Nosocomial Outbreak of Infection With Multidrug-Resistant Acinetobacter baumannii in a Medical Center in Taiwan

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Objective. To investigate a nosocomial outbreak of infection with multidrug-resistant (MDR) Acinetobacter baumannii in the intensive care units at China Medical University Hospital in Taiwan.

Design. Prospective outbreak investigation.

Setting. Three intensive care units in a 2,000-bed university hospital in Taichung, Taiwan.

Methods. Thirty-eight stable patients in 3 intensive care units, all of whom had undergone an invasive procedure, were enrolled in our study. Ninety-four A. baumannii strains were isolated from the patients or the environment in the 3 intensive care units, during the period from January 1 through December 31, 2006. We characterized A. baumannii isolates by use of repetitive extragenic palindromic–polymerase chain reaction (REP-PCR) and random amplified polymorphic DNA (RAPD) fingerprinting. The clinical characteristics of the source patients and the environment were noted.

Results. All of the clinical isolates were determined to belong to the same epidemic strain of MDR A. baumannii by the use of antimicrobial susceptibility tests, REP-PCR, and RAPD fingerprinting. All patients involved in the infection outbreak had undergone an invasive procedure. The outbreak strain was also isolated from the environment and the equipment in the intensive care units. Moreover, an environmental survey of one of the intensive care units found that both the patients and the environment harbored the same outbreak strain.

Conclusion. The outbreak strain of A. baumannii might have been transmitted among medical staff and administration equipment. Routine and aggressive environmental and equipment disinfection is essential for preventing recurrent outbreaks of nosocomial infection with MDR A. baumannii.

Acinetobacter baumannii is an aerobic, non–glucose-fermenting, oxidase-negative, and gram-negative coccobacillus. Because of the simplicity of its growth requirements and its high tolerance of environmental conditions, A. baumannii is ubiquitous in the environment and can be part of the bacterial flora of the human body. In the last decade, it had been increasingly reported as a significant microorganism involved in various nosocomial infections, including pneumonia, septicemia, urinary tract infection, wound infection, and meningitis.

Local environmental contamination during outbreaks of nosocomial infection with A. baumannii has been described, and various typing methods have demonstrated that most patients infected with A. baumannii during an outbreak have the same bacterial strain, suggesting a common origin. Because of its antimicrobial resistance and its resistance to desiccation, A. baumannii can cause outbreaks of multidrug-resistant (MDR) infection. Its capacity to accumulate genes results in high-level antimicrobial resistance, posing a therapeutic challenge.

In 2006, several outbreaks of nosocomial infection with A. baumannii were noted in medical intensive care units. In our study, we collected all the isolates of MDR A. baumannii during a 1-year period to determine, using repetitive extra-
genic palindromic–polymerase chain reaction (REP-PCR), whether there was a predominant clonal strain among these clusters and sporadic events.

METHODS

Bacterial Isolates

A total of 94 isolates of MDR A. baumannii (resistant to all β-lactams [except sulbactam], aminoglycosides, and fluoroquinolones) were recovered from 42 patients in 3 nearby intensive care units (2 medical intensive care units and 1 respiratory care unit of China Medical University Hospital, Taichung, Taiwan), and environmental isolates were recovered throughout 2006, the year in which there were 2 outbreaks. The following data were collected for each patient from whom MDR A. baumannii isolates were recovered: age, sex, hospital ward, and medical procedures (ie, placement of central venous catheter or urinary tract catheter, receipt of mechanical ventilation, surgery, use of nasogastric tube, receipt of parenteral nutrition, or use of antimicrobial agent, during the preceding month).

Bacterial Culture and Antimicrobial Susceptibility

All samples from patients (except blood, which was processed initially with a nonradiometric blood culturing system [Bactec 9000; Becton Dickinson]) or from the environment were streaked across trypticase soy agar with 5% sheep blood (TSA II; Becton Dickinson) and eosin methylene blue agar (Levine EMB agar; Becton Dickinson) and incubated at 35 °C for 18–24 hours. Organisms were identified as A. baumannii by use of an automated microbiologic analysis system (BD Phoenix; Becton Dickinson). The susceptibility of the A. baumannii isolates to various antimicrobial agents was determined by use of the automated system, and these isolates were confirmed to be MDR A. baumannii strains. All MDR A. baumannii strains were stored at −80 °C in Brucella broth (Becton Dickinson) containing 20% glycerol, until experimental procedures were done. The antimicrobial susceptibility of all the isolates (to gentamicin, amikacin, ampicillin-sulbactam, imipenem, ceftazidime, piperacillin-tazobactam, ciprofloxacin, and cefepine) was rechecked by use of the disk diffusion method, following guidelines and criteria from the Clinical Laboratory Standards Institute.9

REP-PCR and Random Amplified Polymorphic DNA (RAPD) Fingerprinting

The genotyping method has been described elsewhere.5,10,11 Stored bacterial isolates were inoculated on 5% sheep blood agar plates and incubated at 37°C. To prepare genomic DNA, bacterial isolates were collected, resuspended in 1 mL of phosphate buffer saline, and centrifuged at 2,000 g for 5 minutes. The bacterial pellets were resuspended in 600 μL of lysis buffer (20 mmol Tris-Cl [pH 7.5], 10 mmol ethylenediaminetetraacetic acid, 40 mmol NaCl, 0.2% sodium dodecyl sulfate, and 200 μg/mL protease K) and incubated at 50°C for 45 minutes. DNA was extracted by use of a phenol-chloroform solution (at a 1:1 ratio). The DNA was precipitated and quantified by use of a spectrophotometer.

REP-PCR uses consensus primers for the REP sequences found in many bacterial chromosomes, including those of A. baumannii. The paired primers REP 1 (5′-IIIGCCGCGICAT-CAGGC-3′) and REP 2 (5′-ACGCTTTAGCAGCCTAC-3′) were used to amplify putative REP-like elements in the bacterial DNA.10 The procedures for amplification by PCR were followed as described elsewhere.10 A negative control containing all components except the DNA extract, which was replaced with 5 μL of sterile distilled H2O, was included in each PCR run, to rule out reagent contamination. The standard strain of A. baumannii (ie, ATCC 19606) was also included, to compare with the isolated strains from patients and from the environment.

The end-products of PCR amplification (12 μL) were subjected to electrophoresis in a 1.5% agarose gel. After electrophoresis, the results were displayed after ethidium bromide staining and photography under UV light. The molecular size of each fragment generated by electrophoresis was determined by comparison with molecular weight standards running simultaneously. The fragments of each strain were compared by visual inspection. If all the visible bands of 2 isolates were the same distance apart, then the fingerprints were considered the same. Both the variations in the intensity of the bands and the shapes of the bands were not taken into account, in accord with previous studies.5,10 Each isolate was run in duplicate, and fingerprint profiles were interpreted without the use of the clinical data.

The RAPD fingerprinting was performed with 0.1 ng of A. baumannii DNA, 0.1 μM of primer p1281 (5′-AACGCGC- AAC-3′) or p1283 (5′-GGATCCCGCA-3′), and standard PCR

FIGURE 1. Representative repetitive extragenic palindromic–polymerase chain reaction (REP-PCR) fingerprints of Acinetobacter baumannii from clinical isolates. Lane M, 100 base-pair DNA ladder; lanes 1–9, REP-PCR genotypes 1–9, respectively. The positions of the size markers are shown at the left margin.
reagents (Protech). The cycling program was as follows: 1 cycle at 94°C for 10 minutes; 36 cycles at 94°C for 1 minute, 45°C for 1 minute, and 72°C for 1 minute 30 seconds; and 1 cycle at 72°C for 10 minutes. The product was analyzed with 2% agarose gel electrophoresis.

**Statistical Analyses**

A comparison of patients with and without MDR *A. baumannii* infection was analyzed by use of the χ² test. A *P* value of less than .05 was considered statistically significant.

**RESULTS**

**Molecular Characterization**

We isolated 94 strains of *A. baumannii* (73 from patients and 21 from the environment) from 3 nearby intensive care units. All of these isolates were found to be MDR strains of *A. baumannii*, as determined by use of an automated microbiologic analysis system and confirmed by use of the disk diffusion method. The 94 isolates of MDR *A. baumannii* were grouped into 9 distinct REP-PCR patterns (Figure 1). Among these 9 genotypes, the most common was type 1, accounting for 75 (79.8%) of the 94 isolates: 66 from 38 different patients and 9 from the environment in the 3 intensive care units in our study. The other 19 samples were determined to be non-type 1 (7 from patients and 12 from the environment). To further assess these strains, we used RAPD to distinguish bacterial chromosomes. Our results showed 9 distinct RAPD profiles of these strains, similar to the observations made with REP-PCR (data not shown). These data suggest that type-1 MDR *A. baumannii* was probably the major causative microorganism of the outbreak in our hospital.

**Isolation of MDR *A. baumannii***

The distribution of bacterial isolates and infected patients during the different months of 2006 is shown in Figure 2. The type 1 strain was more prevalent than the other strains among the environmental isolates, and its numbers increased during the periods from January to March and from August to November. The number of patients infected with the type 1 strain also increased during the same periods (*n* = 38). We further analyzed the sources from which these bacterial isolates were recovered. The most common source was sputum (32 isolates), followed by catheter tips (15 isolates), urine (9 isolates), wounds (8 isolates), blood (6 isolates), and body fluid (3 isolates [from pleural effusion, bile, and ascites]). Within 1 month before a culture positive for MDR *A. baumannii*, these 38 patients had undergone an invasive procedure (placement of a central venous catheter, 32 patients [84.2%]; use of a mechanical ventilator, 33 [86.8%]; placement of a Foley catheter, 34 [89.5%]; and placement of a nasogastric tube, 37 [97.4%]). We then analyzed the causality between common invasive procedures and a later culture positive for MDR *A. baumannii* in these 3 intensive care units.

As shown in the Table, if the proportion of patients with or without MDR *A. baumannii* infection is considered, patients with a central venous catheter or a Foley catheter and patients who underwent hemodialysis were significantly more likely to have an MDR *A. baumannii* infection (*P* < .01). Most patients received broad-spectrum antibiotics: 71.1% of patients had received antipseudomonal β-lactam antibiotics, and 34.2% had been treated with imipenem or meropenem. Regarding underlying diseases, 44.7% of patients had type 2 diabetes mellitus, and 26.3% had end-stage renal disease requiring hemodialysis. These data suggested that colonization or infection with the outbreak strain might be transmitted by healthcare staff and/or equipment.

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**Figure 2.** Time distribution of recovery of multidrug-resistant *Acinetobacter baumannii* isolates and identification of infected patients during 2006.
Several infection control procedures other than standard precautions were implemented after this outbreak occurred, including contact barrier precautions, vigorous environmental cleaning and disinfection, and the cohorting of healthcare personnel. Unlike the effectiveness of the intervention during the outbreak in March 2006 (the number of MDR *A. baumannii* isolates recovered decreased from April to July 2006), the effectiveness of the intervention during the outbreak in October 2006 was only short-lived. The occurrence of MDR *A. baumannii* infection was noted after 1 month. The ineffectiveness of these interventions could be the result of a number of factors. The first factor is partial disinfection. Environmental disinfection was confined to the vicinity of the infected patients and did not include the whole unit or ward. New patients were transferred to the intensive care units from the emergency department, other wards, or the outpatient department. These patients might have harbored strains of *A. baumannii*. This type of cross-transmission could have been avoided by the use of isolation procedures in which culture was used to determine whether a patient was infected or not. The second factor is inadequate disinfection. *A. baumannii* has a high environmental persistence; it can survive under dry conditions for 1–3 weeks. Thus, postdisinfection monitoring or regular environmental sampling might have a role to play in infection control. In addition, contaminated niches in the medical environment or equipment might remain undiscovered, and searching all possible locations is suggested. Thus, the eradication of MDR *A. baumannii* might be difficult to achieve. The persistent promotion of hand hygiene, awareness of the importance of infection control, the cleaning and/or disinfection of medical instruments and the environment, restraining the unnecessary use of antibiotics, and aggressive infection control interventions should be considered, especially for the long-term control of MDR *A. baumannii* infection.

In conclusion, outbreaks of nosocomial infection with *A. baumannii* are serious, because of the organism’s high rate of resistance to currently available antibiotics and the seriousness of infection. Aggressive infection control procedures and the disinfection of medical equipment and the hospital environment are essential for preventing recurrent outbreaks of MDR *A. baumannii* infection in a hospital setting.
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