

Short Communication

Antimicrobial Susceptibility Profiling and Genomic Diversity of Multidrug-Resistant *Acinetobacter baumannii* Isolates from a Teaching Hospital in Malaysia

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SUMMARY: The resistance phenotypes and genomic diversity of 185 *Acinetobacter baumannii* isolates obtained from the intensive care unit (ICU) of a local teaching hospital in Kuala Lumpur from 2006 to 2009 were determined using antimicrobial susceptibility testing and pulsed-field gel electrophoresis (PFGE). Antibiogram analyses showed that the isolates were fully resistant to β -lactam antimicrobials and had high resistance rates to the other antimicrobial agents tested. However, the isolates were susceptible to polymyxin B. Resistance to cefoperazone/sulbactam was only detected in strains isolated from 2007 to 2009. Some environmental isolates and an isolate from the hands of a healthcare worker (HCW) had identical resistance profiles and PFGE profiles that were closely related to patient isolates. Cluster analyses based on the PFGE profiles showed there was a persistent clone of endemic isolates in the ICU environment. The transmission route from HCWs to fomites to patients, which caused a long-term infection in the ICU of the University Malaya Medical Centre, was observed in this study. These data provide a better understanding of *A. baumannii* epidemiology within the hospital and the possible transmission routes. Knowledge of changes in the resistance rates of *A. baumannii* in our local hospital will improve antimicrobial therapy.

Bacteria of the genus *Acinetobacter* are important opportunistic nosocomial pathogens that cause bacteraemia, meningitis, and respiratory and urinary tract infections, particularly in immunocompromised patients (1). *Acinetobacter* spp. are among the most common isolates from intensive care units (ICUs) in most Malaysian hospitals (2). Infection with *Acinetobacter baumannii* is becoming a serious nosocomial problem, particularly in the ICU. Most of the isolates are resistant to multiple antimicrobial agents, and there is a current trend toward increasing resistance to penicillins, β -lactams, aminoglycosides, fluoroquinolones, and carbapenems, which are the drugs of choice for treatment of the infection (3,4). *A. baumannii* is able to acquire antibiotic resistance genes and survive for days on fomites, both in the hospital environment and on the hands of healthcare workers (HCWs), which could lead to possible HCW-fomites-patient transmission and the persistence of endemic *A. baumannii* strains in hospitals (5,6). Appropriate identification of *A. baumannii* and discrimination among isolates during a nosocomial outbreak could lead to a better understanding of the mode of spread, thus enabling better control in an outbreak. Macrorestriction analysis using pulsed-field gel elec-

trohoresis (PFGE) is considered the standard molecular method for epidemiological analyses of *A. baumannii* (7).

In Malaysia, detailed information on the antimicrobial resistance and genetic relationship of *A. baumannii* strains is still lacking. Therefore, the aim of the present study was to determine the antimicrobial susceptibility and genetic relationship of nosocomial *A. baumannii* isolates from the ICU at the University Malaya Medical Centre (UMMC). Documentation of antibiograms and DNA fingerprinting data of *A. baumannii* isolates is important to determine the prevalence of these isolates within the hospital and their transmission in outbreaks in order to provide better outbreak control and effectively manage the patients' infections.

In this study, 185 *A. baumannii* isolates from the ICU at UMMC were used for the analysis. Of these, 170 (92%) were clinical isolates obtained from different sites in 2006 ($n = 61$), 2007 ($n = 25$), 2008 ($n = 47$), and 2009 ($n = 37$) (Table 1). The remaining 15 isolates were from the environment and the hands of HCWs screened in April, August, and September 2006 following the occurrence of increased incidence in March, June, July, and September 2006. The isolates were confirmed as *A. baumannii* by restriction analysis of amplified ribosomal DNA as described by Dijkshoorn et al. (8) (unpublished data).

The antimicrobial susceptibility of *A. baumannii* was determined by the disk diffusion method in accordance with the Clinical and Laboratory Standards Institute

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Table 1. Clinical *A. baumannii* isolates from different types of specimens from the intensive care units of the University Malaya Medical Centre (2006 to May 2009)

Isolation year	No. of isolates from different specimens										Total
	T/sec	T/asp	Sputum	Swab	Catheter tip	Blood	Body fluid	Nasal	Urine	Tissue	
2006	25	2	2	8	10	4	7	1	1	1	61
2007	17	0	0	3	3	1	1	0	0	0	25
2008	21	0	2	7	5	3	6	1	1	1	47
2009	22	0	2	5	2	3	1	0	1	1	37
Total	85	2	6	23	20	11	15	2	3	3	170

T/sec, tracheal secretory; T/asp, tracheal aspirate.

guidelines (9). *Escherichia coli* ATCC 25922 was used as the quality control organism. PFGE was performed by *ApaI* (Promega, Madison, Wis., USA) digestion of chromosomal DNA embedded in 1% SeaKem® Gold Agarose (Cambrex Bio Science, Rockland, Maine, USA) (10). Restriction fragments were obtained by separation using a CHEF DR®III system (Bio-Rad, Hercules, Calif., USA) with $0.5 \times$ TBE buffer for 26 h at 14°C with pulse times of 2–40 s. The PFGE profiles were analyzed using BioNumerics version 6.0 software (Applied Maths, Kortrijk, Belgium).

The present study shows that the *A. baumannii* isolates had very high rates of resistance to the tested antimicrobial agents. All 170 clinical isolates of *A. baumannii* showed 100% resistance to ampicillin, amoxicillin/clavulanic acid, piperacillin/tazobactam, cefuroxime, ceftriaxone, cefoperazone, ceftazidime, and cefepime. These isolates also exhibited high rates of resistance to amikacin (78.8%), gentamicin (85.3%), ampicillin/sulbactam (84.7%), cefoperazone/sulbactam (34.7%), ciprofloxacin (99.4%), imipenem (96.5%), meropenem (98.2%), and trimethoprim/sulfamethoxazole (88.8%).

The rate of resistance to amikacin in the clinical isolates decreased from 70.5% in 2006 to 52.0% in 2007; however, the rate then increased to 91.5 and 97.3% in 2008 and 2009, respectively. Resistance to gentamicin decreased from 91.8% in 2006 to 78.7% in 2008; however, resistance increased slightly to 83.8% in 2009. Similarly, resistance to trimethoprim/sulfamethoxazole decreased to 88.0% in 2007 and 76.6% in 2008 compared to 98.4% in 2006, then increased slightly to 89.2% in 2009. The clinical isolates had a high rate of resistance to ampicillin/sulbactam throughout the 4-year period (82.0% in 2006, 88.0% in 2007, 78.7% in 2008, and 94.6% in 2009). Although no cefoperazone/sulbactam-resistant isolates were observed in 2006, resistance was detected in 2007 (40.0%), and the resistance rates increased to 55.3% in 2008 and 62.2% in 2009. The environmental isolates exhibited 100% resistance to cefoperazone, and had high resistance rates to ampicillin (83.3%) and cefuroxime (61.1%). These isolates had intermediate resistance rates, at varying levels, to the other antimicrobial agents except cefoperazone/sulbactam. All the clinical and environmental isolates of *A. baumannii* were fully susceptible to polymyxin B.

Twenty-seven different resistance profiles (R1–R27) were observed. The predominant resistance profile, R11 (35.1%), comprised isolates resistant to all antimicrobi-

al agents except polymyxin B and cefoperazone/sulbactam. Resistance profiles R23 to R27 comprised 7 non-multidrug resistant (non-MDR) isolates from the environment and the hands of HCWs. Seven MDR environmental isolates shared a similar resistance profile with the clinical isolates (R11).

PFGE analysis generated 98 profiles and the number of DNA fragments ranged from 17 to 29 with sizes from 25.9 kb to 680.1 kb. A dendrogram based on representatives of each PFGE profile was generated (Fig. 1). On the basis of 70% similarity, 8 clusters, A–H (5 isolates or more in each cluster), with 27 clones were observed. A clone was defined as a collection of bacterial isolates independently isolated from different sources and at different times that shared a similar PFGE profile (11). Clones X, XVI, and XXVI were the predominant clones observed in clusters D, G, and H, respectively.

Cluster A consisted of 3 different clones (I, II, and III) with 92.9% similarity, and was comprised of 24 isolates obtained in 2006. These 3 clones were closely related, with less than 4 band differences ($F_{\text{value}} = 0.91\text{--}1.00$). An isolate from the hands of a HCW (ACIBA 47) and 3 MDR environmental isolates were clustered together with the clinical isolates at more than 80% similarity. Clone X (2006 cefoperazone/sulbactam-susceptible isolates) and clone XI (2007 cefoperazone/sulbactam-resistant isolates) were grouped in cluster D with 5 DNA band differences ($F_{\text{value}} = 0.86\text{--}1.00$).

Cluster G comprised 9 clones (XVI–XXIV) with 72.8% similarity. Clones XVI and XVII consisted of 2006 clinical isolates and 3 environmental MDR isolates that were obtained in August 2006 with a similar resistance profile (R11). Clones XVIII to XXIV consisted of 2008 isolates. Cluster H comprised 3 clones (XXV–XVII) of 28 isolates from 2009 with 84.7% similarity and R11 and R13 resistance profiles. The non-MDR environmental isolates were distinctly different from the clinical isolates and had unique profiles.

In the present study, all the clinical *A. baumannii* isolates exhibited high resistance to all the antimicrobial agents tested except for polymyxin B. There was an emergence of imipenem-resistant and amikacin-resistant isolates, which were not found in a previous study from the same hospital (12). A similar increase in the resistance of *A. baumannii* isolates to imipenem and amikacin from 1996 to 2006 was reported in Greece (13). In addition, *A. baumannii* isolates (obtained in 2006–2007) from Singaporean hospitals were also highly resistant to carbapenems (14). Routine use of the same

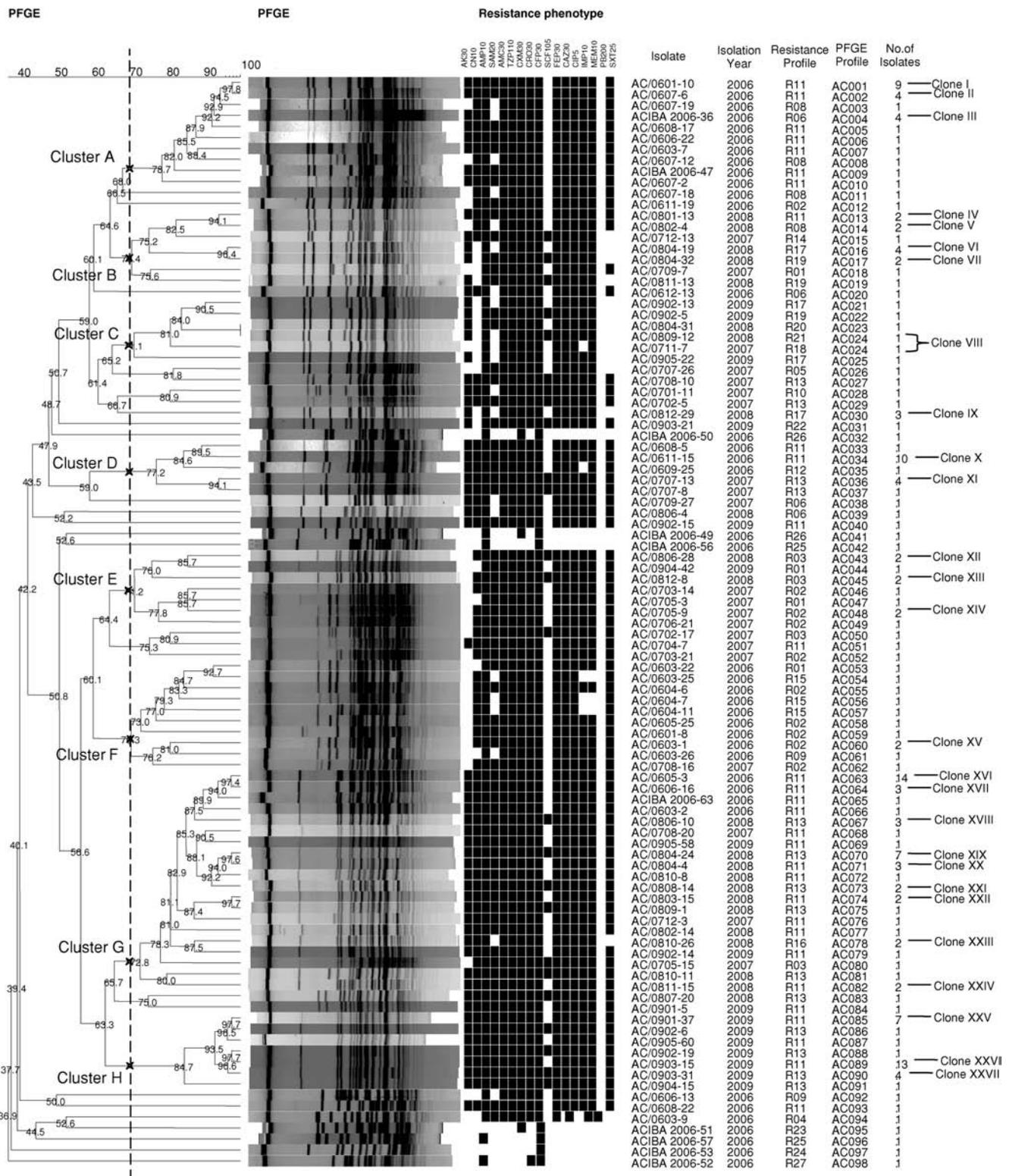


Fig. 1. Dendrogram of representative *A. baumannii* isolates with 27 different resistance profiles using the unweighted pair group arithmetic means methods (UPGMA). The dotted vertical line indicates 70% similarity.

antimicrobial agents for treatment might have contributed to the selective pressure for resistance build-up.

Williams (15) reported that addition of sulbactam, tazobactam, and clavulanic acid as β -lactamase inhibitors could increase the susceptibility of *A. baumannii* to penicillins and cephalosporins. However, in this study, the isolates were 100% resistant to both amoxicillin

clavulanic and piperacillin/tazobactam, and had a high resistance to ampicillin/sulbactam (84.1%). Although the combination of cefoperazone and sulbactam were effective against *A. baumannii* isolated in 2006, there was evidence of diminished effectiveness, since cefoperazone/sulbactam-resistant isolates were detected in the period 2007–2009.

Ballow and Schentag (16) reported that replacement with piperacillin plus an aminoglycoside resulted in recovery of penicillin susceptibility and reduction of ceftazidime resistance in cephalosporin resistant *Enterobacter cloacae* isolates. In our study, substituting cefoperazone/sulbactam for therapeutic treatment might have decreased resistance to aminoglycosides and trimethoprim/sulfamethoxazole. However, increased use of cefoperazone/sulbactam might have resulted in the increased resistance observed in 2007. Therefore, properly scheduled antibiotic class changes are needed to select appropriate drugs for treatment. Polymyxins have been used as the therapeutic option for treatment of MDR *A. baumannii* infections (13,17); however, there have been reports of polymyxins-resistant *A. baumannii* in the US (18) and Korea (19). Fortunately, the *A. baumannii* isolates in our hospital remain sensitive to polymyxin B.

Isolates in this study with PFGE profiles AC034 and AC063 were believed to be endemic in the ICU area throughout the year of 2006. An indication of an endemic clone of *A. baumannii* isolates in the ICU at UMMC was the observed persistence of environmental and multiple subtypes throughout the study period. An isolate from the hands of HCW had a closely related PFGE profile and similar resistance profile to isolates from patients, suggesting likely transmission from HCWs to patients via the hands. The transmission route among HCW-fomites-patient in the ICU at UMMC may have occurred; however, the direction was unknown. Careful hand hygiene and environmental cleaning should contribute to the successful control of MDR *A. baumannii* outbreaks in ICUs (20).

In conclusion, *A. baumannii* isolates from the ICU at UMMC from 2006 to 2009 had high resistance rates to the majority of antimicrobials commonly used in hospitals. Only polymyxin B remains effective for the treatment of infections caused by *A. baumannii*. PFGE analysis confirmed a persistent clone of MDR *A. baumannii* in the ICU environment, which might have led to an increased incidence of infection throughout the year of 2006. Hence, proper management and careful selection of antimicrobial agents for the treatment of *A. baumannii* infection is important for preventing the build-up of resistance.

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Conflict of interest None to declare.

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