Jpn. J. Infect. Dis., 66, 245-248, 2013

Short Communication

A Single Clone *Acinetobacter baumannii* Outbreak in a State Hospital in Turkey

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(Received June 27, 2012. Accepted February 25, 2013)

SUMMARY: *Acinetobacter baumannii* is an important pathogen in hospitalized patients, particularly those in the intensive care unit (ICU). A total of 21 *A. baumannii* (6 from 5 patients and 15 from environmental samples) were isolated in the ICU and the isolation room of a state hospital in June 2011. The possible source of the outbreak was investigated. *A. baumannii* isolates were identified using conventional biochemical tests, BBL Crystal Identification Systems, OXA-51 specific PCR, and 16S rDNA sequencing. All the isolates were multidrug-resistant, showing resistance to cephalosporins, carbapenems, fluoroquinolones, and the aminoglycoside group of antibiotics. Pulsed-field gel electrophoresis suggested that all *A. baumannii* isolates were derived from a common source.

Acinetobacter baumannii isolates are important opportunistic pathogens in different units of hospitals, particularly the intensive care unit (ICU). They can cause various infections such as pneumonia associated with endotracheal tubes or tracheostomies, endocarditis, meningitis, skin and wound infections, peritonitis, and urinary tract infections (1–5). The major risk factors for *A. baumannii* infections include trauma, mechanical ventilation, immunosuppression, surgical procedures, intravenous catheterization, tracheostomy, enteral nutrition, and the use of antibiotics such as third-generation cephalosporins, fluoroquinolone, or carbapenem (4–8). *A. baumannii* outbreaks mainly originate from an environmental source such as patient's beds, air conditioners, monitors, telephone receivers, or ventilation equipments (5,8). Information regarding the clonal relationship between *A. baumannii* isolates may aid in preventing the spread of resistant strains among patients and improving the efficiency of control measures in hospitals.

This study aimed to analyze the clinical and bacteriological properties of an *A. baumannii* outbreak observed among patients in the ICU and isolation room (IR) of 82.Yil Rize State Hospital, a 250-bed facility, in June 2011.

We analyzed 6 clinical samples from 5 patients and 43 environmental samples from the 10-bed ICU and 5-bed IR, which is next to the ICU. The type of samples is shown in Table 1. All the samples were plated on eosin-methylene blue (EMB) agar and blood agar. The presumptive *Acinetobacter* isolates, which were oxidase-negative, non-lactose fermenting, and Gram-negative diplococci, were identified to the species level using conventional biochemical tests, growth ability at 37°C and 44°C, and BBL Crystal Identification Systems (Enteric/Nonfermenter ID Kit; Becton Dickinson and Co., Paramus, N.J., USA) (9). The isolates were also identified using both OXA-51 specific PCR (10) and 16S rDNA sequencing (11).

Antibiotic susceptibility of the isolates was determined by the Kirby Bauer disk diffusion method (12,13). Ampicillin-sulbactam (10 μg/10 μg), piperacillin (100 μg), netilmicin (30 μg), cefepime (30 μg), ceftazidime (30 μg), ceftriaxone (30 μg), imipenem (10 μg), meropenem (10 μg), gentamicin (10 μg), amikacin (30 μg), levofloxacin (5 μg), trimethoprim-sulfamethoxazole (1.25 μg/23 μg), colistin (10 μg), and tigecycline (15 μg) antibiotic disks (Oxoid, Hampshire, UK) were used. *Pseudomonas aeruginosa* (ATCC 27853) was included as a quality control strain. For colistin and tigecycline, the interpretive criteria of Gales et al. (10) and Jones et al. (14), respectively, were applied. The imipenem-resistant strains detected by the disk diffusion method were confirmed by the imipenem E-test (Oxoid).

The clonal relationship of the isolates was determined by pulsed-field gel electrophoresis (PFGE) using the *Apa*I restriction enzyme (15). The PFGE results were evaluated according to the interpretation criteria of Tenover et al. (16). Molecular typing and evaluation of the PFGE results were performed at the Turkish National Public Health Agency, Molecular Microbiology Research and Application Laboratory, Ankara, Turkey.

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Table 1. Epidemiological markers of the 21 *A. baumannii* isolates

Origin of isolates	Date of isolation	No. of isolate	Antibiotype			PFGE type
			AK	GN	AK + GN	
Isolation Room						
Edge of the bed of Patient-1	10.06.2011	37	R	R	a	I
Irrigation solution of Patient-3	10.06.2011	13	R	R	a	I
Mattress of the bed of Patient-3	10.06.2011	10	R	R	a	I
Sputum of Patient-1	04.06.2011	587	R	R	a	I
Sputum of Patient-2	08.06.2011	591	R	R	a	I
Sputum of Patient-3	09.06.2011	627	R	R	a	I
File of Patient-3	10.06.2011	15	S	S	b	I
Surface of the bed of Patient-3	10.06.2011	9	R	R	a	I
Surface of the medicine cabinet	10.06.2011	41	S	S	b	I
Surface of the mechanical ventilation of Patient-1	10.06.2011	38	R	R	a	I
Surface of the mechanical ventilation of Patient-3	10.06.2011	17	S	S	b	I
Surface of the shelf of Patient-1	10.06.2011	40	I	S	c	I
Surface of the shelf of Patient-3	10.06.2011	16	R	R	a	I
Tip of the aspirator of Patient-1	10.06.2011	42	R	R	a	I
Tip of the aspirator of Patient-3	10.06.2011	14	R	R	a	I
Intensive Care Unite						
Sputum of Patient-4	20.06.2011	642	R	R	a	I
Wound of Patient-5	24.06.2011	641	R	R	a	I
Urine of Patient-5	15.06.2011	629	R	R	a	I
File of Patient-4	10.06.2011	35	R	R	a	I
Surface of the adjustable table of Patient-5	10.06.2011	6	R	R	a	I
Surface of the medicine cabinet	10.06.2011	30	R	R	a	I

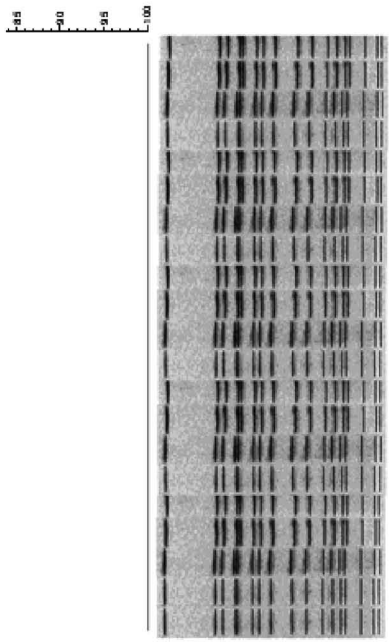
AK, amikacin; GN, gentamicin; S, susceptible; R, resistant; I, intermediate resistant.

A. baumannii clinical isolates were cultured from wound (1 isolate), urine (1 isolate), and sputum (4 isolates) samples from 5 patients, 2 of which had respiratory failure associated with pneumonia, one had chronic obstructive lung disease (COLD), one had hemorrhagic infarction and respiratory failure, and one underwent surgery due to a traffic accident. Four of the patients were relatively older, received long-term antibiotic treatment, had prolonged hospitalization, and were mechanically ventilated. All the patients died as a result of *A. baumannii* sepsis. A total of 15 *A. baumannii* isolates were recovered from the 43 environmental samples. Of the 15 isolates, 12 were isolated from the IR and 3 were isolated from the ICU. Detailed information regarding the isolation source and epidemiological information of the isolates are provided in Table 1.

Antimicrobial susceptibility testing of the patient isolates revealed that all the isolates were susceptible to colistin, tigecycline, and netilmicin, but resistant to piperacillin, cefepime, ceftazidime, ceftriaxone, imipenem, meropenem, gentamicin, amikacin, levofloxacin, trimethoprim-sulfamethoxazole, and ampicillin-sulbactam. Three of the 15 environmental isolates, which were recovered from the mechanical ventilator, medicine cabinet, and patient file in the IR, were susceptible to amikacin, gentamicin, colistin, tigecycline, and netilmicin, but resistant to all the other antibiotics. One isolate recovered from the surface of a shelf in the IR was susceptible to gentamicin, colistin, tigecycline, and netilmicin; intermediate resistant to amikacin; and resistant to all the other antibiotics. The remaining environmental isolates were susceptible to colistin, tigecycline, and netilmicin but resistant to the other antibiotics. All the isolates from the patient and environmental samples in our study were determined to be multidrug resistant (MDR) according to the description of Peleg et al. (17).

Based on the resistance patterns to the antibiotics (amikacin and gentamicin), which showed variation among the isolates tested, 3 different antibiotypes (a, b, and c) were identified (Table 1). PFGE typing of the 21 *A. baumannii* isolates yielded a unique PFGE profile, which comprised 14 DNA fragments resulting from restriction of the chromosome with *Apa*I enzyme. As shown in Fig. 1, regardless of the isolation source and date, all the clinical and environmental isolates exhibited indistinguishable pulsotypes.

A. baumannii is an emerging opportunistic pathogen that causes a wide range of nosocomial infections and exhibits MDR worldwide (18,19). In this study, MDR *A. baumannii* isolates were recovered over a relatively short period (from June 4 to June 25, 2011) from various surfaces in the IR and ICU as well as from patient samples. This was thought to be an outbreak in this hospital due to the failure to comply with rules regarding contact isolation precautions and inadequate hygiene, which resulted in cross-contamination. PFGE confirmed that the *A. baumannii* environmental and clinical isolates originated from a single clone. In accordance with our results, *A. baumannii* outbreaks originating from patient environments have been reported from different hospitals (5,7,9,18,20). In contrast to PFGE typing results, antibiotyping revealed one main group including 80.1% of the isolates (type a, 17 isolates) and 2 small groups, type b including 3 isolates and type c including 1 isolate. Thus, it may be possible



Number of strains	Source	Date of isolation	Place of isolation
41	Surface of the medicine cabinet	10.06.2011	Isolation room
17	Surface of the mechanical ventilation of Patient-3	10.06.2011	Isolation room
16	Surface of the shelf of Patient-3	10.06.2011	Isolation room
37	Edge of the bed of Patient-1	10.06.2011	Isolation room
10	Mattress of the bed of Patient-3	10.06.2011	Isolation room
38	Surface of the mechanical ventilation of Patient-1	10.06.2011	Isolation room
642	Sputum of patient 4	20.06.2011	intensive care unit
30	Surface of the medicine cabinet	10.06.2011	intensive care unit
641	Wound of patient 5	24.06.2011	intensive care unit
587	Sputum of patient 1	07.06.2011	Isolation room
13	Irrigation solution of Patient-3	10.06.2011	Isolation room
9	Surface of the bed of Patient-3	10.06.2011	Isolation room
591	Sputum of patient 2	07.06.2011	Isolation room
35	File of patient 4	10.06.2011	intensive care unit
6	Surface of the adjustable table of patient 5	10.06.2011	intensive care unit
42	Tip of the aspirator of Patient-1	10.06.2011	Isolation room
629	Urine of patient 5	15.06.2011	intensive care unit
14	Tip of the aspirator of Patient-3	10.06.2011	Isolation room
15	File of patient 3	10.06.2011	Isolation room
627	Sputum of patient 3	16.06.2011	Isolation room
40	Surface of the shelf of Patient-1	10.06.2011	Isolation room

Fig. 1. Pulsed-field gel electrophoresis (PFGE) profiles of the 21 *A. baumannii* isolates. All the clinical isolates such as strain nos. 587, 591, 629, 627, 641, and 642 had indistinguishable PFGE profiles, and all of these shared an identical DNA band profile with the environmental isolates such as strain nos. 10, 14, 15, 16, 17, 37, 38, 40, 41, and 627 in the isolation room and 6, 30, 35, and 42 in the intensive care units.

that the loss of resistance genes results in the production of clonally related strains exhibiting different antibiotic resistance patterns. In accordance with our data, previous studies also indicated discrepancies between antibiotyping and PFGE typing of the *A. baumannii* isolates (9,21).

Because of the intrinsic or acquired-resistance mechanisms, it is difficult to treat *A. baumannii* infections (22). Previously, carbapenems were known to be the most effective antibiotics; however, recently, the use of broad-spectrum cephalosporins such as ceftazidime and cefotaxime, carbapenems, and fluoroquinolones has led to an increase in MDR among *Acinetobacter* spp. (18,23). In particular, in the last few years, carbapenem-resistant *A. baumannii* strains have become prevalent worldwide (18). In the present study, the *A. baumannii* isolates were MDR. All the patient and environment isolates were susceptible to tigecycline, colistin, and netilmicin, few were also susceptible to amikacin and gentamicin, whereas all were resistant to other antibiotics, including carbapenem.

A. baumannii isolates can survive for several weeks in the hospital environment (24). The prevention of nosocomial infections caused by these bacteria can be accomplished by active surveillance and contact isolation of colonized and infected patients, aseptic care of endotracheal and vascular tubes, and general improvements in hand hygiene of healthcare workers, which is the most difficult measure to implement. Despite the presence of standard infection control measures, the emergence of this outbreak was thought to be caused by several factors. These mainly included the insufficient number of personnel and lack of compliance with rules regarding entrance into the ICU. Thus, infection control training for all hospital staff, particularly new personnel who work in the ICU, was provided by the hospital infection control committee. This has contributed to the reduction of bacterial transmission between the staff and patient; patient and patient; staff, equipment, and patient; and patient units. As a result of these attempts, no new outbreak has been recorded in facility.

Acknowledgments This study was presented at the 1st National Clinical Microbiology Congress, Antalya, Turkey, November 12–16, 2011.

Conflict of interest None to declare.

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