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Epidemiology and resistance features of *Acinetobacter baumannii* isolates from the ward environment and patients in the burn ICU of a Chinese hospital

Yali Gong[1,2], Xiaodong Shen[3], Guangtao Huang[2], Cheng Zhang[2], Xiaoqiang Luo[2], Supeng Yin[2], Jing Wang[1], Fuquan Hu[1], Yizhi Peng[2*], and Ming Li[1*]

[1]*Department of Microbiology, Third Military Medical University, Chongqing 400038, P. R. China*
[2]*Institute of Burn Research, Southwest Hospital, Third Military Medical University, Chongqing 400038, P. R. China*
[3]*Department of Biochemistry and Molecular Biology, Third Military Medical University, Chongqing 400038, P. R. China*

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Acinetobacter baumannii **is an important opportunistic pathogen that causes severe nosocomial infections, especially in intensive care units (ICUs). Over the past decades, an ever-increasing number of hospital outbreaks caused by *A. baumannii* have been reported worldwide. However, little attention has been directed toward the relationship between *A. baumannii* isolates from the ward environment and patients in the burn ICU. In this study, 88 *A. baumannii* isolates (26 from the ward environment and 62 from patients) were collected from the burn ICU of the Southwest Hospital in Chongqing, China, from July through December 2013. Antimicrobial susceptibility testing results showed that drug resistance was more severe in isolates from patients than from the ward environment, with all of the patient isolates being fully resistant to 10 out of 19 antimicrobials tested. Isolations from both the ward environment and patients possessed the β-lactamase genes $bla_{\text{OXA-51}}$, $bla_{\text{OXA-23}}$, bla_{AmpC}, bla_{VIM}, and bla_{PER}. Using pulsed-field gel electrophoresis (PFGE) and multi-locus sequence typing (MLST), these isolates could be clustered into 4 major PFGE types and 4 main sequence types (ST368, ST369, ST195, and ST191) among which, ST368 was the dominant genotype. Epidemiologic and molecular typing data also revealed that a small-scale outbreak of *A. baumannii* infection was underway in the burn ICU of our hospital during the sampling period. These results suggest that dissemination of β-lactamase genes in the burn ICU might be closely associated with the high-level resistance of *A. baumannii*, and the ICU environment places these patients at a high risk for nosocomial infection. Cross-contamination should be an important concern in clinical activities to reduce hospital-acquired infections caused by *A. baumannii*.**

Keywords: *Acinetobacter baumannii*, nosocomial infection, burn ICU, epidemiology, resistance

Introduction

Acinetobacter baumannii has emerged worldwide as an important opportunistic pathogen and is responsible for a variety of hospital-acquired infections in patients with multiple risk factors, such as surgery, burns, major trauma, and is especially prevalent in intensive care units (ICUs) (Peleg *et al*., 2008; Howard *et al*., 2012). This pathogen is highly capable of surviving and spreading in the hospital environment and developing resistance to antimicrobial agents (Antunes *et al*., 2014; Tiwari *et al*., 2015), which may have contributed to the high incidences of *A. baumannii* infection in the last 10 years and its increased transmission between hospitals (Higgins *et al*., 2010; Peleg and Hooper, 2010). As a consequence, considerable attention has been focused on the remarkable ability of *A. baumannii* to cause outbreaks of infection, engage in nosocomial colonization, and acquire resistance to most antibiotics (Dijkshoorn *et al*., 2007; Perez *et al*., 2007; Potron *et al*., 2015).

ICU patients are more vulnerable to the risk of acquiring a nosocomial infection than non-ICU patients. Due to various factors, such as the loss of skin barrier, prolonged hospital stays, and complicated treatment protocols, burn ICU patients are markedly more susceptible to nosocomial infections and present a challenging problem in treatment (Rezaei *et al*., 2011; Oncul *et al*., 2014). However, few studies have focused on the relationship between *A. baumannii* isolates from the ward environment and patients in the burn ICU.

The aim of this study was to characterize β-lactam antibiotic resistance profiles and the genetic relationships among strains isolated from the ward environment and patients in a burn ICU from 1-Jul-2013 to 31-Dec-2013. Epidemiologic and molecular typing data revealed that a small-scale outbreak of *A. baumannii* infection occurred in early August of 2013, and suggest that the ward environment of the burn ICU places these patients at a high risk for nosocomial infection. A multifaceted intervention should be implemented for the treatment, prevention and control of hospital-acquired infections caused by multidrug-resistant *A. baumannii*.

*For correspondence. (Y. Peng) E-mail: yizhipen@sina.com; Tel.: +86-23-65461677; Fax: +86-23-65461677 / (M. Li) E-mail: liming@tmmu.edu.cn; Tel.: +86-23-68772241; Fax: +86-23-68752834

Materials and Methods

Collection and identification of bacterial isolates

Ward environmental *A. baumannii* isolates were collected by swabbing from the burn ICU of the Southwest Hospital from July through December 2013. Clinical *A. baumannii* strains were isolated from patient samples in the burn ICU during the same window period. All non-duplicate samples were processed according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) (Pfaller *et al.*, 2005; Hindler and Stelling, 2007), and isolates were identified using the Biomerieux API20NE system and 16S rRNA gene sequence analysis.

Antimicrobial susceptibility testing

Antibiotic resistance profiles of all *A. baumannii* isolates were determined by the Kirby-Bauer (K-B) disc diffusion method, according to the recommendations and interpretive breakpoints of the CLSI guidelines. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as controls in the antimicrobial susceptibility testing.

Detection of β-lactamase genes in *A. baumannii* isolates

All *A. baumannii* strains were screened for the presence of the β-lactamase genes most frequently reported in China, using standard PCR conditions and the primers listed in Table 1. These genes represent the four classes of β-lactamase sequences identified thus far (denoted A to D). Specifically targeted, were the class A bla_{PER} and bla_{SHV} genes, the class B bla_{IMP}, bla_{VIM}, bla_{SIM}, and bla_{NDM-1} genes, the class C bla_{AmpC} gene, and the class D carbapenemase genes bla_{OXA-23}, bla_{OXA-24}, bla_{OXA-51}, bla_{OXA-58}, and $bla_{OXA-143}$. Purified PCR products

Table 1. Primers used in this study for PCR analysis

Category	Target gene	Sequence (5′→3′)	Size (bp)
Resistance genes	bla_{PER}	ATGAATGTCATTATAAAAGC AATTTGGGCTTAGGGCAGAA	925
	bla_{SHV}	ATTTGTCGCTTCTTTACTCGC TTTATGGCGTTACCTTTGACC	1,051
	bla_{IMP}	GGAATACAGTGGCTTAACTCTC CCAAACCACTAGGTTATCT	188
	bla_{VIM}	GATGTGTTTGGTCGCATA CGAATGCGCAGCACCAG	390
	bla_{SIM}	TACAAGGGATTCGGCATCG TAATGGCCTGTTCCCATGTG	570
	bla_{NDM-1}	GAATGTCTGGCAGCACACTT TTGGCCTTGCTGTCCTTGAT	1,067
	bla_{AmpC}	CGGGCAATACACCAAAAGAC CCTTAATGCGCTCTTCATTTGG	1,049
	bla_{OXA-23}	GATGTGTCATAGTATTCGTCG TCACAACAACTAAAAGCACTG	1,067
	bla_{OXA-24}	ATGAAAAAATTTATACTTCCTATATTCAGC TTAAATGATTCCAAGATTTTCTAGC	828
	bla_{OXA-51}	TAATGCTTTGATCGGCCTTG TGGATTCGACTTCATCTTGG	353
	$bla_{OXA-143}$	AACCTGACACGAGCACATAC CCAGGCATTCCTTGCTTCAT	569
	bla_{OXA-58}	AAGTATTGGGGCTTGTGCTG CCCCTCTGCGCTCTACATAC	599
Housekeeping genes	*gltA*	AATTACAGTGGCACATTAGGTCC C GCAGAGATACCAGCAGAGATACACG	722
	gyrB	TGAAGGCGGCTTATCTGAGT GCTGGGTCTTTTTCCTGACA	594
	gdhB	GTTAACCGAACGTGCAACTG GCATAGGCATAACCACTGTC	717
	recA	CCTGAATCTTCCGGTAAAAC GTTTCTGGGCTGCCAAACATTAC	425
	cpn60	GGTGCTCAACTTGTTCGTGA CACCGAAACCAGGAGCTTTA	640
	gpi	GAAATTTCCGGAGCTCACAA TCAGGAGCAATACCCCACTC	456
	rpoD	ATCGAAATTACCAAACGAAGGTT ACGACAGACCCTGTACGTATGTA	921

Table 2. Detection rate of *A. baumannii* from the burn ICU environment

Sampling points	Sampling quantity	No. of positive isolates	Detection rate (%)
Bedside	13	5	38.5
ECG monitor button	12	5	41.6
Pump button	12	4	33.3
Bed wards	22	3	13.6
Hands (medical staff)	25	2	8.0
Medical vaporizer	7	1	14.3
Doorknob	12	1	8.3
Breathing machine	14	1	7.1
Grill button	10	1	10.0
Drinking straw	6	1	17.0
Mattress	6	1	17.0
Restraint strap	4	1	25.0
Air	14	0	0.0
Elevator button	2	0	0.0
Phone	2	0	0.0
Total	161	26	16.1

were sequenced and the data was subjected to BLAST analysis using the tools provided at the NCBI (National Center for Biotechnology Information) server (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

PFGE analysis

Genomic DNA from *A. baumannii* isolates was extracted and digested with the restriction enzyme *Apa*I, and the digestion products subjected to PFGE using a CHEF DR III apparatus (Bio-Rad) at 14°C, 6 V/cm for 20 h. Gels were stained with ethidium bromide and visualized using the Quantity One package (Bio-Rad). PFGE patterns were analyzed using the Bionumerics platform (Applied Maths). Strains having genomic fingerprints with at least 80% similarity were considered as identical in genotype (Povilonis *et al*., 2013).

MLST

MLST (multi-locus sequence typing) analysis was performed as previously reported (Bartual *et al*., 2005). DNA fragments from seven housekeeping genes (*gltA*, *gyrB*, *gdhB*, *recA*, *cpn60*, *gpi*, and *rpoD*) were PCR-amplified using the primers previously described (Ruan *et al*., 2014). After the purified PCR products were sequenced, the results were submitted to PubMLST (http://www.pubmlst.org) to assign allele numbers and retrieve the corresponding ST types from the PubMLST database.

Results

A. baumannii strains isolated from the burn ICU

Based on the API20NE identification system and 16S rRNA gene sequence analysis, a total of 26 (16.1%) non-duplicate *A. baumannii* strains (numbered 1 to 26) were isolated from 161 different samples collected from the ward environment in the burn ICU of the Southwest Hospital from July through December 2013. The overall detection rate was lower than that reported in a previous study, in which *A. baumannii* was isolated in 22 (39.3%) of 56 ICU environmental samples (Aygun *et al*., 2002). The ward environmental strains were most frequently isolated from the electrocardiogram (ECG) monitor button, bedside, and pump buttons, with detection rates of 41.6%, 38.5%, and 33.3%, respectively (Table 2). Meanwhile, 62 non-duplicate *A. baumannii* strains (numbered 27 to 88) were isolated from 165 patients hospitalized in the burn ICU. Of the 62 strains, 34 were from wound secretions (55%), 16 from sputum (25.8%), 9 from blood (14.5%),

Table 3. Antibiotic susceptibility of *A. baumannii* strains isolated from the ward environment and patients in the burn ICU

Antibiotics	Ward environmental isolates (n=26)			Patient isolates (n=62)		
	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)
Piperacillin	84.6	3.8	11.5	100.0	0.0	0.0
Cefoperazone/sulbactam	76.9	7.7	15.4	96.7	1.7	1.7
Ampicillin/sulbactam	84.6	0.0	15.4	100.0	0.0	0.0
Piperacillin/azobactam	84.6	0.0	15.4	100.0	0.0	0.0
Ceftazidime	84.6	0.0	15.4	100.0	0.0	0.0
Cefotaxime	84.6	11.5	3.8	100.0	0.0	0.0
Cefepime	84.6	0.0	15.4	100.0	0.0	0.0
Imipenem	80.8	0.0	19.2	100.0	0.0	0.0
Meropenem	80.8	0.0	19.2	100.0	0.0	0.0
Amikacin	84.6	0.0	15.4	100.0	0.0	0.0
Gentamicin	84.6	0.0	15.4	98.3	0.0	1.7
Netilmicin	84.6	0.0	15.4	98.3	0.0	1.7
Tobramycin	84.6	0.0	15.4	98.4	0.0	1.6
Ciprofloxacin	84.6	0.0	15.4	98.4	0.0	1.6
Levofloxacin	84.6	0.0	15.4	91.9	6.5	1.6
Sulphamethoxazole/trimethoprim	80.8	3.8	15.4	100.0	0.0	0.0
Polymyxin B	0.0	0.0	100	0.0	10.7	89.3
Minocycline	0.0	11.5	88.5	27.1	50.8	22
Tetracycline	76.9	3.8	19.2	98.4	1.6	0.0

R, resistant; I, intermediate-resistant; S, sensitive

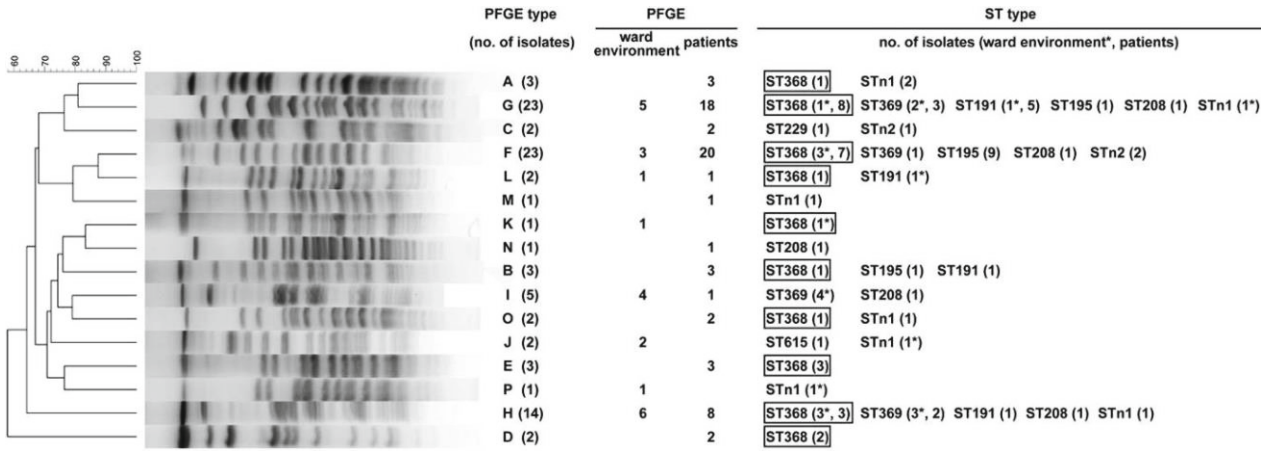

Fig. 1. PFGE and MLST analysis of genomic DNA from ward environmental and patient isolates of *A. baumannii* in the burn ICU. A dendrogram of representative *Apa*I-digested PFGE profiles is shown to the left. Details of the ST types (no. of isolates) are shown to the right. The asterisk indicates the number of ward environmental isolates. The predominant ST type (ST368) is highlighted (boxed).

2 from catheter (3.2%), and 1 from pus (1.5%).

Antimicrobial susceptibility patterns of *A. baumannii* isolates

All *A. baumannii* isolates were subjected to antimicrobial susceptibility testing and the results are shown in Table 3. The ward environmental isolates showed a resistance rate of more than 80% to most of the antibiotics tested (15 out of 19). More importantly, drug resistance was even more severe among the 62 *A. baumannii* isolates from patients hospitalized in the burn ICU. All the patient isolates were found to be fully resistant to 10 out of 19 antimicrobials tested, and over 90% resistant to 7 others. Of note, although none of the ward environmental isolates exhibited resistance to either minocycline or polymyxin B, 27.1% of the patient isolates were resistant to minocycline, and 10.7% of the patient isolates exhibited intermediate resistance to polymyxin B.

Molecular characterization of β-lactamase genes

To characterize the resistance to β-lactamase antibiotics among these *A. baumannii* isolates, selected β-lactamase-encoding genes (specifically, those prevalent in China) were amplified by PCR and then sequenced (Ji *et al.*, 2014; Huang *et al.*, 2015; Liu and Liu, 2015). All four classes of β-lactamase genes were identified in both the ward environmental and patient isolates, including bla_{PER} (class A), bla_{VIM} (class B), bla_{AmpC} (class C), and $bla_{OXA\text{-}51}$ and $bla_{OXA\text{-}23}$ (class D). The detection rates for these genes were 61.5% (16/26), 92.3% (24/26), 88.5% (23/26), 100% (26/26), and 80.8% (21/26) in the ward envi-

Table 4. The relationship between PFGE types and β-lactamase-encoding genes

PFGE type	No. of strains carrying $bla_{OXA\text{-}23}$, bla_{VIM}, and bla_{AmpC} genes											
A	41*#	85*	31*#									
B	70*#	77*#	68*#									
C	49	53*#										
D	25*#	26*#										
E	71*#	82*	84*									
F	7*#	22*#	23*#	47*#	79	54*#	65*#	75*#	80*	42*#	74*#	78*#
	28*#	46*#	55*#	72*#	76*#	86*	87*	50	35	88*	60	
G	10*#	11*#	15*#	16*#	24*#	59	18*#	39*#	40	45*#	48*#	66
	38	30*#	61	73*#	27*#	36*#	56*#	34*#	51*#	44*#	57*#	
H	5*#	13#	14*#	17*#	19*#	21*#	69*#	67	33*#	81*	37*#	58*#
	29*#	32										
I	1*#	3*#	4*#	9*#	64*#							
J	2*	12*#										
K	8*#											
L	6*#	43*#										
M	52											
N	63*#											
O	62*#	83*										
P	20											

The underlined strains are bla_{VIM}-positive isolates. The asterisk indicates $bla_{OXA\text{-}23}$-positive isolates.
The pound sign indicates bla_{AmpC}-positive isolates.

ronmental isolates, respectively. The corresponding rates in the patient isolates were 40.3% (25/62), 70.9% (44/62), 80.6% (50/62), 100% (62/62), and 83.9% (52/62). However, no bla_{SHV}, bla_{IMP}, bla_{SIM}, bla_{NDM-1}, bla_{OXA-24}, or bla_{OXA-58} genes were amplified from any *A. baumannii* strain.

Epidemiologic typing by PFGE and MLST analysis

Genotypic analysis by PFGE revealed the presence of 16 different patterns (designated A to P) that share < 80% similarity among the 88 *A. baumannii* isolates. Types G, F, I, and H were the predominant types (≥ 5 strains) accounting for 73.9% of the strains tested. Figure 1 shows representative PFGE patterns and the distribution of the PFGE types among these *A. baumannii* isolates. The relationship between PFGE types and β-lactamase genes detected in this study are shown in Table 4.

Molecular analysis by MLST revealed 7 sequence types (STs) and 2 newly identified types (STn1 and STn2) among the 88 isolates. Four major sequence types (ST368, ST369, ST195, and ST191) comprise 78.4% of the isolates, with ST368 being the most prevalent (10 out of 16 PFGE patterns). MLST results are summarized in Fig. 1 together with the PFGE data.

Discussion

Previously, we reported the primary isolation rate and the high-level resistance of *A. baumannii* in the burn ICU of our hospital (Gong *et al.*, 2014; Huang *et al.*, 2014). The data presented here provide further evidence of the widespread distribution of multidrug-resistant *A. baumannii* in both the ward environment and among patients in the burn ICU. Of note, *A. baumannii* isolates from hospitalized patients exhibited a greater resistance to antibiotics than those from the ICU environment, this has created significant challenges for effective clinical treatment due to limited therapeutic options. The susceptibility tests show that minocycline and polymyxin are the most effective drugs against *A. baumannii* isolates. Recently it has been suggested that combinations of colistin with meropenem or doripenem have *in vitro* synergistic activity, and should be considered when treating multidrug-resistant *A. baumannii* infections (Le Minh *et al.*, 2015; Park *et al.*, 2016). Unfortunately, antibiotics with a high resistance rate in *A. baumannii*, such as carbapenems, cephalosporins, aminoglycosides, and quinolones, are widely employed in clinical therapy. Overuse and misuse of antibiotics can contribute to the incidence and persistence of hospital outbreaks caused by multidrug-resistant *A. baumannii* (Corbella *et al.*, 2000; Takahashi *et al.*, 2000; Gallego and Towner, 2001; Mahgoub *et al.*, 2002). Global efforts should be made to promote more judicious use of broad-spectrum antibiotics to reduce the emergence of multidrug-resistant *A. baumannii* infections.

The β-lactams are one of the most important and frequently used classes of antibiotics in clinical practice and are the mainstay in the treatment of serious Gram-negative infections (Papp-Wallace *et al.*, 2011; Zervosen *et al.*, 2012). However, some bacteria can produce β-lactamase enzymes which catalyze the hydrolytic cleavage of the β-lactam ring, thus providing resistance to β-lactam antibiotics (Drawz and Bonomo, 2010). Examples include the extended-spectrum β-lactamases (ESBL) of class A, the metallo-β-lactamases (MBLs) of class B, the AmpC-type β-lactamases of class C and the oxacilinases of class D. In the current work, *A. baumannii* isolates from both the ward environment and patients were frequently found to possess β-lactamase genes, including the class A bla_{PER} gene, the class B bla_{VIM} gene, the class C bla_{AmpC} gene, and the class D bla_{OXA-23} and bla_{OXA-51} genes. bla_{OXA-23} and bla_{OXA-51} were previously reported to be the most prevalent class D β-lactamase genes in China (He *et al.*, 2011; Gao *et al.*, 2014; Ma *et al.*, 2015; Wu *et al.*, 2015; Zhou *et al.*, 2015). In our study, the bla_{OXA-51} gene, which is naturally occurring and chromosomally located in *A. baumannii* (Evans and Amyes, 2014), was detected in all 88 *A. baumannii* strains. The high detection rates for these β-lactamase-encoding genes, whose products confer resistance to almost all of the antibiotics used in this study, provide a genetic explanation for the high level of antibiotic resistance among *A. baumannii* isolates from the burn ICU.

Interestingly, we noticed that the detection rates of β-lactamase genes from the ward environmental sources were higher than those from the patient isolates. However, the resistance rate was higher in the patient isolates. We think that the reason for this discrepancy is multifaceted. Production of β-lactamases is only one of the principal resistance mechanisms in *A. baumannii*. Besides this, *A. baumannii* may develop drug resistance through other various mechanisms, including overexpression of efflux pumps, decreased permeability, biofilm formation, and acquisition of mobile genetic elements carrying resistance determinants (Coyne *et al.*, 2011). Previously, it was reported that although *A. baumannii* isolates were related and possessed similar plasmids carrying the carbapenem-hydrolyzing oxacillinase gene, they might show variable levels of resistance to carbapenems (Bertini *et al.*, 2007). The authors attributed this phenomenon mainly to an IS*26*-mediated transposition or recombination process. Similarly, this genotypic-phenotypic discrepancy between antibiotic resistance characteristics has also been reported in other common nosocomial pathogens, such as *E. coli* (Davis *et al.*, 2011) and *Staphylococcus aureus* (Martineau *et al.*, 2000). At present, we don't know what accounts for the discrepancy in this study, and we will investigate this issue further.

PFGE is generally regarded as the "gold standard" of epidemiological typing, and is used to determine the monoclonal pattern of nosocomial outbreaks, the intra- and inter-hospital spread of pathogens, and their persistence in the environment (Tenover *et al.*, 1995). Our PFGE results identified 4 major PFGE types (G, F, I, and H) among the 88 *A. baumannii* isolates. Most notably, 3 non-duplicate strains (Nos. 40, 41, and 46) of type G, which showed indistinguishable genomic fingerprints, were isolated from different hospitalized burn patients in early August of 2013. Using the definition of hospital infection outbreaks, according to which there are more than three cases of homologous infection at the same medical institution within a short time window (Chen *et al.*, 2013), we can infer that a small-scale nosocomial outbreak of *A. baumannii* infection was underway in the burn ICU of our hospital during the sampling period.

Moreover, a single PFGE clonal type can contain different resistance genes, and a given resistance gene can be found in different PFGE types (Table 4).

It should be noted that different molecular typing methods may not corroborate each other (Hamouda *et al*., 2010; Bai *et al*., 2016; Tomaschek *et al*., 2016). Each method has its own advantages and limitations. Despite strenuous efforts at standardization, PFGE data generated in various laboratories are difficult to compare. In contrast, the sequence-based MLST method can be standardized for uniform application in distant laboratories and subsequent data are more easily disseminated and unambiguously compared. Therefore, we have compared the results reported here with the *A. baumannii* MLST database to enhance the PGFE data. In this study, ST368, ST369, ST191, and ST195 were identified as the predominant genotypes by MLST analysis. ST368, ST191, and ST195 were previously reported to be widespread in other areas of China (Chang *et al*., 2015; Ying *et al*., 2015; Zhou *et al*., 2015), our data suggest that these three genotypes may be dispersed throughout China. However, ST92, which is the most frequently isolated and widespread type across the world especially in China (Fu *et al*., 2010; Runnegar, *et al*., 2010; Alvargonzalez *et al*., 2014; Chang *et al*., 2015), was not detected in the current work.

Tracing the sources of ward environmental isolates, we notice that most of them are from the buttons on various medical devices (ECG monitors, pumps, and breathing machines), followed by the bed unit. It should also be emphasized that the hands of medical personnel cannot be ignored as significant vectors for transmission of multidrug-resistant *A. baumannii*, and probably serve as an important reservoir for antibiotic resistance strains in hospitals. These observations indicate that cross-contamination events are very likely to occur between the ward environmental and patient isolates during routine clinical activities. In our burn ICU ward, strict disinfection and sterilization procedures are followed to reduce the transmission of infectious diseases, and include precautions such as three daily environmental decontaminations using chlorine, the use of air disinfection machines, requirements that healthcare workers use alcohol-based hand disinfectants, and the implementation of contact isolation precautions for patients colonized or infected with multidrug-resistant organisms. However, *A. baumannii* remains one of the most frequently encountered nosocomial pathogen in the burn ICU (Gong *et al*., 2014). A multifaceted intervention was reported to be able to reduce multidrug-resistant *A. baumannii* colonization and infection, including strict infection-control measures, enhanced contact isolation, improvement in hand hygiene adherence and rational use of antibiotics (Apisarnthanarak *et al*., 2008). Because of the unique challenges presented by burn ICU patients, periodic epidemiological monitoring and antimicrobial susceptibility testing are extremely important for the effective control of nosocomial *A. baumannii* infections, and can provide critical evidence linking ward environmental exposures to the incidence and prevalence of infection as well as valuable clues for improved clinical practice (Zorgani *et al*., 2015).

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