure was defined as a serum creatinine value of $\geq$2 mg/dL, a 50% reduction in creatinine clearance, or as

a decline in renal function that prompted the initiation of renal replacement therapy.

Data collection

Patient medical records were reviewed in order to extract epidemiologic data and clinical information, including age, gender, durations of ICU and hospital stays, sites of infection or colonization, time from admission to acquisition, underlying diseases, reasons for ICU admission, date and type of antimicrobial therapy received, mechanical ventilation required, and clinical outcome. The severity of each patient's illness was evaluated using the acute physiology and chronic health evaluation II (APACHE II) score[20] and the sequential organ failure assessment (SOFA) score[21]; these scores were recorded at the time of admission to the ICU and at the first time of IRAcb isolation from each patient during the study period. All of the procedures used were in accordance with the recommendations found in the Helsinki Declaration of 1975. Guidelines for human experimentation and the appropriate conduct for clinical research were followed, as required by the institutional review board of Taipei Veterans General Hospital.

Species identification and antimicrobial susceptibilities

The identification of *A. baumannii* was performed using the multiplex-PCR method with two pairs of primers: 1) Ab-ITSF and Ab-ITSB and 2) rA1 and rA2 (Table 1).[22] Other isolates identified as other *Acinetobacter* species were identified at the genomic species level using 16S-23S ribosomal DNA intergenic spacer (ITS) sequence analysis.[23] The minimum inhibitory concentrations (MICs) for tigecycline and colistin were determined using the E-test (AB BIODISK, Solna, Sweden) or agar dilution with colistin sulfate (Sigma Aldrich, St. Louis, MO, USA), respectively. All other MICs were determined by broth dilution using the automated Sensititre Susceptibility Plate (TREK Diagnostic Systems Ltd., West Sussex, UK). The results were interpreted according to the guidelines of the Clinical Laboratory Standards Institute (CLSI),[24] except for tigecycline's breakpoints which were interpreted according to the criteria of the U.S. Food and Drug Administration (susceptibility is defined as $\leq$2 μg/mL; resistance as $\geq$8 μg/mL).

Molecular typing

The clonal relationships of the IRAcb isolates were determined by pulsed-field gel electrophoresis (PFGE). PFGE of *Apa*I-digested genomic DNA was performed using the Bio-Rad CHEF-Mapper apparatus (Bio-Rad Laboratories, Hercules, CA, USA). DNA restriction patterns were interpreted according to the criteria of Tenover et al[25] and cluster analysis was performed using GelCompar II v. 3.5 (Applied Maths, Sint-Martens-Latem, Belgium) and the unweighted pair-group method with arithmetic averages (UPGMA). The Dice correlation coefficient was used with a tolerance of 1% in order to analyze any similarities between banding patterns. In brief, isolates showing more than three DNA fragment differences and a similarity of $<$85% following dendrogram analysis were considered to represent different PFGE types.

Table 1 Primers used in this study

Primer	Target	Sequence (5′to 3′)
rA1	*recA*	CCTGAATCTTCTGGTAAAAC
rA2		GTTTCTGGGCTGCCAAACATTAC
Ab-ITSF	internal fragment	CATTATCACGGTAATTAGTG
Ab-ITSB	of 16S-23S *rRNA* intergenic spacer region	AGAGCACTGTGCACTTAAG
IS*Aba1*F	IS*Aba*1	CACGAATGCAGAAGTTG
IS*Aba1*R		CGACGAATACTATGACAC
IS*Aba2*A	*tnpA* of IS*Aba2*	AATCCGAGATAGAGCGGTTC
IS*Aba2*B		TGACACATAACCTAGTGCAC
IS*Aba3*A	*tnpA* of IS*Aba3*	CAATCAAATGTCCAACCTGC
IS*Aba3*B		CGTTTACCCCAAACATAAGC
IS*Aba4*A	*tnpA* of IS*Aba4*	ATTTGAACCCATCTATTGGC
IS*Aba4*B		ACTCTCATATTTTTTCTT
IS*18*A	*tnpA* of IS*18*	CACCCAACTTTCTCAAGATG
IS*18*B		ACCAGCCATAACTTCACTCG
IS*1008*F	*tnpA* of IS*1008*	TCTAGATCGGCACTTTCAAGGT GAAAT
m23F	$bla_{\text{OXA-23-like}}$	GATCGGATTGGAGAACCAGA
m23R		ATTTCTGACCGCATTTCCAT
m24F	$bla_{\text{OXA-24-like}}$	GGTTAGTTGGCCCCCTTAAA
m24R		AGTTGAGCGAAAAGGGGATT
m51F	$bla_{\text{OXA-51-like}}$	TAATGCTTTGATCGGCCTTG
m51R		TGGATTGCACTTCATCTTGG
m58F	$bla_{\text{OXA-58-like}}$	CCCCTCTGCGCTCTACATAC
m58R		AAGTATTGGGGCTTGTGCTG
IMP-F	$bla_{\text{IMP-like}}$	GGAATAGRGTGGCTTAAYTCTC
IMP-R		TGGCCAAGCTTCWAHATTTGC
VIM1-F	$bla_{\text{VIM-1,4,5,13,14}}$	GGTTGTATACGTCCCGTCAG
VIM1-R		TGCTTTGACAACGTTCGC
VIM2-F	$bla_{\text{VIM-2,3,6,8,9,10,11}}$	CTATCATGGCTATTGCGAG
VIM2-R		ATCGCACAACCACCATAG
SIM1-F	$bla_{\text{SIM-1}}$	TACAAGGGATTCGGCATCG
SIM1-R		TAATGCTCCTGTTCCCATGTG
SPM1-F	$bla_{\text{SPM-1}}$	AGACCGCGATTTCTATTCTT
SPM1-R		AGTTCCTTCGGCTTTATCAT
GIM1-R	$bla_{\text{GIM-1}}$	AGAACCTTGACCGAACGCAG
GIM1-F		ACTCATGACTCCTCACGAGG

Detection of CHDLs, MBLs, and genes upstream of CHDL

Detection of the CHDL genes ($bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-51-like}}$, and $bla_{\text{OXA-58-like}}$) was performed using a multiplex PCR assay.[26] The upstream locations of the insertion sequences (ISs) were mapped by PCR using forward primers within the ISs and reverse primers within the carbapenemase genes (Table 1).[13,27,28] MBLs were detected by phenotypic tests and PCR assays. MBL phenotypic detection used the imipenem and imipenem-EDTA combined-disk test and the imipenem and EDTA-sodium mercaptoacetic acid (SMA) double-disk synergy test, as previously described.[17] PCR analysis was used to confirm the presence of MBL genes with primers specific to

the bla_{IMP}, bla_{VIM}, bla_{SIM}, bla_{SPM} and bla_{GIM-1} genes (Table 1).[29–32]

Statistical analysis

The Chi-square tests with Yates' correction or Fisher's exact test were used to compare discrete variables. Fisher's exact test was used instead of the Chi-square test with Yates' correction when one or more expected values in the 2 × 2 contingency table were less than 5. The comparison of two quantitative variables was made using the Mann-Whitney test for nonparametric variables and the Student t test for parametric variables. Two-sided tests were used for all analyses. A p-value <0.05 was considered statistically significant. All analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 15.0 (SPSS, Chicago, IL, USA).

Results

Clinical characteristics of case patients

During the study period, 27 ICU patients became infected (17 patients) or colonized (10 patients) with IRAcb. The infected/colonized patients represented approximately 16.6% (27/163) of the total population of ICU patients admitted during the outbreak. No patient was found to be positive for IRAcb before ICU admission or on their first screening upon admission to the ICU. The main reasons for ICU admission were pneumonia and postsurgical complications. All patients were mechanically ventilated. The sites of infection included the respiratory tract ($n = 12$), wound sites ($n = 4$), and urinary tract ($n = 1$). Three patients had concomitant bacteremia. Of the infected and colonized patients, 12 and four patients died, respectively. No statistical significance was observed between infected and colonized patients in terms of the all-cause mortality (70.6% vs. 40.0%, $p = 0.224$).

Species identification and antimicrobial susceptibilities

During the study period, 46 isolates of IRAcb were recovered from one or more clinical samples that were obtained from the case patients. Forty-two isolates were identified as *A. baumannii* and the other four isolates were recognized as *Acinetobacter* genomic species 13TU. Analysis of 72 samples obtained from the patients' direct environment, including equipment, yielded four imipenem-resistant *A. baumannii* isolates (from the bed headboards, support stands of the infusion pump, pulseoxymeters, and ventilation hoses) and five imipenem-resistant isolates of *Acinetobacter* genomic species 13TU (from the door handles, curtains, surfaces of the ventilator, water taps, and procedure trolleys). In addition, one isolate of *A. baumannii* and one isolate *Acinetobacter* genomic species 13TU were recovered from cultures of samples obtained from the hands of 70 hospital care workers (from one surgeon and one respiratory therapist, respectively).

Imipenem MICs of the isolates ranged between 16 and 64 μg/mL. All of the study isolates were resistant to piperacillin/tazobactam. All of the *A. baumannii* isolates were resistant to ceftazidime, cefepime, ampicillin/sulbactam, and ciprofloxacin, for which two, one, one, and all 10 of the isolates from *Acinetobacter* genomic species 13TU, respectively, were defined as susceptible. All but four clinical isolates of *A. baumannii* showed resistance to all three aminoglycosides tested (gentamicin, amikacin, and tobramycin). The four aminoglycoside susceptible isolates were all from the same pulsotype: pulsotype B.

Molecular epidemiology and distributions of carbapenemase gene-associated genetic structures

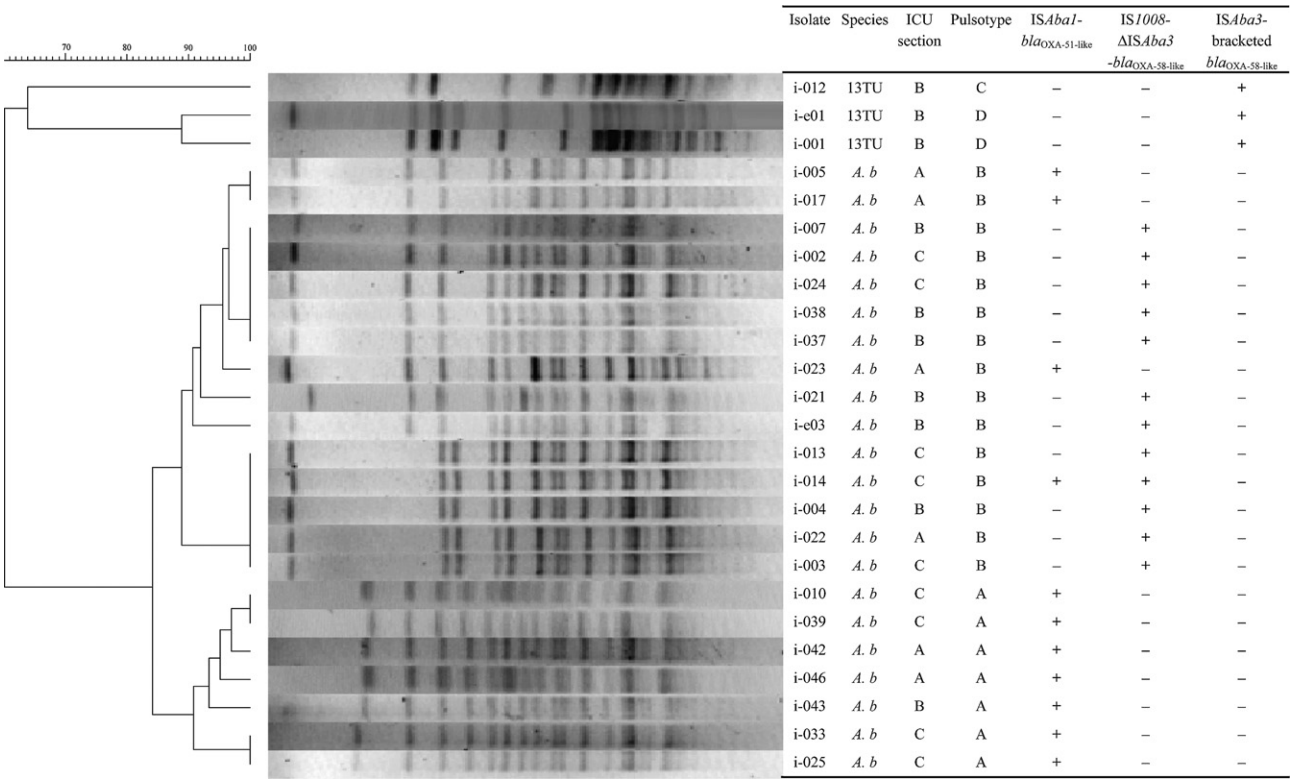
The clinical isolates of *A. baumannii* identified in this study were grouped into two pulsotypes, designated pulsotypes A and B, all which were relatively similar (83%). All environmental isolates of *A. baumannii* belonged to pulsotype B. The clinical isolates of *Acinetobacter* genomic species 13TU were classified as pulsotypes C and D (each pulsotype included two isolates). All of the environmental isolates of *A. baumannii* and *Acinetobacter* genomic species 13TU belonged to pulsotype B and D, respectively. All of the environmental IRAcb isolates, except one, were obtained in proximity to the patient with the same strain or from the hands of a hospital care worker who cared for the patient with the same strain. The PFGE results of half of the clinical isolates belonging to the four different pulsotypes and one representative strain of environmental isolates belonging to each species are shown in Fig. 1.

The $bla_{OXA-58-like}$ gene was detected in 22 clinical isolates of imipenem-resistant *A. baumannii* (all belonging to pulsotype B), in all of the four clinical isolates of *Acinetobacter* genomic species 13TU (pulsotypes C and D), and in all 11 of the environmental isolates of IRAcb. All of the $bla_{OXA-58-like}$ genes found in *A. baumannii* had an upstream insertions of the truncated IS*Aba3* and IS*1008* versions of this gene (IS*1008*-ΔIS*Aba3*-$bla_{OXA-58-like}$),[27] while IS*Aba3* was found both upstream and downstream of the $bla_{OXA-58-like}$ gene in all isolates of *Acinetobacter* genomic species 13TU. In one of the *A. baumannii* isolates carrying IS*1008*-ΔIS*Aba3*-$bla_{OXA-58-like}$ and 20 clinical isolates of *A. baumannii* without $bla_{OXA-58-like}$ gene (belonging to either pulsotype A or B), IS*Aba1* was found upstream of $bla_{OXA-51-like}$. $Bla_{OXA-23-like}$, $bla_{OXA-24-like}$ genes, and MBL genes were not detected in any isolate identified in this study. Phenotypic assays also failed to identify MBL production.

Different isolates collected from the same patient had identical pulsotypes and carried the same carbapenemase gene-associated genetic structures, except in two patients (patient No. 9 and No. 19; Fig. 2), for which additional details are provided below.

Outbreak investigation

A timeline was constructed to follow the occurrence of new cases in relation to patient movements and locations (Fig. 2). The first isolate carrying IS*1008*-ΔIS*Aba3*-$bla_{OXA-58-like}$ was identified in patient No. 4 in section C, but the origin of *A. baumannii* could not be traced. The emergence of



Isolate	Species	ICU section	Pulsotype	IS*Aba1*-$bla_{\text{OXA-51-like}}$	IS*1008*-ΔIS*Aba3*-$bla_{\text{OXA-58-like}}$	IS*Aba3*-bracketed $bla_{\text{OXA-58-like}}$
i-012	13TU	B	C	–	–	+
i-e01	13TU	B	D	–	–	+
i-001	13TU	B	D	–	–	+
i-005	*A. b*	A	B	+	–	–
i-017	*A. b*	A	B	+	–	–
i-007	*A. b*	B	B	–	+	–
i-002	*A. b*	C	B	–	+	–
i-024	*A. b*	C	B	–	+	–
i-038	*A. b*	B	B	–	+	–
i-037	*A. b*	B	B	–	+	–
i-023	*A. b*	A	B	+	–	–
i-021	*A. b*	B	B	–	+	–
i-e03	*A. b*	B	B	–	+	–
i-013	*A. b*	C	B	–	+	–
i-014	*A. b*	C	B	+	+	–
i-004	*A. b*	B	B	–	+	–
i-022	*A. b*	A	B	–	+	–
i-003	*A. b*	C	B	–	+	–
i-010	*A. b*	C	A	+	–	–
i-039	*A. b*	C	A	+	–	–
i-042	*A. b*	A	A	+	–	–
i-046	*A. b*	A	A	+	–	–
i-043	*A. b*	B	A	+	–	–
i-033	*A. b*	C	A	+	–	–
i-025	*A. b*	C	A	+	–	–

Figure 1. Pulsed-field gel electrophoresis profiles, genomic species identification, isolation locations, and carbapenemase genes of representative clinical and environmental strains in this study. i-e01, a representative environmental strain of *Acinetobacter* genomic species 13TU; i-e03, a representative environmental strain of *A. baumannii*; *A. b*, *Acinetobacter baumannii*; 13TU, *Acinetobacter* genomic species 13TU.

isolates with IS*1008*-ΔIS*Aba3*-$bla_{\text{OXA-58-like}}$ in section B could be traced to the transfer of patient No. 4, but no patient transfer could account for the appearance of isolates with IS*1008*-ΔIS*Aba3*-$bla_{\text{OXA-58-like}}$ in section A (patient No. 10).

An isolate with IS*Aba1*-$bla_{\text{OXA-51-like}}$ was first identified in a female patient (case No. 15) in section A. This patient had been previously colonized with multidrug-resistant (but not imipenem-resistant) *A. baumannii* for 2 months and was transferred to the ICU from a local hospital upon admission. Then, imipenem-resistant *A. baumannii* with IS*Aba1*-$bla_{\text{OXA-51-like}}$ appeared in section C after patient No.16 was transferred from section A to section C. Similarly, an isolate with IS*Aba1*-$bla_{\text{OXA-51-like}}$ was identified in section B (patient No. 21) after patient No. 20 was moved from section A to section B.

In patient No. 9, the first two imipenem-resistant *A. baumannii* isolates (i-013 and i-014) were identified, which were collected on the same day, and these isolates carried different carbapenemase genes (i-013 carried IS*1008*-ΔIS*Aba3*-$bla_{\text{OXA-58-like}}$ and i-014 contained both IS*Aba1*-$bla_{\text{OXA-51-like}}$ and IS*1008*-ΔIS*Aba3*-$bla_{\text{OXA-58-like}}$), although they exhibited identical pulsotypes (Fig. 1). Around the time when i-014 was identified in patient No. 9 in section C, clusters of isolates with either IS*Aba1*-$bla_{\text{OXA-51-like}}$ or IS*1008*-ΔIS*Aba3*-$bla_{\text{OXA-58-like}}$ were noted in section C.

In patient No. 19, an isolate harboring IS*Aba1*-$bla_{\text{OXA-51-like}}$ was initially isolated in section C (i-010). Fourteen days after patient No. 19 was transferred from section A to section B, an isolate carrying IS*1008*-ΔIS*Aba3*- $bla_{\text{OXA-58-like}}$ (i-021) was discovered. These two isolates belonged to different pulsotypes. Just before i-021 was identified, isolates with IS*1008*-ΔIS*Aba3*- $bla_{\text{OXA-58-like}}$ were discovered in three patients in section B in the vicinity of patient No. 19. However, patient No. 19 might also have been colonized/infected during his short stay in section C (i002, i003, and i013).

Comparisons of patients whose first imipenem-resistant *A. baumannii* isolate carried different carbapenemase gene-associated genetic structures

Patients whose first imipenem-resistant *A. baumannii* isolate carried IS*Aba1*-$bla_{\text{OXA-51-like}}$ or IS*1008*-ΔIS*Aba3*-$bla_{\text{OXA-58-like}}$ are compared in Table 2. Patient No. 9 was excluded because one of his first two imipenem-resistant *A. baumannii* isolates simultaneously harbored two types of genetic structures. No significant differences were observed between the two patient groups in terms of age, underlying diseases, clinical presentations, disease severity, mortality, mechanical ventilation, or the lengths of their respective hospital stays. Compared to the patients whose first imipenem-resistant *A. baumannii* isolate carried IS*1008*-ΔIS*Aba3*-$bla_{\text{OXA-58-like}}$, those whose isolates carried IS*Aba1*-$bla_{\text{OXA-51-like}}$ tended to have been treated with carbapenems or cefepime. Prior treatment with piperacillin/tazobactam, however, was observed more frequently in the patients whose first imipenem-resistant *A. baumannii*

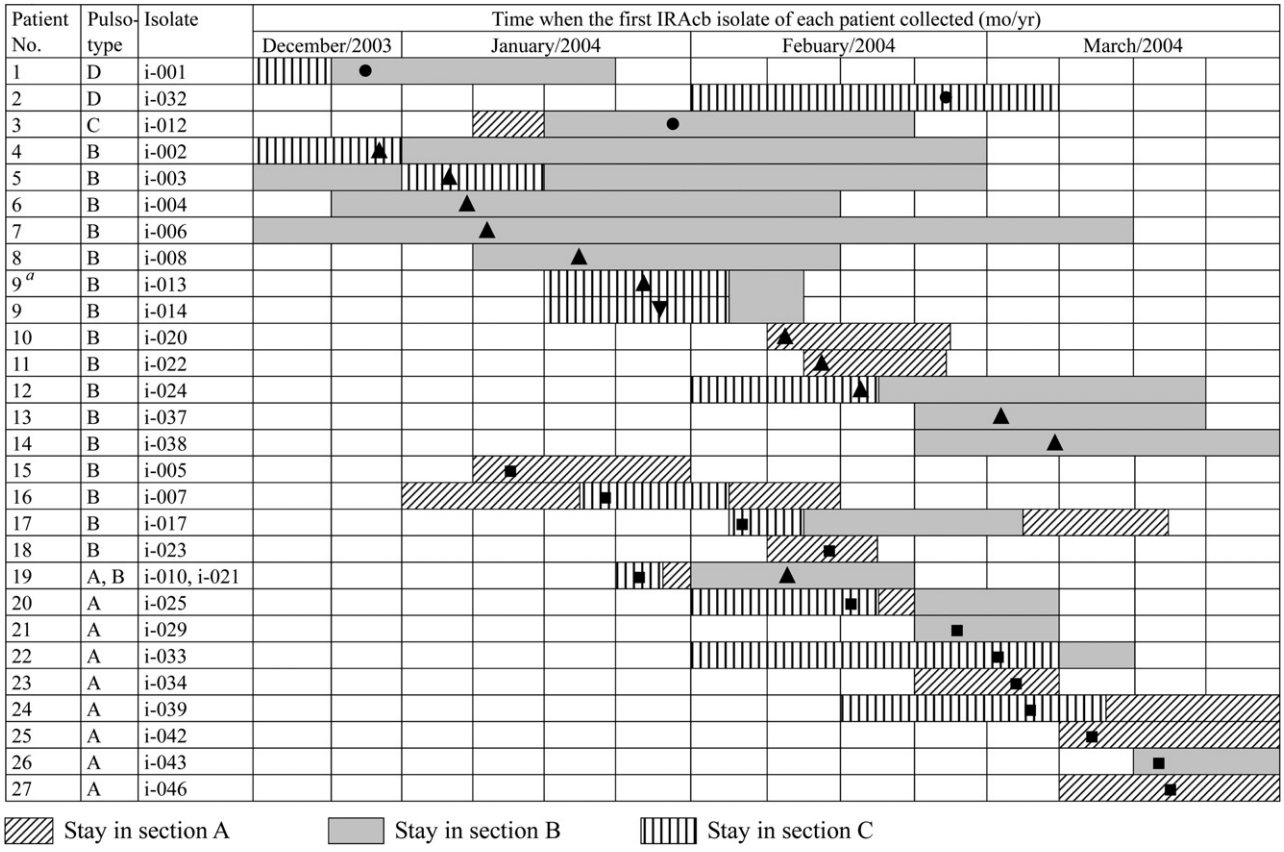


Figure 2. Timeline of the colonization or infection of the 27 patients with imipenem-resistant *A. baumannii* or *Acinetobacter* genomic 13TU who were identified during the outbreak. ■ Date of the first imipenem-resistant *A. baumannii* isolate with IS*Aba1*-$bla_{\text{OXA-51-like}}$. ● Date of the first imipenem-resistant isolate of *Acinetobacter* genomic species 13TU with IS*Aba3*-bracketed $bla_{\text{OXA-58-like}}$. ▲ Date of the first imipenem-resistant *A. baumannii* isolate with IS*1008*-ΔIS*Aba3*-$bla_{\text{OXA-58-like}}$. ▼ Date of the first imipenem-resistant *A. baumannii* isolate from patient No. 9 with both IS*Aba1*-$bla_{\text{OXA-51-like}}$ and IS*1008*-ΔIS*Aba3*-$bla_{\text{OXA-58-like}}$. [a] For patient No. 9, the first two imipenem-resistant *A. baumannii* isolates were collected on the same day.

isolate carried IS*1008*-ΔIS*Aba3*-$bla_{\text{OXA-58-like}}$. When confined to just those patients whose first imipenem-resistant *A. baumannii* isolate belonged to pulsotype B, there were no significant differences regarding any of the above-mentioned parameters between the two patient groups whose first imipenem-resistant *A. baumannii* isolate carried IS*1008*-ΔIS*Aba3*-$bla_{\text{OXA-58-like}}$ and IS*Aba1*-$bla_{\text{OXA-51-like}}$, respectively. During the outbreak period, no preferences in the use of certain antimicrobial agents were noted in any section of the ICU and there were no differences in antimicrobial agents used among the three sections.

Infection control measures

Infection control measures were applied to all affected patients, including isolation in a private room, strict hand hygiene, and the required use of gowns and gloves before entering rooms. The minimum number of staff required for care was allowed to enter each affected room, with medical staff seeing affected patients last on their rounds around each ward. Nurses were limited to caring for either affected or unaffected patients. Movement between the sections was restricted. The antibiotic policy was not changed because the controlled administration of prescriptions was routine practice in the ICU. Potentially contaminated medical equipment was cleaned and disinfected with disinfectants using an effective concentration for the provisioned amount of time. The performance of personnel was closely monitored. No additional cases were found after the last positive patient left the ICU at the end of April 2004.

Discussion

In this study, different carbapenemase gene-associated genetic structures were observed among isolates belonging to the identical pulsotypes. Similar findings have been reported during outbreaks of carbapenem-resistant *A. baumannii*.[8] So-called "micro-variation," as revealed by PFGE profiling, coupled with extra information obtained by the detection of additional genes, is thought to be helpful for investigating individual outbreaks of *A. baumannii*.[33] A careful review of the intra-ICU movements of our study patients and the progression of clones revealed that, within a single clone of *A. baumannii*, infection or colonization with isolates carrying different genetic structures followed different epidemiologic patterns of hospital acquisition and patient-to-patient transmission. Our findings further emphasize that PFGE and the detection of carbapenemase gene-associated genetic structures are warranted when investigating an outbreak caused by carbapenem-resistant acinetobacters.

Table 2 Comparisons of patients whose first imipenem-resistant *Acinetoabacter baumannii* isolate carried IS*Aba1-bla*$_{OXA-51-like}$ and patients whose first imipenem-resistant *A. baumannii* isolate carried IS*1008*-ΔIS*Aba3-bla*$_{OXA-58-like}$

	Patients whose first imipenem-resistant *A. baumannii* isolate carried IS*Aba1-bla*$_{OXA-51-like}$ (n = 13)			Patients whose first imipenem-resistant *A. baumannii* isolate carried IS*1008*-ΔIS*Aba3-bla*$_{OXA-58-like}$ (n = 10)			*p*-value
	n	%	Mean ± SD	n	%	Mean ± SD	
Age (y)			69.1 ± 14.4			60.9 ± 18.1	0.263
APACHE II score upon ICU admission			32.6 ± 9.4			29.6 ± 6.3	0.248
SOFA score upon ICU admission			15.0 ± 10.7			15.0 ± 9.6	0.333
APACHE II score at culture date [a]			33.2 ± 9.8			28.7 ± 7.2	0.305
SOFA score at culture date [a]			10.3 ± 5.4			7.9 ± 3.4	0.349
Length of hospital stay (d)			60.2 ± 29.7			86.0 ± 39.6	0.088
Length of ICU stay (d)			32.2 ± 15.5			51.2 ± 25.6	0.051
Admission to culture date (d)			25.5 ± 21.8			23.2 ± 15.0	0.964
ICU admission to culture date (d)			10.0 ± 8.9			15.2 ± 10.3	0.151
Length of mechanical ventilation (d)			33.5 ± 24.8			54.8 ± 36.1	0.171
Transfer from other hospital [b]	5	38.5		2	20.0		0.405
Mortality	9	69.2		5	50.0		0.417
Presentation							
Infection	7	53.8		6	60.0		1.000
Colonization	6	46.2		4	40.0		1.000
Hypotension or shock [c]	9	69.2		4	40.0		0.222
Acute renal failure	9	69.2		5	50.0		0.417
Underlying disease							
Diabetes mellitus	4	30.8		5	50.0		0.417
Chronic pulmonary disease	3	23.1		0	0.0		0.604
Malignancy	4	30.8		4	40.0		0.685
First isolate discovered at							
Section A/B/C	5/2/6			2/5/3			0.323
Previous use of [d]							
Carbapenem[e]	6	46.2		0	0		0.019
Cefepime[f]	5	38.5		0	0		0.046
Piperacillin/tazobactam[g]	1	7.7		6	60.0		0.019
Ciprofloxacin	4	30.8		5	50.0		0.417
Teicoplanin	7	53.8		7	70.0		0.669

[a] Time at which the first isolate of imipenem-resistant *A. baumannii* was collected from each case patient.
[b] Patient had been hospitalized in another hospital and then transferred to our emergency room just prior to admission.
[c] Occurred in the 48-hour period around the time of the first isolate of imipenem-resistant *A. baumannii* was collected.
[d] One of the antibiotics as listed below (carbapenem, cefepime, piperacillin/tazobactam, ciprofloxacin or teicoplanin) has been used within 30 days before the first imipenem-resistant *A. baumannii* isolate was discovered and the antibiotic has been used for at least 72 hours.
[e] Carbapenem included imipenem and meropenem. The duration of carbapenem therapy in these six patients ranged from 4—11 days (average 7.2).
[f] The duration of cefepime therapy in these five patients ranged from 4—12 days (average 6.8).
[g] The duration of piperacillin/tazobactam therapy in the patient whose first imipenem-resistant *A. baumannii* isolate carried IS*Aba1-bla*$_{OXA-51-like}$ was 10 days and the duration of piperacillin/tazobactam therapy in the 6 patients whose first imipenem-resistant *A. baumannii* isolate carried IS*1008*-ΔIS*Aba3-bla*$_{OXA-58-like}$ were 5—23 days (average 14.2).

According to the timeline, following the occurrence of new cases in relation to patient movements and locations, patient transfer between ICU sections was suggested as one of the reasons for the rapid progression of the clones within the three ICU sections. Patient-to-patient transmission via patient transfer explains most cases of transmission, especially for imipenem-resistant *A. baumannii* isolates with IS*Aba1-bla*$_{OXA-51-like}$. Environmental contamination may also have played a role in disseminating the outbreak strains. Interestingly, IS*1008*-ΔIS*Aba3-bla*$_{OXA-58-like}$, but not IS*Aba1-bla*$_{OXA-51-like}$, was detected in all of the environmental isolates of *A. baumannii*. A possible explanation is that the common source of the isolates with IS*Aba1-bla*$_{OXA-51-like}$ was not derived from the environment, or that there may be differences in the abilities of isolates with IS*1008*-ΔIS*Aba3-bla*$_{OXA-58-like}$ and IS*Aba1-bla*$_{OXA-51-like}$ to survive in an inanimate environment. The high prevalence of IS*1008*-ΔIS*Aba3-bla*$_{OXA-58-like}$ and IS*Aba3*-bracketed *bla*$_{OXA-58-like}$ genes in environmental isolates of *A. baumannii* and *Acinetobacter* genomic species 13TU, respectively, also suggest that environmental contamination may explain the transmission of isolates with IS*1008*-ΔIS*Aba3-bla*$_{OXA-58-like}$ to

section A, and this may be the main mode of transmission for isolates of *Acinetobacter* genomic species 13TU with IS*Aba3*-bracketed $bla_{OXA-58-like}$ gene.

It has been reported that the use of carbapenem or certain classes of antibiotics is associated with the subsequent evolution of multidrug-resistant or extensive drug-resistant *A. baumannii*.[34,35] To the best of our knowledge, this is the first report to identify the correlation between the use of different antimicrobial agents and the subsequent acquisition of imipenem-resistant *A. baumannii* isolates that carry different carbapenemase gene-associated genetic structures. Although only a small number of patients were included, statistical significance was detected. In a recent study, introducing IS*Aba1*-$bla_{OXA-51-like}$ failed to transform piperacillin/tazobactam-susceptible *A. baumannii* to a piperacillin/tazobactam-resistant strain,[36] indicating that IS*Aba1*-$bla_{OXA-51-like}$ does not confer resistance to piperacillin/tazobactam and that isolates carrying only this genetic structure may succumb to piperacillin/tazobactam. It has also been shown that the overexpression of the bla_{OXA-58} gene contributes to resistance to piperacillin/tazobactam.[37] It remains to be seen if the acquisition of different carbapenemase genes in *A. baumannii* can be determined by using different antimicrobial agents.

The genetic structure IS*1008*-ΔIS*Aba3*-$bla_{OXA-58-like}$ was first reported by one of our team members in a study on one of the *A. baumannii* strains identified during this outbreak. This structure was identified in a plasmid, and acquisition of this plasmid-borne genetic structure conferred a high level of carbapenem resistance to *A. baumannii*.[27] IS*Aba1*-$bla_{OXA-51-like}$ also appeared to be involved in the carbapenem resistance of *A. baumannii*.[28,36] However, what the actual mechanism of carbapenem resistance is in our *Acinetobacter* genomic species 13TU remains in doubt. The IS*Aba3*-bracketed $bla_{OXA-58-like}$ gene alone is not sufficient to provide a high degree of carbapenem resistance in *A. baumannii*,[38] and this genetic structure has been identified in a plasmid of a clinical isolate of *Acinetobacter* genomic species 13TU for which the imipenem MIC was 6 μg/mL.[39] Furthermore, no known MBL genes were detected in our isolates by PCR or phenotypic assays. It is still under investigation whether there may be other carbapenemase-independent mechanisms involved.

In conclusion, epidemiologic clarification of an outbreak caused by carbapenem-resistant Acinetobacter species required not only genomic species identification and PFGE analysis but also the detection of carbapenemase gene-associated genetic structures. Patient transfers between wards and environmental contamination might play different roles in the transmission of the closely related *A. baumannii* pulsotypes that harbor different carbapenemase-associated genetic structures. It remains to be investigated whether the isolates that carry different carbapenemase gene-associated genetic structures differ based on their abilities to survive in the environment or if they can be selected using different antibiotics.

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