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ORIGINAL ARTICLE

Emergence in Taiwan of novel imipenem-resistant *Acinetobacter baumannii* ST455 causing bloodstream infection in critical patients

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KEYWORDS

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imipenem resistance;
mortality;
sequence type

Background: *Acinetobacter baumannii* is one of the most important noscomial pathogens worldwide. The study aimed to use multilocus sequence typing (MLST) for epidemiological surveillance of *A. baumannii* isolates in Taiwan and analyze the clinical presentations and patients' outcomes.

Methods: MLST according to both Bartual's PubMLST and Pasteur's MLST schemes was applied to characterize bloodstream imipenem-resistant *A. baumannii* (IRAB) infection in intensive care units in a medical center. A total of 39 clinical IRAB bloodstream isolates in 2010 were enrolled. We also collected 13 imipenem-susceptible *A. baumannii* bloodstream isolates and 30 clinical sputum isolates (24 IRAB and 6 imipenem-susceptible *A. baumannii*) for comparison. Clinical presentations and outcome of the patients were analyzed.

Results: We found that infection by ST455^B/ST2^P and inappropriate initial therapy were statistically significant risk factors for mortality. More than one third of the IRAB isolates belonged to ST455^B/ST2^P. Most ST455^B/ST2^P (80%) carried IS_{Aba1}-bla_{OXA-23}, including 10 (66.7%) with Tn2006 (IS_{Aba1}-bla_{OXA-23}-IS_{Aba1}) in an AbaR4-type resistance island. ST455^B/ST2^P appears to evolve from ST208^B/ST2^P of clonal complex (CC) 92^B/CC2^P. In this hospital-based study, *A. baumannii* ST455 accounted for 38.5% of IRAB bacteremia, with a high mortality of 86.7%.

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Approximately 85% of ST455^B/ST2^P bacteremia had a primary source of ventilation-associated pneumonia.

Conclusion: We report the emergence in Taiwan of IRAB ST455^B/ST2^P, which is the current predominant clone of IRAB in our hospital and has been causing bacteremia with high mortality in critical patients.

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Introduction

Acinetobacter baumannii is one of the most important noscomial pathogens worldwide, causing bacteremia, pneumonia, urinary tract infection, soft tissue infection, or intra-abdominal infection in hospitalized patients.¹ Bacteremia caused by imipenem-resistant *A. baumannii* (IRAB) has been associated with a higher mortality (46.0%) than bacteremia by imipenem-susceptible *A. baumannii* (ISAB; 28.3%) and other diagnoses of *A. baumannii* infection.² The most common source of *A. baumannii* infection was the respiratory tract,¹ however, the relationship between respiratory tract infection and IRAB bacteremia remains unknown.

Multilocus sequence typing (MLST) is a widely used technique for bacterial typing, proven to provide unambiguous typing data for long-term and global *A. baumannii* epidemiological surveillance.³ Two MLST schemes, Bartual's PubMLST and Pasteur's MLST, are available in delineating the population structure of *A. baumannii*.^{3–6} However, there are no published Bartual's MLST data in clinical *A. baumannii* isolates in Taiwan.

This study using MLST was designed to characterize bloodstream IRAB infection of intensive care unit (ICU) patients in 2010 in a single medical center. We compared the result with those from bloodstream ISAB, and also IRAB and ISAB from sputum of patients with clinically confirmed ventilation-associated pneumonia (VAP).

Materials and methods

Study patients and inclusion criteria

This study was approved by the Institutional Review Board (100-3592B) of Chang Gung Memorial Hospital (CGMH), Taiwan. Charts were reviewed for ICU patients who were treated in CGMH in 2010 and had VAP, at least one positive blood or sputum culture for *A. baumannii*, and symptoms and signs of infection. For patients with multiple episodes, only the first episode was included. Patients with incomplete medical records, aged < 18 years, or with polymicrobial infection were excluded.

Clinical data collection and definitions

Clinical data were collected retrospectively. VAP was defined as pneumonia with pulmonary infiltrates on chest radiographs, purulent tracheal secretions not less than 48

hours after intubation, and the start of mechanical ventilation.^{7,8} VAP as a primary source was defined by the condition when at least one *A. baumannii* sputum isolate was found from patients with VAP before the development of bacteremia, which was proved by the isolation of an *A. baumannii* in blood. Mortality was defined as infection-attributable death, wherein the criteria of death were met before the symptoms and signs of bacteremia or pneumonia were resolved, and with at least a blood or sputum culture positive for *A. baumannii*.⁹ Appropriate antimicrobial therapy was defined as administering patients with at least one antimicrobial agent, except aminoglycoside, susceptible *in vitro*, within 2 days after bacteremia onset.¹⁰ Culture detecting time was the interval (days) from culture sampling to reporting.

Bacterial isolates and antimicrobial susceptibility

Conventional biochemical tests and 16S–23S rRNA intergenic spacer region sequencing as described previously were used to characterize bacterial genospecies.¹¹ Antimicrobial susceptibility was determined by the disk diffusion method according to Clinical and Laboratory Standards Institute standards.¹² Minimum inhibitory concentration (MIC) of imipenem was further determined using Etest (Biomérieux, La Balmes les Grottes, France). The breakpoint for defining ISAB was an MIC ≤ 4 mg/L, and a breakpoint of ≥ 8 mg/L for IRAB, including both intermediately-resistant and resistant strains.¹² The tigecycline disk diffusion breakpoints were based on the FDA criteria (susceptible: ≥ 16 mm; resistant: ≤ 12 mm).¹³

MLST

In order to survey clonal relationships, Bartual's PubMLST and Pasteur's MLST schemes were used.^{4,5} By Bartual's PubMLST method, seven standard housekeeping loci [citrate synthase (*gltA*), gyrase B (*gyrB*), glucose dehydrogenase B (*gdhB*), recombination A (*recA*), chaperone 60 (*cpn60*), glucose-6-phosphate isomerase (*gpi*), and RNA polymerase (*rpoD*)] were checked.⁴ Sequences were compared with the *A. baumannii* database at the MLST database (<http://pubmlst.org/abaumannii/>) and conducted by the Basic Local Alignment Search Tool (BLAST, National Library of Medicine, Bethesda, MD 20894, USA). By the Pasteur's MLST scheme (www.pasteur.fr/recherche/genopole/PF8/mlst/Abaumannii.html), internal fragments of seven housekeeping genes [*cpn60*, protein elongation factor EF-G (*fusA*), *gltA*, CTP synthase (*pyrG*), *recA*, 50S

ribosomal protein L2 (*rplB*) and RNA polymerase subunit B (*rpoB*) were amplified and sequenced for each isolate.⁵

The eBURST algorithm (<http://eburst.mlst.net/>) was used to analyze the genetic relationships of sequence types (STs).¹⁴ Clonal complexes (CCs) were stringently defined as a cluster of STs having the same alleles at six of the seven loci and containing at least two STs.¹⁴ Novel CCs were named by the STs of their founders.

Pulsed-field gel electrophoresis

Isolates were analyzed by pulsed-field gel electrophoresis (PFGE) using methods as described elsewhere.¹⁵ The fragment patterns obtained were interpreted as described by Tenover et al.¹⁶ Briefly, isolates with no less than seven-band difference were considered to be different genotypes, which were arbitrarily designated in alphabetical order. Isolates with identical fingerprints and less than two-band difference were considered as the same genotype, whereas those with two-band to six-band difference were considered subtypes of an existing genotype.

Polymerase chain reaction and sequencing

Primers specific for imipenem resistance genes (*bla*_{OXA-23}, *bla*_{OXA-40}, *bla*_{OXA-54}, *bla*_{OXA-58}, *bla*_{OXA-51}, *bla*_{ADC}, *bla*_{IMP-1}, *bla*_{IMP-2}, *bla*_{VIM-1}, *bla*_{VIM-2}, and *bla*_{NDM-1}), insertion sequences (*ISAb*₁, *ISAb*₂, *ISAb*₃, *ISAb*₄ and *IS1008*), and the *AbaR4*-type islands were designed, and polymerase chain reaction amplification and sequence determination were performed as described previously.^{17–19}

Statistical analysis

Data analyses were performed using SPSS software, v. 17.0 (SPSS Inc., Chicago, IL, USA). Student *t* test, Chi-square test, or Fisher exact test were used when appropriate to compare proportions. Variables with a *p* value < 0.2 in the univariate analysis were added in a forward stepwise manner and selected to create the final model for multivariable analysis. All statistical analyses were two-sided, and significance was set at *p* < 0.05.

Results

Clinical isolates

A total of 39 clinical IRAB bloodstream isolates in 2010 met the inclusion criteria and were enrolled in this cohort study (Table 1). For comparison, we collected 13 ISAB bloodstream isolates and 30 clinical sputum isolates (24 IRAB and 6 ISAB) from patients who were treated in the same year and also met the inclusion criteria (Tables 1 and 2).

Sequence types, antimicrobial susceptibilities, and PFGE genotypes

There were 26 novel STs according to Bartual's PubMLST scheme in this study, including ST455, ST456, ST544 to ST556, and ST673 to ST683. These novel STs were newly

found in Chang Gung Memorial Hospital in Taiwan, and registered in the PubMLST web site (<http://pubmlst.org/>). Most IRAB isolates (38.5% from bloodstream and 45.8% sputum) belonged to ST455. ST455 isolates were susceptible to colistin (100%), and tigecycline (66.7%), but were 86.7% resistant to ampicillin–sulbactam and 100% resistant to all other antibiotics tested.

Most IRAB isolates belonged to CC92 and CC455 (bloodstream, CC455 48.7%, CC92 28.2%, and sputum, CC455 62.5%, CC92 20.8%). In contrast to IRAB, few ISAB isolates (15.4% from bloodstream and 16.7% sputum) belonged to CC92 and no ISAB was in CC455. STs of ISAB were so diverse that 13 bloodstream isolates belonged to 11 different STs and six sputum isolates belonged to six different STs.

No difference was found in the ratio of ST455 among IRAB isolated from blood and sputum (15/39 vs. 11/24, *p* = 0.564). Similar results were also found in all other STs (all *p* > 0.05).

More than half of the IRAB clinical isolates were found in the main evolutionary tract of CC92 to the novel CC455, descending from the founder ST208 to ST544, and then to ST455 (Figure 1). It is notable that the attributable mortality in patients infected by these STs appeared higher and higher along the evolution (ST208: 33.3%; ST544: 75%; ST455: 86.7%) but this should be evaluated further. ST544 is a one-locus variant of ST455 and two-locus variant of ST92 (1-3-3-2-2-7-3). ST544 therefore belonged to CC455 but not CC92.

By contrast, among 39 IRAB isolates, 34 isolates (87.2%) belonged to ST2, three (7.7%) to ST129, one (2.6%) to ST217, and one (2.6%) novel STN1 (Table 1) according to Pasteur's MLST scheme.

Based on Tenover et al's¹⁶ criteria, the 39 bloodstream IRAB isolates included 15 different PFGE genotypes and their subtypes. Sixty percent (9/15) ST455^B and 50% (17/34) ST2^P belonged to PFGE genotype 1 or its seven subtypes.

Imipenem resistance genes

All isolates carried *bla*_{OXA-51-like}. The *bla*_{OXA-23} gene (GenBank accession No. GQ861439.1) was found in 54 isolates (65.9%, 54/82). The *bla*_{OXA-58} gene appeared in three isolates.

Among the 39 bloodstream IRAB isolates, the upstream *ISAb*₁ was found in seven (17.9%) with *bla*_{OXA-51-like} and in 25 (64.1%) with *bla*_{OXA-23}, including 18 with Tn2006 (*ISAb*₁–*bla*_{OXA-23}–*ISAb*₁) in an *AbaR4*-type resistance island and seven with Tn2008 (*ISAb*₁–*bla*_{OXA-23}) (Table 1). All of these isolates showed high-level resistance to imipenem (all MIC > 32 mg/L). Two isolates (ST455 and ST208) carried *bla*_{IMP-1}. None carried *bla*_{OXA-40}/*bla*_{OXA-24}, *bla*_{IMP-2}, *bla*_{VIM-1}, *bla*_{VIM-2}, *bla*_{NDM-1}, *bla*_{ADC}, *ISAb*₂, *IS1008*, or Tn2007 (*ISAb*₄–*bla*_{OXA-23}).

Among the 24 IRAB isolates from sputum, the upstream *ISAb*₁ was found in two isolates (8.3%) with *bla*_{OXA-51-like} and in 20 (83.3%) with *bla*_{OXA-23}, including 18 Tn2006 (*ISAb*₁–*bla*_{OXA-23}–*ISAb*₁) in an *AbaR4*-type resistance island and two Tn2008 (*ISAb*₁–*bla*_{OXA-23}) (Table 2). All of these isolates showed high-level resistance to imipenem (all MIC > 32 mg/L). Tn2007 (*ISAb*₄–*bla*_{OXA-23}) was not found in these isolates. The upstream *ISAb*₃ was found in one

Table 1 Sequence types (ST), allelic profiles, clonal complex (CC), imipenem resistance genes, primary source of infection, and attributable mortality of *Acinetobacter baumannii* derived from patients with imipenem-resistant *Acinetobacter baumannii* (IRAB) bacteremia or imipenem-susceptible *Acinetobacter baumannii* (ISAB) bacteremia

ST MLST (B/P)	Bartual's PubMLST allelic profiles	CC	No. of isolates (%)	Carbapenem resistance genes					VAP as a source No. (%)	Mortality (%)
				Tn2006 No. (%)	Tn2008 No. (%)	ISAba1–bla _{OXA-51} No. (%)	ISAba3–bla _{OXA-58} –ISAba3, No. (%)	bla _{IMP} No. (%)		
IRAB										
455/2	1-87-3-2-2-83-3	455	15 (38.5)	10 (66.7)	2 (13.3)	3 (20)	0 (0)	0 (0)	13 (86.7)	13/15 (86.7)
544/2	1-87-3-2-2-97-3	455	4 (10.2)	1 (25)	2 (50)	0 (0)	0 (0)	0 (0)	3 (75)	3/4 (75)
545/2	1-12-3-2-2-104-3	545	4 (10.2)	3 (75)	0 (0)	0 (0)	0 (0)	0 (0)	3 (75)	3/4 (75)
208/2	1-3-3-2-2-97-3	92	3 (7.7)	0 (0)	1 (33.3)	0 (0)	0 (0)	0 (0)	3 (100)	1/3 (33.3)
436/2	1-3-3-2-2-103-3	92	2 (5.1)	0 (0)	0 (0)	2 (100)	0 (0)	0 (0)	2 (100)	1/2 (50)
191/2	1-3-3-2-2-94-3	92	2 (5.1)	1 (50)	1 (50)	0 (0)	0 (0)	0 (0)	1 (50)	0/2 (0)
473/2	1-3-3-2-2-99-3	92	2 (5.1)	1 (50)	0 (0)	0 (0)	0 (0)	0 (0)	2 (100)	1/2 (50)
456/217	11-65-14-20-37-83-15	456	1 (2.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	1 (100)
546/2	1-3-3-2-2-66-3	92	1 (2.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
547/2	1-3-3-2-2-157-3	92	1 (2.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
548/N1	1-15-3-2-2-157-3	—	1 (2.6)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)
549/129	18-3-3-2-1-99-4	555	1 (2.6)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	1 (100)	0 (0)
550/129	18-3-3-2-1-157-4	555	1 (2.6)	0 (0)	1 (100)	1 (100)	0 (0)	0 (0)	1 (100)	0 (0)
551/129	18-15-3-2-1-106-4	—	1 (2.6)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)
ISAB										
208/2	1-3-3-2-2-97-3	92	2 (15.4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (50)	1/2 (50)
552	21-35-2-28-1-145-4	552	2 (15.4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (100)	1/2 (50)
485	33-12-59-11-32-158-5	—	1 (7.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
546/2	1-3-3-2-2-66-3	92	1 (7.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
553	21-35-2-28-1-157-4	552	1 (7.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	1/1 (100)
554	1-31-12-11-4-103-3	—	1 (7.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
679	56-100-137-7-51-99-74	—	1 (7.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
680	21-101-3-28-32-172-5	—	1 (7.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
681	1-102-59-28-1-83-45	—	1 (7.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
683	2-38-42-1-1-185-49	—	1 (7.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

MLST = multilocus sequence typing; VAP = ventilator-associated pneumonia; B/P = Bartual's PubMLST and Pasteur's MLST.

Table 2 Sequence types (ST), allelic profiles, clonal complex (CC), imipenem resistance genes, and attributable mortality of *Acinetobacter baumannii* derived from patients with ventilator-associated pneumonia caused by imipenem-resistant *A. baumannii* (IRAB) or imipenem-susceptible *A. baumannii* (ISAB)

ST (B/P)	Allelic profiles	CC	Number of isolates (%)	Carbapenem resistance genes						Mortality (%)
				Tn2006 No. (%)	Tn2008 No. (%)	ISAb ₁ –bla _{OXA-51} No. (%)	ISAb ₃ –bla _{OXA-58} ISAb ₃ No. (%)	bla _{IMP} No. (%)		
IRAB										
455/2	1-87-3-2-2-83-3	455/2	11 (45.8)	10 (90.9)	1 (9.1)	0 (0)	0 (0)	0 (0)	3/11 (27.3)	
544/2	1-87-3-2-2-97-3	455/2	4 (16.7)	4 (100)	0 (0)	0 (0)	0 (0)	0 (0)	2/4 (50)	
208/2	1-3-3-2-2-97-3	92/2	2 (8.3)	1 (50)	1 (50)	0 (0)	0 (0)	0 (0)	1/2 (50)	
556/N2	1-12-3-2-2-144-3	545	1 (4.2)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	1/1 (100)	
456/217	11-65-14-20-37-83-15	456	1 (4.2)	0 (0)	0 (0)	0 (0)	1 (100)	1 (100)	1/1 (100)	
473/2	1-3-3-2-2-99-3	92/2	1 (4.2)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	
545/2	1-12-3-2-2-104-3	545/2	1 (4.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
555/129	18-3-3-2-1-144-4	555	1 (4.2)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
673/129	18-3-3-2-1-144-73	555	1 (4.2)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	
676/2	1-3-3-2-2-184-3	92/2	1 (4.2)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
ISAB										
436	1-3-3-2-2-103-3	92	1 (16.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
556	1-12-3-2-2-144-3	545	1 (16.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
674	11-20-14-20-37-83-15	456	1 (16.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
675	11-65-14-21-37-83-15	456	1 (16.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
677	55-65-136-65-27-172-57	—	1 (16.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	
678	26-99-43-20-25-83-28	—	1 (16.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	

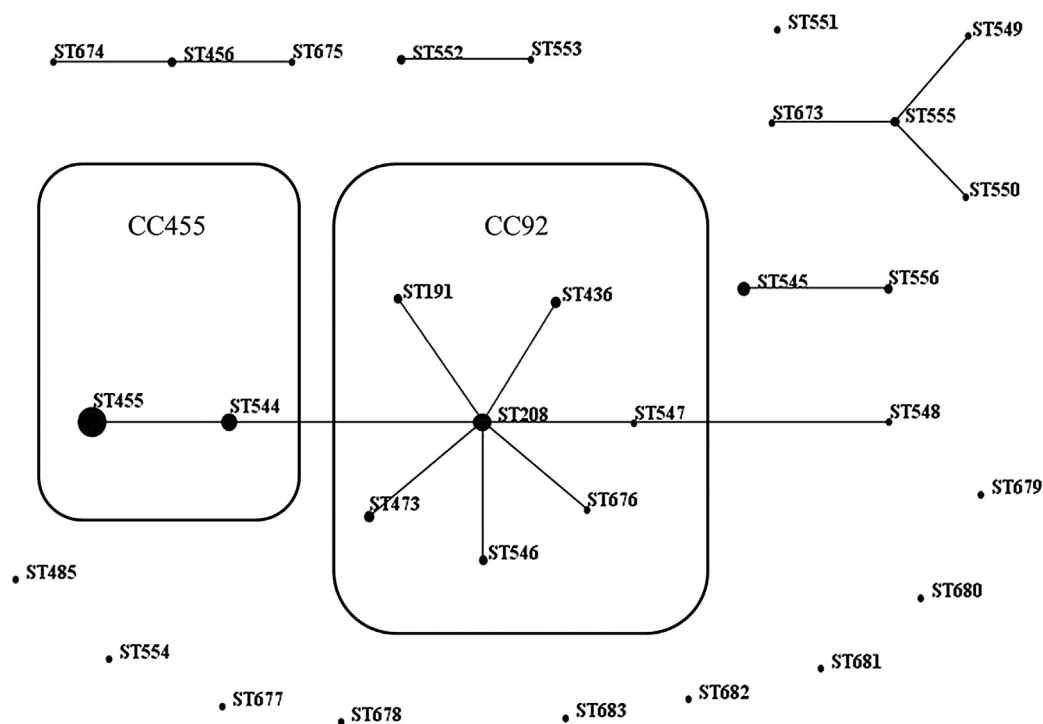


Figure 1. Population snapshot of 82 *Acinetobacter baumannii* isolates derived from patients in this study. Current existing clones in Bartual's PubMLST database analyzed using the eBURST algorithm were shown. The radial diagram reflects the predicted evolutionary descent from the sequence type of the founder. A circle represents a sequence type, and its size corresponds to the number of isolates. CC92 included ST208 with its derivative STs (ST191, ST436, ST473, ST546, ST547, and ST676). Novel CC455, including ST455 and ST544, have a double-locus-variant relationship with ST92 and derived from CC92 following the evolutionary line from ST208 to ST455. In addition to CC455, four other new clonal complexes (CC456, CC545, CC552, and CC555) descent from ST456, ST545, ST552, and ST555 were also found and newly named by this study.

Table 3 Comparison of clinical presentations of patients with ST455^B/ST2^P infections and those with non-ST455^B/ST2^P infections^a among the study population of all patients with bloodstream imipenem-resistant *Acinetobacter baumannii* ST2^P infections

Characteristic	Patients with ST455 ^B /ST2 ^P n = 15	Patients with non-ST455 ^B /ST2 ^P n = 19	p
Demographic characteristics			
Age (y)	64.8 ± 18.1	66.6 ± 18.0	0.930
Male	7 (46.7)	11 (57.9)	0.516
Length of stay in hospital (d)	32.3 ± 26.8	24.3 ± 19.8	0.327
Length of stay in ICU (d)	12.1 ± 4.3	8.8 ± 3.0	0.014
Underlying diseases			
Charlson score	3.7 ± 2.7	4.1 ± 3.0	0.700
Neoplastic disease ^b	7 (46.7)	11 (57.9)	0.516
Metastatic malignancy ^b	3 (20.0)	4 (21.1)	0.940
Cardiac disease	3 (20.0)	6 (31.6)	0.451
Cerebrovascular disease	1 (6.7)	4 (21.1)	0.264
Diabetes	5 (33.3)	1 (5.3)	0.059
Pulmonary disease	3 (20.0)	2 (10.5)	0.445
Hepatic disease	1 (6.7)	2 (10.5)	0.696
Renal disease	7 (46.7)	7 (36.8)	0.564
Peptic ulcer	1 (6.7)	5 (26.3)	0.165
Clinical severity			
APACHE II score	26.6 ± 4.7	23.0 ± 5.0	0.040
Pitt bacteremia score	5.7 ± 1.9	4.2 ± 2.0	0.032
Attributable mortality	13 (86.7)	9 (47.3)	0.026
Inappropriate initial therapy	10 (66.7)	11 (57.9)	0.827

Data are presented as mean ± standard deviation for continuous variables, and number of cases (%) for categorical variables.

APACHE II = Acute Physiology and Chronic Health Evaluation II; ICU = intensive care unit.

^a Isolates of non-ST455^B/ST2^P are those belonging to ST2 in the Pasteur's scheme but not ST455 in the Bartual's scheme. They belonged to ST544, ST545, ST208, ST436, ST191, ST473, ST546, and ST547 in the Bartual's scheme.

^b Neoplastic disease included metastatic malignancy and other primary neoplastic diseases.

isolate with *bla*_{OXA-58}. The isolate with *ISAb3-bla*_{OXA-58} also carried *ISAb3* in the downstream and showed high-level resistance to imipenem (MIC ≥ 16 mg/L). This isolate belonged to ST456. One isolate belonging to ST456 harbored

*bla*_{IMP-1} that offered all these isolates with high-level resistance to imipenem as well. None of the isolates carried *bla*_{OXA-40}/*bla*_{OXA-24}, *bla*_{IMP-2}, *bla*_{VIM-1}, *bla*_{VIM-2}, or *bla*_{NDM-1}, *bla*_{ADC}, *ISAb2*, *IS1008*, or *Tn2007 (ISAb4-bla*_{OXA-23}*)*.

Table 4 Logistic regression analysis of risk factors for mortality of patients with bloodstream imipenem-resistant *Acinetobacter baumannii* infection

Characteristic	Nonsurvivors n = 24	Survivors n = 15	Univariate analysis	p	Multivariate analysis*	p
			Odds ratio (95% CI)		Odds ratio (95% CI)	
MLST genotypes						
ST455 ^B	13 (54.2)	2 (13.3)	7.68 (1.42–41.69)	0.018	11.26 (1.45–87.78)	0.021
ST2 ^P	22 (91.7)	13 (86.7)	2.75 (0.40–18.80)	0.302		
Demographic characteristics						
Age (y)	64.4 ± 17.6	68.9 ± 20.0	0.99 (0.95–1.02)	0.456		
Male	13 (54.1)	10 (66.7)	0.59 (0.16–2.26)	0.442		
Underlying diseases						
Charlson score	3.62 ± 2.90	3.60 ± 2.67	1.003 (0.79–1.27)	0.978		
Clinical severity						
APACHE II score	24.0 ± 5.2	22.1 ± 4.5	1.18 (1.02–1.37)	0.030		
Pitt bacteremia score	5.3 ± 1.9	4.0 ± 2.2	1.39 (0.99–1.95)	0.058		
Inappropriate initial therapy	18 (79.2)	4 (26.7)	10.45 (2.31–47.30)	0.002	14.31 (2.34–87.57)	0.004

Data are presented as mean ± standard deviation for continuous variables, and number of cases (%) for categorical variables.

* All variables with a p value < 0.20 in the univariate analysis were considered to be included in the logistic regression model in the multivariate analysis. A forward stepwise selection process was utilized. We found that only infection by ST455 and inappropriate initial therapy were statistically significant risk factors for mortality.

APACHE II = Acute Physiology and Chronic Health Evaluation II; CI = confidence interval.

There was no significant difference in the number of isolates carrying Tn2006 between blood and sputum IRAB (25/39 vs. 18/24, $p = 0.416$). Similar results were also found in other carbapenem resistance genes (all $p > 0.05$).

Clinical outcomes

Among 34 patients with ST2^P infection (87.2% of IRAB), 15 patients infected by ST455^B/ST2^P had higher attributable mortality ($p = 0.026$), higher APACHE II score ($p = 0.040$), higher Pitt bacteremia score ($p = 0.032$), and longer ICU stay ($p = 0.014$) than 19 patients with non-ST455^B/ST2^P infection (Table 3).

In multivariate logistic regression analysis for mortality of the patients, infection by ST455^B and inappropriate initial antimicrobial therapy were statistically significant risk factors for mortality (Table 4). For IRAB bacteremia, patients given appropriate therapy (early effective antibiotics administered in 2 days) had a lower mortality (31.2%) than those given inappropriate therapy (late therapy or therapy with ineffective agents; 82.6%; $p = 0.002$). Mean culture reporting time was 3 days.

Patients with IRAB bacteremia showed higher mortality (26/39, 66.7%) than those with ISAB bacteremia (3/13, 23.1%; $p = 0.009$). Patients with IRAB bacteremia showed higher mortality (26/39, 66.7%) than those with only IRAB pneumonia (8/24, 33.3%; $p = 0.018$). Patients with ST455 bacteremia showed higher mortality (13/15, 86.7%) than those with non-ST455 bacteremia (16/37, 43.2%; $p = 0.005$).

Discussion

Although Pasteur's scheme is considered to be the gold standard of the MLST method in typing *A. baumannii*,⁶ we found in this study that ST455^B/ST2^P can only be differentiated from non-ST455^B/ST2^P by using Bartual's scheme. ST455^B was first reported in Taiwan, however, it has been reported to be the predominant clone among *A. baumannii* isolates collected from 12 hospitals throughout Japan.²⁰ ST455 also appeared in southern Taiwan (C.H. Chiu, unpublished data). Since the population structure of *A. baumannii* seems to be highly diverse,²¹ it is important to use an MLST scheme with a higher level of resolution to identify any tiny but significant difference. In our study, nine different STs (ST455, ST544, ST545, ST208, ST436, ST191, ST473, ST546, and ST547) by Bartual's scheme could not be differentiated by Pasteur's scheme and thus all nine different ST^P belonged to ST2^B. However, ST455^B/ST2^P infection showed a significantly higher attributable mortality than non-ST455^B/ST2^P (Table 3). The important clinical difference can only be found using Bartual's scheme rather than Pasteur's scheme. All ST455^B/ST2^P isolates were collected from patients in ICUs in our hospital during a 12-month period. We have observed no temporal or geographic association among these isolates. No outbreak was found. By contrast, PFGE genotypes 1–7 could only be differentiated by different MLST genotypes using Bartual's scheme rather than Pasteur's scheme.

Although Bartual's scheme was an important MLST method for genotyping *A. baumannii* worldwide,^{4,6} no data

were found by this method in Taiwan. Fifteen isolates in 2006 were analyzed using only Pasteur's scheme in the Taiwan Surveillance of Antimicrobial Resistance study and all belonged to ST2^P.²² In another study, a total of 19 MLST types defined by Pasteur's scheme were found among the 87 isolates examined in 2010.²³ The predominant MLST genotype, ST2^P, was identified in 42 isolates and ST129^P in 25 isolates.²³ Likewise, ST2^P and ST129^P were the major sequence types in isolates in this study. Again, Bartual's scheme has never been used in previous studies, and identification of novel, clinically significant STs by Bartual's scheme was not possible in previous as well as current studies.^{22,23}

Most isolates in this study belonged to CC92^B/CC2^P, which is currently the most prevalent clonal complex worldwide.^{6,24} Currently, most clinical isolates in Asia, USA, and Australia belong to CC92 and carried *bla*_{OXA-23}.^{25–29} AbaR4-type resistance islands that carried *bla*_{OXA-23} have been detected in IRAB isolates from most Asian countries.²⁵ In China, ST92 was the most prevalent clone and was found in every province of the country.^{26,27} It is apparent that the reason for the rapidly increasing carbapenem resistance in China was the wide dissemination of imipenem-resistant, *bla*_{OXA-23}-like-producing CC92, especially ST92, ST75, and ST138.²⁷ By contrast, carbapenem-susceptible isolates in China had a more diverse genetic background.²⁷ In USA, ST122 and ST208 are the most common and were found in four of the six hospitals surveyed in previous studies.^{28,29} Carbapenemase-encoding genes *bla*_{OXA-23} and/or I *SAb*1-*bla*_{OXA-51}-like were present among the majority of these isolates. First reported in Pittsburgh, St Louis, Los Angeles, and Las Vegas in the USA in 2008–2009,²⁸ ST208 was the founder of our isolates belonging to CC92. ST208 was also found in Denmark,³⁰ Egypt, and China (<http://pubmlst.org/abaumannii/>). In Australia, most clinical isolates of *A. baumannii* also belong to ST92 and carry *bla*_{OXA-23}.²⁴

IRAB appears to be more diverse in South America.^{31–33} However, *bla*_{OXA-23}-like gene and AbaR-type genomic islands widely distributed in CC92 were also found in different CCs. In Brazil, CC104, CC109, and CC113 were found as predominant clones.³³ Most IRAB isolates (72%) carried *bla*_{OXA-23}-like gene in Brazil.³¹ CC104 and CC113 were also found common in Argentina. AbaR-type genomic islands have been detected in most multidrug resistant *A. baumannii* isolates (36/51) in Argentina, Uruguay, and Chile since 1986.³² CC113, CC103, and ST25 were the major clones in Buenos Aires and Rosario and most of these clones carried *bla*_{OXA-23}-like.³³

We identified 26 novel ST^B and five novel CC^B in this study. Novel ST455^B, contributing to 38.5% of IRAB bacteremia, resulted in 86.7% attributable mortality, which was much higher than previously reported.^{19,34} A possible explanation for the successful spread of ST455 in the hospitals is that they have a selective advantage over other strains. Antimicrobial resistance is one such advantage, and we found that all ST455 isolates showed high imipenem resistance (MIC > 16 mg/L) and also resistance to all other tested antibiotics except colistin and tigecycline. In addition to 33.3% of isolates carrying Tn2008 or I *SAb*1-*bla*_{OXA-51}, 66.7% of ST455 bloodstream isolates carried Tn2006 in an AbaR4-type resistance island. It is difficult to give effective antibiotics for treating ST455

infection in the 2 days before culture reporting (mean, 3 days). No administration of appropriate drugs in time apparently resulted in high mortality among patients infected by ST455. This clone might become a threat to public health because higher attributable mortality, higher APACHE II score, and higher Pitt bacteremia score were found from patients with ST455^B/ST2^P infection. Increased virulence of this clone deduced from this result needs further study.

The majority of IRAB ST455 isolates showed a common imipenem resistance genetic determinant, IS*Aba1*–*bla*_{OXA-23}–IS*Aba1* (Tn2006), located in an AbaR4-type resistance island. Our previous study indicated that in Taiwan this transposon had become the most common carbapenem resistance gene in *A. baumannii* since 2009 and has replaced IS*Aba1*–*bla*_{OXA-51}, the predominant resistance mechanism from 1993 to 2007.^{17,19}

ST455 bacteremia appeared to originate from VAP based on three findings of this study: firstly, > 85% of ST455 bacteremia had a primary source of VAP; secondly, no significant difference was found in the distribution of STs and carbapenemase genes between blood isolates from bacteremic patients and sputum isolates from patients with VAP; and thirdly, all three paired isolates from blood and sputum of the same patients showed the same MLST and PFGE genotype, including two patients infected by ST455 (PFGE type 1) and one by ST544 (PFGE type 2).

In conclusion, *A. baumannii* has become the most common pathogen for nosocomial bloodstream infection in ICUs in Taiwan since 2009.¹⁹ ST455^B/ST2^P, the predominant clone of IRAB identified in this study, has been posing a significant threat to patients' health and has the potential to cause a regional or global spread, given its highly pathogenic and resistant nature. Continued surveillance is necessary. It is also important to detect and use effective drugs to treat ST455^B/ST2^P infection earlier at the stage of VAP rather than in the bacteremic stage to decrease mortality.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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