BED RAILS AND ENDOTRACHEAL TUBE CONNECTORS AS POSSIBLE SOURCES FOR SPREADING *ACINETOBACTER BAUMANNII* IN VENTILATOR-ASSOCIATED PNEUMONIA PATIENTS

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Abstract. This study aimed to determine molecular patterns of *Acinetobacter baumannii* using a PCR-based technique with REP-1, REP-2 and M13 primers to distinguish the patients' strains and the environmental strains (condensate, endotracheal tube connector, bed rail and nurses hands). There were 67 cases of ventilator-associated pneumonia (VAP) among 600 patients using mechanical ventilators in 10 wards from March to July 2006. The incidence of VAP was 11.2% or 8.9/1,000 ventilator days with a 54.5% fatality rate. Among 19 of 22 *A. baumannii* VAP patients, 68.4% (13/19) had their environmental samples contaminated with *A. baumannii* and the most common contaminated sites were bed rails and endotracheal tube connectors (36.8% each). Multidrug resistant (MDR) *A. baumannii* were involved in 77.3% of *A. baumannii*-VAP. Molecular typing of 96 *A. baumannii* isolates was able to differentiate *A. baumannii* isolates into 7 types. Type 2 was the most common and found in 77.3% (17/22) of *A. baumannii* VAP patients admitted in 6 of 7 wards. Identical fingerprints were found in clinical isolates and their bed rails, endotracheal tube connectors and condensates of 5 patients. The results demonstrate that multiple clones of MDR *A. baumannii* were widely spread in the hospital. Bed rails and contaminated endotracheal tube connectors could be potential sources of *A. baumannii* spread.

INTRODUCTION

Acinetobacter has emerged as an important nosocomial pathogen and commonly isolated from patients, healthcare workers, medical equipment and the hospital environment (Corbella *et al*, 2000). It is also able to develop resistance to the majority of antimicrobial agents (Cisneros and Rodriguez-Bano, 2002; Abbo *et al*, 2005). At Maharaj Nakhon Si Thammarat Hospital, a tertiary healthcare located in the southern Thailand, *A. baumannii* is the most common pathogen causing ventilator associated pneumonia (VAP). It accounts for 23.4-29.9% of all isolated pathogens (Songuman *et al*, 2004). Recently, isolation of multidrug resistant (MDR) *A. baumannii* in a high proportion of cases raised infection control concerns of the spread of the organism to other wards of the hospital. Molecular typing has enabled investigators to distinguish and follow the spread of specific antimicrobial resistant strains among different hospital strains (Swaminathan and Matar, 1993; Bonten, 1999).

There are different molecular typing methods that are currently applied to *Acinetobacter*

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spp (Gouby *et al*, 1992; Seifert *et al*, 1994), among them a PCR-based method using either specific or random primers, such as M13 (Graser *et al*, 1993), repetitive ERIC1 and ERIC2 (Struelens *et al*, 1993), or REP-1 and REP-2 (Reboli *et al*, 1994; Vila *et al*, 1996) are being used increasingly due to the simplicity and rapidity or its usefulness in tracing epidemic strains during outbreaks on a day-to-day basis (Bergogne-Bérézin and Towner, 1996). The objective of the study was to determine the molecular patterns of *A. baumannii* by using a PCR-based technique with REP-1, REP-2 and M13 primers to distinguish the patient strains and hospital environmental strains and their relationship among VAP patients.

MATERIALS AND METHODS

Study design and sample collection

A prospective surveillance study of the occurrence of VAP was conducted at Maharaj Nakhon Si Thammarat Hospital, a 1,000-bed tertiary care hospital, from March 2006 to July 2006. A VAP case was identified by a nosocomial surveillance system (CDC, 2004). A sample size of 600 patients was calculated with a confidence level of 95% with an estimated incidence of nosocomial pneumonia of 50% (Bowton, 1999) and an 8% acceptable error. The endotracheal aspirate was collected aseptically with a sterile mucus extractor (Unomedical, USA) and taken to the laboratory for bacterial isolation and identification using standard microbiological procedures (Vandepitte, 1991). Endotracheal aspirate sampling was repeated in some cases if there was no clinical improvement.

Identification of *A. baumannii* isolates was performed using Gram staining and biochemical tests: gram negative bacilli, non-motile, oxidase negative, glucose O/F +/-, citrate test positive and ability to grow at 44ºC (Bouvet and Grimont, 1987; Bergogne-Bérézin, 2003). When *A. baumannii* VAP was identified, the patient's environmental samples were collected to identify *A. baumannii* contamination from the patient's condensate in the respirator circuit, endotracheal tube connector, bed rail (ventilator site) and the designated nurse's hands. For each *A. baumannii*-positive sample, 2 to 4 isolates were collected for molecular study. A total of 96 isolates, 52 from clinical isolates and 44 from the environmental isolates were kept in glycerol-LB broth at -80ºC for molecular typing. This study was approved by the Ethics Committee of Mahidol University (No. MU2006-031) and the administrator of Maharaj Nakhon Si Thammarat Hospital.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of 48 clinical and environmental *A. baumannii* isolates was determined using the disk diffusion method according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2007). The following nine antibiotics were tested: amikacin, ampicillin, amoxicillin/clavulanic acid, cefotaxime, ceftazidime, cefazolin, gentamicin, imipenem and netilmicin (Oxoid, England). MDR *A. baumannii* was defined as an isolate that was resistant to amikacin, ceftazidime and at least one other third generation cephalosporin (Department of Pathology, 2006).

PCR-based typing method

The genomic DNA was extracted using a modified spin filter-silica based method (NucleoSpin® Tissue kit). The primer sets used in this study were REP-1 5′-IIIGCGCCGIC ATCAGGC-3′, REP-2 5′-ACGTCTTATCAGG CCTAC-3′ (Vila *et al*, 1996), and M13, 5′-GAGGGTGGCGGTTCT-3′ (Graser *et al*, 1993). Amplification reactions were performed in a final volume of 25 µl. Each reaction mixture contained 2.5 µl of 10X PCR buffer, 1 U of Dynazyme Taq EXT (Finzyme, USA), and 200 µM each dNTP. The $MgCl_2$ concentration

was 3 mM and the primers were used at 0.5 µM concentration. Five hundred nanograms of chromosomal DNA was used as a template. A negative control (distilled water) was included in every reaction set. Amplification reactions were carried out in a PTC 100 Thermal cycler (MJ, USA), with initial denaturation of 10 minutes at 94°C, followed by 30 cycles of denaturation (1 minute at 94°C), annealing (1 minute at 45°C) and extension (2 minutes at 72°C), with a single final extension of 16 minutes at 72°C. Amplified products were analyzed by electrophoresis in 1.2% agarose gel containing ethidium bromide (0.2 µg/ml) and detected by an UV transilluminator (BIS 303 PC, Jerusalem, Israel). The PCR fingerprint patterns visualized on gel were saved and analyzed on the basis of similarity in numbers and matching positions of all major bands. Similarity analysis of the data was carried out by the UPGMA clustering method (Geneious 2.5.2; Biomatters, New Zealand).

RESULTS

Incidence and causes of VAP

During the study period, there were 67 cases of VAP among 600 patients using mechanical ventilators in 7 of 10 wards at Maharaj Nakhon Si Thammarat Hospital (Table 1). The incidence rate of VAP was 11.2% (67/600). The incidence density rate was 8.9 per 1,000 ventilator days with a 54.5% fatality rate. Bacteria were identified as causes of VAP in 97% of all cases. Gram-negative bacteria accounted for 59.7% followed by mixed bacterial infection in 25.4% and gram-positive bacteria in 11.9 %. There were 23.9% (16/67) of VAP cases that had *A. baumannii* as a causative agent and 8.9% (6/67) as a co-infection with other bacteria. Thus, *A. baumannii* was the most common causative agent of VAP (32.8%). *Pseudomonas aeruginosa* was the second leading cause of VAP, accounting for 29.9% of all cases, followed by *Klebsiella pneumoniae* in 20.9% (14/67), and *Staphylococcus aureus* in 16.4% (11/67); mixed infection with *A. baumannii* and *K. pneumoniae* in 4.5% and *A. baumannii* and *P. aeruginosa* in 3.0% of all cases.

Environmental sample contamination

Among 22 *A. baumannii* VAP cases, 3 patients (pediatric case numbers 4, 16 and

Table 1
Distribution of VAP and *A. baumannii* VAP patients at Maharaj Nakhon Si Thammarat Hospital during March-July 2006.

Ward[a]	Number of VAP patients	%	Number of *A. baumannii* VAP patients	%
ICU I (General)	6	8.9	3	13.6
ICU II (Medicine)	6	8.9	1	4.5
Male surgical ward (Trauma)	15	22.4	5	22.7
Male surgical ward (General)	1	1.5	0	0.0
Female surgical ward (FS)	4	6.0	1	4.5
Male orthopedic ward	1	1.5	0	0.0
Medical Sub-ICU (Med I)	29	43.3	9	40.9
Female medical (FM) ward	1	1.5	0	0.0
Neonatal ICU I (NICU I)	3	4.5	2	9.1
Neonatal ICU II (NICU II)	1	1.5	1	4.5
Total	67	100	22	100

[a]Wards with patient using a mechanical ventilator

Table 2
Distribution of *A. baumannii* in the environments of 19 *A. baumannii* VAP patients[a].

Sampling sites	Number of samples	Number of *A. baumannii* positive samples	% Positive
Bed rails	19	7	36.8
Nurse's hands	19	3	15.8
Condensate	19	3	15.8
Endotracheal tube connectors	19	7	36.8
Total	76	20	26.3

[a]Three patients died before environmental samples were obtained.

Table 3
Antibiotic sensitivities of 48 *A. baumannii* isolates from the patients (26 isolates) and their environments (22 isolates).

Antibiotics	Resistant (%)			Intermediate (%)			Sensitive (%)		
	Patient	Environ	Total	Patient	Environ	Total	Patient	Environ	Total
Amikacin	73.0	32.0	52.5	0.0	0.0	0.0	27.0	68.0	47.5
Ampicillin	100.0	95.5	97.8	0.0	0.0	0.0	0.0	4.5	2.2
Amoxcillin/clavulanic acid	84.6	59.1	71.8	7.7	22.7	15.2	7.7	18.2	13.0
Cefotaxime	84.6	59.1	71.8	11.6	22.7	17.2	4.0	18.2	11.0
Ceftazidime	84.6	59.1	71.8	0.0	0.0	0.0	15.4	40.9	28.2
Cefazolin	100.0	90.9	95.5	0.0	0.0	0.0	0.0	9.1	4.5
Gentamicin	80.0	50.0	65.0	8.0	6.6	7.3	12.0	43.4	27.7
Imipenem	73.0	36.4	54.7	3.9	0.0	2.0	23.1	63.6	43.3
Netilmicin	53.8	31.8	42.8	0.0	0.0	0.0	46.2	68.2	57.2

17) died before their environmental samples were collected. Therefore, 76 environmental samples were collected from 19 *A. baumannii* VAP cases. Thirteen of 19 patients (68.4%) had environmental samples positive for *A. baumannii*. Table 2 shows there were 20 (26.3%) *A. baumannii*-positive samples among 76 environmental samples. Bed rails and endotracheal tube connectors were the two most common sites (36.8%) for *A. baumannii* contamination, followed by condensate (15.8%) and nurse's hand (15.8%).

Antimicrobial susceptibility testing

All clinical isolates (100%) were resistant to ampicillin and cefazolin, 84.6% were resistant to amoxycillin/clavulanic acid, cefotaxime and ceftazidime. More than 70% of isolates were resistant to gentamicin, amikacin and imipenem. Netilmicin resistance was found the least (53.8%). The environmental isolates were less resistant to the tested antibiotics (31.8-95.5%) than the clinical isolates (53.8-100%). However, antibiotic resistant patterns from the environmental samples were similar to those of the clinical isolates. The antibiotics arranged from the most to the least resistance were ampicillin, cefazolin, ceftazidime, cefotaxime, amoxycillin/clavulanic acid, gentamicin, imipenem, amikacin and netilmicin (Table 3).

The antibiograms of the 48 *A. baumannii* isolates showed 21 different patterns. The most common pattern (39.6% of isolates) was

Table 4
Distribution of *A. baumannii* molecular type by sources of isolation.

Case number	*A. baumannii* molecular type					Ward[b]
	Patient's tracheal aspirate	Bed rail	Endotracheal tube connectors	Condensate	Nurse's hands	
1[a]	1,1	1,2	-	-	1,1	ICU I
2	4,4	-	-	-	-	MS
3	2,2	2,2	-	2,2	-	Med I
4[d]	2,2	ND	ND	ND	ND	NICU II
5	2[c],2[c]	-	-	-	-	Med I
6	2,2	2,2	-	-	-	Med I
7	2,2/ 2,2	-	-	-	7,7	MS
8	1,2[c]	-	2,2	-	-	MS
9[a]	2,2	2,2/2,2	2,2/2,2	2,6	-	Med I
10	2,3	-	2,2	-	-	Med I
11[a]	2,2	2,2	2,2	-	-	Med I
12	1,1	1,2	-	-	-	MS
13[a]	2[c],2[c]/ 1,2[c]/ 2[c],5	5,5	5,5	-	-	ICU I
14[a]	5,5	-	-	1,5	-	ICU II
15	5,5	-	-	-	-	MS
16[d]	2[c],2[c]	ND	ND	ND	ND	NICU I
17[d]	2[c],2[c]	ND	ND	ND	ND	NICU I
18	2,2	-	2,2	-	-	ICU I
19	1,1/ 2[c],2[c]	-	-	-	-	Med I
20	2[c],2[c]	-	-	-	-	Med I
21	2[c],2[c]	-		-	-	Med I
22	2,2	-	2,2	-	2,2	FS
Total isolates	52	16	16	6	6	96

[a]Five patients had *A. baumannii* with identical fingerprints in the patients and their environmental isolates (bold number).
[b]Seven wards with *A. baumannii* VAP cases - ICU I, intensive care unit (general); ICU II, intensive care unit (medicine); MS, male surgical ward (trauma); FS, female surgical ward (general); Med I, Medical sub- ICU; NICU, Neonatal intensive care unit.
[c]Molecular type 2 with identical fingerprints in 8 cases admitted to 4 wards (Med I, MS, ICU I, and NICU I).
[d]Three cases died before collection of environmental samples. (ND, not determined).

resistant to all 9 tested antibiotics, followed by resistance to 8 antibiotics (20.8%). About 54% of the 48 isolates were MDR *A. baumannii*, which were isolated from 17 cases (77.3%) and their environments. Among them, 88.5% were resistant to imipenem.

PCR-based typing

A total of 96 isolates were molecular typed using PCR-based technique with REP-1, REP-2 and M13 primers and differentiated into 7 types with 85% similarity (Fig 1). Type 2 was the most common isolate found in 17/22

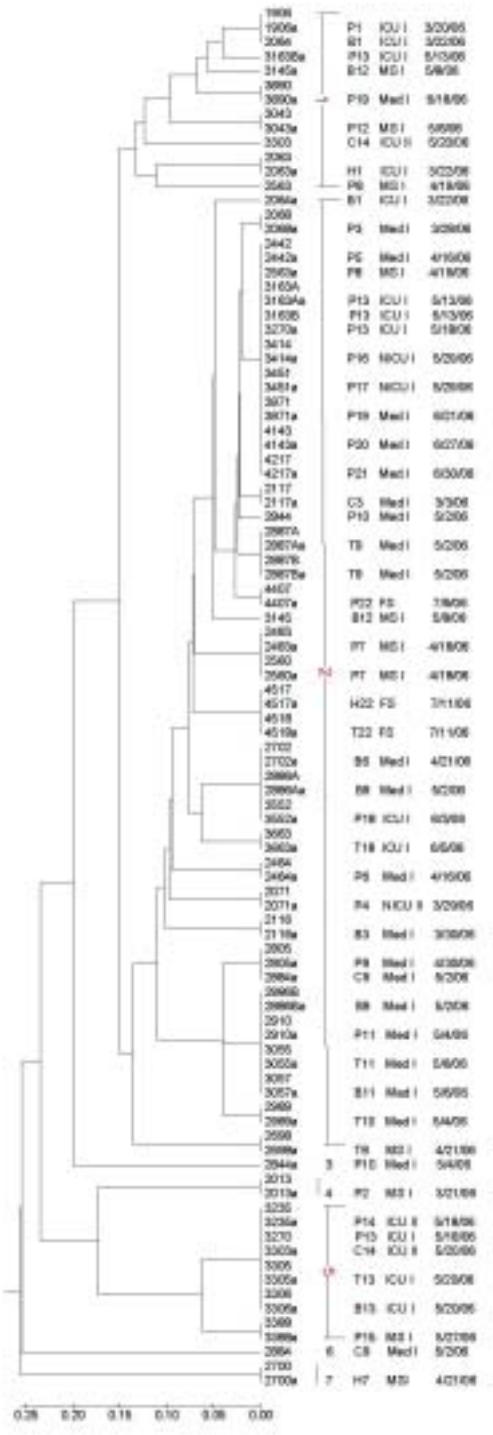

Fig 1–Dendrogram of similarity among patient and environmental isolates of *A. baumannii*, based on REP-PCR and AP-PCR analysis and 7 established types with 85% similarity. The patient's numbers were run according to occurrence of VAP on the ward and date of sample collection. P=patient; B=bed rail; T=endotracheal tube connector; C=condensate; H=nurse's hand.

cases (77%) and 69.8% of 96 isolates. Four patients were infected with 2 or more molecular types. Types 1, 2 and 5 belonged to the clinical and environmental isolates while types 6 and 7 were obtained from environmental isolates only. Type 2 isolates were found in 6 out of 7 wards with *A. baumannii* VAP patients. Types 1 and 5 were found in 3 wards. The medical sub-ICU (Med I) had the highest number of *A. baumannii* VAP patients (9/22), all of whom were infected with type 2 *A. baumannii*. Two (case numbers 10 and 19) of nine cases in this ward also had co-infections with type 3 and type 1, respectively. There were 8 cases infected with type 2 *A. baumannii* having identical fingerprints found in 4 different wards. Of these 8 cases, case numbers 19, 20, and 21 were admitted to the same ward (Med I) at the same period of time (Fig 1 and Table 4).

The most common molecular type in the environmental isolates was type 2 (70%, 31/44 isolates). Fourteen and 12 isolates of type 2 were from endotracheal tube connectors and bed rails, respectively. Identical fingerprints were found in patients, bed rails and endotracheal tube connectors isolated from of 2 patients (case numbers 11 and 13); they were found in patients and bed rail isolates in case number 1, and in patients and condensates isolates case numbers 9 and 14 (Table 4). Three of 19 nurses hand swab samples were positive for *A. baumannii* and one was identical to an endotracheal tube connector isolate.

DISCUSSION

In the past decade, MDR *A. baumannii* has emerged as a significant organism in hospital outbreaks and has caused increasing concern in hospitals worldwide (Abbo *et al*, 2005; Guducuoglu *et al*, 2005; Jeong *et al*, 2006; Poirel *et al*, 2006). In this study, MDR *A. baumannii* was involved in 77.3% of *A. baumannii* VAP cases. Antibiotic sensitivity patterns of microorganisms may alert us to the emergence of MDR *A. baumannii*, but distinction between strains with slight differences in resistance profiles may be difficult to evaluate. Moreover, unrelated strains may exhibit the same antibiograms and changes in the sensitivity patterns may occur during episodes of infection (Dijkshoorn *et al*, 1993). We observed multiple molecular types of *A. baumannii* in our VAP patients which also had different patterns of antibiograms. Together with polymicrobial infection, it explained the difficulty in case management of the VAP patients. The co-infections of different strains or different pathogens can increase the problem of treatment, especially multidrug resistant strains (Joly-Guillou *et al*, 1990).

Molecular typing of *A. baumannii* was able to differentiate *A. baumannii* isolates into 7 types with type 2 as the major type commonly found on several wards in the hospital. This may represent a clonal dissemination of an endemic strain in the hospital during the study period. It is possible this strain was transmitted to patients via the hands of healthcare personnel working in different wards at the same time. Mutation of this endemic strain may also give rise to different molecular types. Bed rails and endotracheal tube connectors were the most common sites contaminated with *A. baumannii* (36.8%) in this study. Identical fingerprints were found in paired isolates (patient and bed rail isolates) in 3 patients. Moving bed rails up or down or leaning over contaminated bed rails, the healthcare staff may easily transfer microorganism from one bed to another. Similar studies have found that bed rails and other objects in the patient environment, such as bedside tables, surfaces of ventilators and infusion pumps, water for nasogastric tubes and sinks are contaminated with *A. baumannii* (Catalano *et al*, 1999; Wang *et al*, 2003). These contaminated objects may be reservoirs for epidemic spread of bacteria in the hospital. *A. baumannii* is able to persist

in a dry environment, such as on bed rails, for up to 9 days after the infected patient is discharged (Catalano *et al*, 1999).

Procedures such as endotracheal suction and manipulation of ventilator circuits increase the opportunity for cross contamination. The risk for cross-contamination can be reduced by using aseptic techniques and sterile or disinfected equipment when appropriate and eliminating pathogens from the hands of personnel (Healthcare Infection Control Practices Advisory Committee, 2004). The contaminated condensate within the ventilator circuits may predispose to VAP or serve as a reservoir for the spread of nosocomial pathogens in the intensive care unit (Craven *et al*, 1984). Our study found 15.8% of condensate samples were contaminated with *A. baumannii*. Identical genotypes with those of the patients were found in 2 of 3 cases. Hence, our study gave evidence to support the recommendation of preventing VAP by routinely monitoring ventilator circuits for the accumulation of condensate (Healthcare Infection Control Practices Advisory Committee, 2004).

Multiple clones of MDR *A. baumannii* were widely spread in all the ICUs and several wards with ventilated patients and their environments in this hospital. One possible mechanism for spread of these strains was via the hands of healthcare personnel who working in different wards at the same time. Infection control measures, particularly hand hygiene and contact isolation, should therefore be emphasized for personnel coming into contact with patients.

A. baumannii is becoming an increasingly important nosocomial pathogen in Thailand and worldwide. It is capable of rapid adaptation to the hospital environment. It will continue causing problems, especially in the intensive care unit, because of increasing antibiotic resistance and persist in the environment for long periods. A combination of infection control measures is required to contain these bacteria. Target surveillance, maintaining good housekeeping, equipment decontamination, routine gloving and gowning, alcohol based hand washing and isolation as well as control of antibiotic usage are the combined approaches most likely to control the spread and emergence of MDR *A. baumannii* in the hospital.

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