Epidemiologic characterization of nosocomial *Acinetobacter baumannii* infections in a Turkish university hospital by pulsed-field gel electrophoresis

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Background: Although members of the *Acinetobacter* genus are not commonly part of the human flora, their relatively high prevalence in hospital environment frequently results in colonization of the skin and respiratory tract.
Objectives: The present investigation was carried out to elucidate epidemiologic characteristics of nosocomial *Acinetobacter baumannii* infections in a teaching hospital.
Methods: Epidemiologic, clinical, and demographic features of the 66 patients with *A baumannii* infection during a 14-month period were recorded. Antibiotic susceptibilities of the isolates were determined by the standardized disk-diffusion method, and the clonal relationship of the isolates was analyzed by pulsed-field gel electrophoresis (PFGE).
Results: The incidence of *A baumannii* infection was especially high in January, April, May, and June 2006. The isolates were most frequently obtained from blood and tracheal aspirates sent from the intensive care unit and neurosurgery ward. Although the most frequently identified predisposing factors were cerebrovascular disease and surgical operation, the main risk factors identified in these patients were catheterization and mechanical ventilation. Genotype analysis of the 66 *A baumannii* strains by PFGE revealed the circulation of 36 different PFGE types, of which type A (12) and K (17) accounted for 44% of the isolates. We found high clonal relationship (80.3%) among the typed strains. Thirteen antibiotypes were observed. Most of the isolates were multidrug resistant. Resistance to imipenem, meropenem, gentamicin, amikacin, tobramycin, netilmicin, ampicillin-sulbactam, trimethoprim-sulfamethoxazole, piperacillin-tazobactam, cefoperazone-sulbactam, ciprofloxacin, and levofloxacin were found in 44%, 47%, 47%, 84.8%, 21.2%, 3%, 62.1%, 57.6%, 94%, 62.1%, 95.5%, and 95.5% of the isolates, respectively.
Conclusion: The epidemiologic data obtained suggested that the increase in the number of *A baumannii* infections in our hospital was caused by the interhospital spread of especially 2 epidemic clones. We determined that clonally related strains can survive for a long time in our hospital and cause nosocomial infections in the predisposed patients. (Am J Infect Control 2009;37:56-64.)

Although members of the *Acinetobacter* genus are not commonly part of the human flora, their relatively high prevalence in hospital environment frequently results in colonization of the skin and respiratory tract of hospitalized patients.[1,2] *Acinetobacter* species had been considered as relatively harmless organisms in the past, but in recent years increasing numbers of hospital-acquired infections, especially in the patients with underlying disease and those who are hospitalized in intensive care units (ICU), caused by these organisms have been recorded. Outbreaks involving multidrug-resistant *Acinetobacter* strains have been reported worldwide.[3-6] The prevalence of these organisms is evidenced by the fact that *Acinetobacter baumannii* is the second most frequent nonfermenter encountered in clinical laboratories.[7]

Apart from being intrinsically resistant to certain classes of antibiotics, *A baumannii* strains can develop or acquire resistance easily to a wide variety of antibacterial agents.[8,9] Extensive use of antimicrobial chemotherapy within hospitals has contributed to the emergence and increase in the number of *A baumannii* strains that are resistant to a wide range of antibiotics, including broad-spectrum β-lactams, aminoglycosides, and fluoroquinolones.[10,11] Because of this multiple antibiotic resistance, physicians usually struggle with therapeutic difficulties during the management of nosocomial infections caused by these organisms. *A baumannii* outbreaks are also difficult

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to control because this microorganism easily spreads and persists in hospital settings, thus favoring the transmission between patients, either via human reservoirs or via inanimate materials.[12] Understanding of the epidemiology of nosocomial *A baumannii* infections is essential to develop effective strategies to control their spread. The use of modern molecular techniques, such as pulsed-field gel electrophoresis (PFGE) and polymerase chain reaction-based typing, has shown to be suitable for the investigation of hospital outbreaks.[13,14]

An increase in the number of cases of *A baumannii* infection was observed from January 2006 to July 2006 and from October 2006 to February 2007 at the Research and Implementation Hospital of Suleyman Demirel University Medical Faculty, Isparta, Turkey. The objectives of the present study were (1) to investigate the clinical characteristics and predisposing factors of patients from whom *A baumannii* had been isolated; (2) to assess the genetic relatedness of *A baumannii* isolates at this medical center during the study periods; and (3) to analyze the antimicrobial susceptibility of the *A baumannii* isolates.

MATERIALS AND METHODS

Patients and bacterial isolates

A total of 66 *A baumannii* strains were isolated from 66 patients hospitalized in a 440-bed capacity university hospital during a period from January 2006 to February 2007. Epidemiologic, clinical, and demographic features of these patients were recorded by following chart reviews and conversations with physicians. The collected clinical data included the patient's age, gender, days of hospital stay, sites of infection, time from admission to acquisition, medical comorbidities, and major risk factors (eg, urinary catheters, intravenous catheterization, mechanical ventilation). Nosocomial infections were defined by standard Centers for Diseases Control and Prevention definitions.[15]

All presumptive *A baumannii* isolates, which were oxidase-negative, nonlactose fermentative, and gram-negative diplococci, were identified to species level by using BBL Crystal Identification Systems (Enteric/Nonfermenter ID Kit; Becton Dickinson and Company, Sparks, MD) and were stored at −80°C in nutrient broth containing glycerol 20% vol/vol until performing the genotyping analysis.

Of the 66 *A baumannii* strains, 24 were obtained from adult ICU, 10 from neurosurgery, 7 from orthopedics, 7 from respiratory medicine, 5 from neurology, 10 from other internal medicine services, and 3 from other surgery departments. Clinical specimens included blood, endotracheal aspirate, pleural fluid, postoperative wounds, sputum, and urine (Table 1).

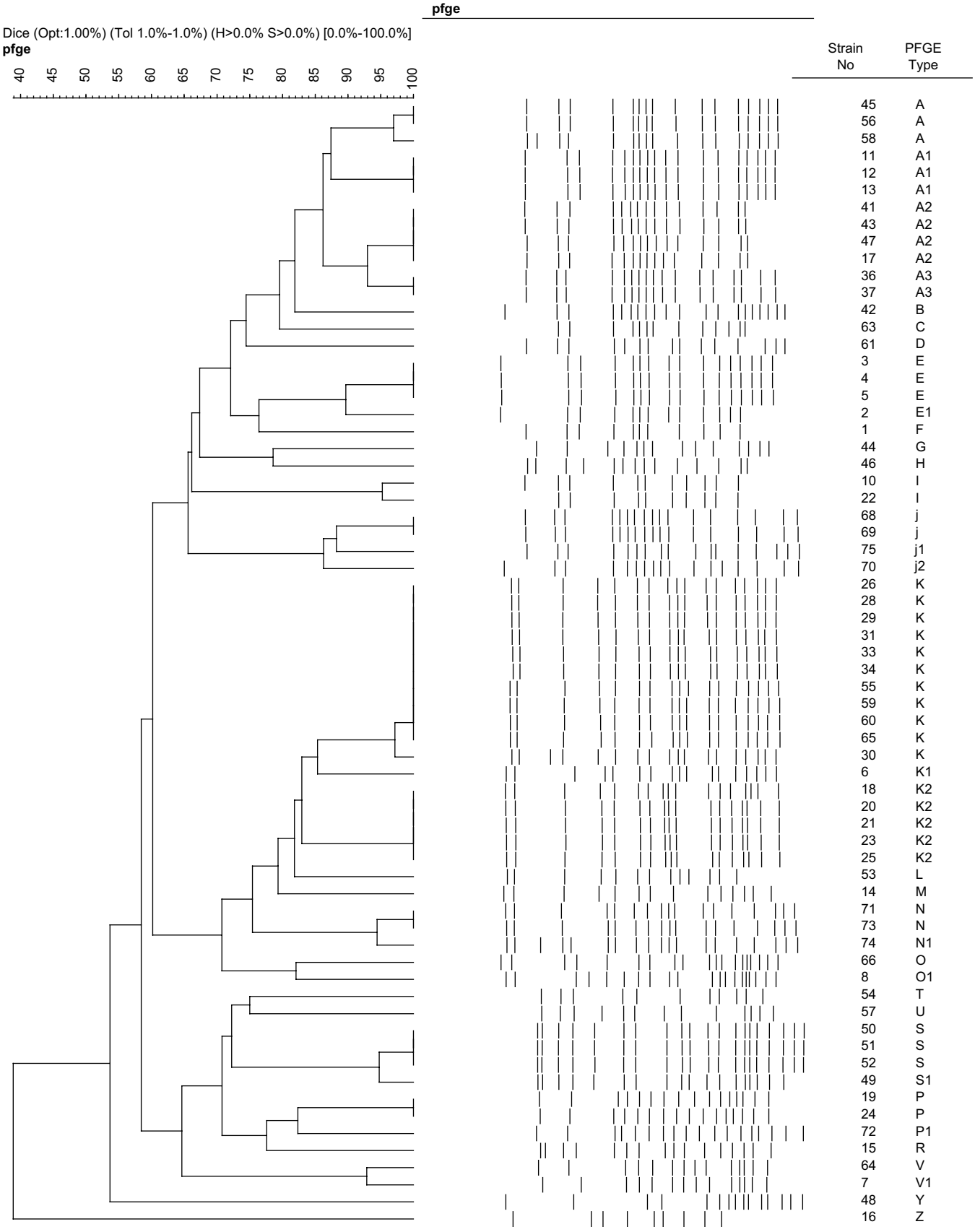
Table 1. Distribution of the 66 *A baumannii* strains according to specimen type and clinics

Clinics	Blood	TA	Wound	Sputum	Urine	PF	No. of total
ICU	12	9	-	1	2	-	24
Neurosurgery	3	2	-	3	2	-	10
Orthopedics	-	-	6	-	1	-	7
Respiratory medicine	-	1	1	5	-	-	7
Neurology	2	2	-	-	-	1	5
Internal medicine	4	-	3	-	3	-	10
Other surgery	-	1	2	-	-	-	3
Total	21	15	12	9	8	1	66

TA, Tracheal aspirate; *PF*, Pleural fluid; *ICU*, intensive care unit.

Typing of the strains

Molecular typing by PFGE and dendrogram analysis. We used a rapid PFGE protocol developed by Durmaz et al.[16] Briefly, the bacterial cells in 2% low-melting point agarose (Gibco BRL, Paisley, United Kingdom) plugs were lyzed in the cell lysis solution 1 (CLS 1) (CLS 1: 50 mmol/L Tris-HCl, 50 mmol/L EDTA, lysosim [2.5 mg/mL], proteinase K [1.5 mg/mL], pH 8.0) and the CLS 2 (CLS 2: 0.5 mol/L EDTA [pH 8.0], 1% sarcosyl, and proteinase K [400 μg/mL]). The plugs were washed 3 times with sterile ultrapure water (Reagent Grade Type 1) and additionally 3 times with TE buffer (10 mmol/L Tris-HCl, 0.1 mmol/L EDTA, pH 7.6), 4 mL for each washing step. Bacterial DNA in one fourth of each plug was restricted with 30 U *Apa*I enzyme (Promega Corporation, Madison, WI). Fragmented DNA was electrophorezed in 1% pulsed-field certified agarose (Bio-Rad Laboratories, Hercules, CA) by using the CHEF-DR II system (Bio-Rad Laboratories, Nazareth, Belgium). The electrophoresis conditions were 14°C at 6 V/cm^2 for 20 hours. The initial and final switch times were 5 seconds and 30 seconds, respectively. The gel was stained with ethidium bromide (5 μg/mL) for 20 minutes, visualized under ultraviolet (UV) light, and photographed by using gel logic 2200 imaging system (resolution: 1708 × 1280 pixel; Kodak Company, Rochester, NY). The DNA band profiles were analyzed with GelCompar software (version 3.0; Applied Maths, Sint-Martens-Latem, Belgium). DNA profiles on each gel were normalized using the external reference strains running on 3 lanes on each gel. A 1% band tolerance was used for comparison of DNA profiles. According to criteria of Tenover et al,[17] the strains were evaluated as indistinguishable, closely related, possibly related, or different.


pfge
Dice (Opt:1.00%) (Tol 1.0%-1.0%) (H>0.0% S>0.0%) [0.0%-100.0%]
pfge
40 45 50 55 60 65 70 75 80 85 90 95 100
Strain No
PFGE Type
45 A
56 A
58 A
11 A1
12 A1
13 A1
41 A2
43 A2
47 A2
17 A2
36 A3
37 A3
42 B
63 C
61 D
3 E
4 E
5 E
2 E1
1 F
44 G
46 H
10 I
22 I
68 j
69 j
75 j1
70 j2
26 K
28 K
29 K
31 K
33 K
34 K
55 K
59 K
60 K
65 K
30 K
6 K1
18 K2
20 K2
21 K2
23 K2
25 K2
53 L
14 M
71 N
73 N
74 N1
66 O
8 O1
54 T
57 U
50 S
51 S
52 S
49 S1
19 P
24 P
72 P1
15 R
64 V
7 V1
48 Y
16 Z

Antimicrobial susceptibility testing and antibiotyping. Antimicrobial susceptibility of the strains was investigated by the standardized disk-diffusion method following the criteria of the Clinical and Laboratory Standards Institute.[18] *Pseudomonas aeruginosa* ATCC 27853 was used as an internal control. The antibiotic disks (Oxoid Limited, Basingstoke, Hampshire, England) used were ticarcillin (75 μg), piperacillin (100 μg), ampicillin-sulbactam (10/10 μg), piperacillin-tazobactam (75/10 μg), cefoperazone-sulbactam (75/30 μg), cefotaxime (30 μg), ceftriaxone (30 μg), ceftazidime (30 μg), aztreonam (30 μg), meropenem (10 μg), imipenem (10 μg), gentamicin (10 μg), amikacin (30 μg), tobramycin (10 μg), netilmicin (30 μg), tetracyclin (30 μg), minocyclin (30 μg), ciprofloxacin (5 μg), levofloxacin (5 μg), trimethoprim-sulfamethoxazole (1.25/23.75 μg), colistin (10 μg), and tigecycline (15 μg). Isolates showing an intermediate level of susceptibility were classified as resistant except minocyclin, colistin, and tigecycline. Susceptibilities to colistin and tigecycline were evaluated according to the zone criteria suggested by Jones at al[19,20] (≤11-mm inhibition zone for resistance and ≥17 mm for susceptible to colistin; ≤12 mm for resistance and ≥16 mm for susceptible to tigecycline). Susceptibility to cefoperazone-sulbactam was evaluated according to the zone criteria suggested by Bradford and Sanders (≤15 mm for resistance and ≥20 mm for susceptible).[21]

Antibiogram typing profiles were defined by using the results, which showed differences in susceptibilities among the isolates, and arbitrarily designated as I through XIII. Briefly, the isolates were at first classified according to their resistance to carboxi-ureidopenicillins, third-generation cephalosporins (cefotaxime, ceftriaxone, and ceftazidime), monobactams (aztreonam), and carbapenems (imipenem, meropenem). On the second step, resistance to aminoglycosides (gentamicin, amikacin, tobramycin, and netilmicin) was determined. Finally, resistance to ampicillin-sulbactam, piperacillin-tazobactam, tetracyclin, ciprofloxacin, levofloxacin, trimethoprim-sulfamethoxazole, and cefoperazone-sulbactam were evaluated. Resistance to minocyclin, colistin, and tigecyclin were also evaluated. The isolates displaying the same resistance profile were classified in the same antibiotype.

RESULTS

A baumannii isolates and patients

A total of 66 *A baumannii* strains were isolated from the 66 patients hospitalized in a 14-month period. Of the 66 *A baumannii* strains, 43 were isolated from January 2006 to July 2006, and 23 were isolated from August 2006 to the end of February 2007. The incidence of *A baumannii* infection was especially high in January, April, May, and June 2006. *A baumannii* strains were most frequently isolated from blood, tracheal aspirates, and postoperative wound specimens sent from ICU, neurosurgery, and orthopedics wards (Table 1). The mean age of the patients was 42.5 (range, 20-72) years, and there was a predominance of males (42/24). The length of hospital stay ranged from 10 to >155 days. Average of the bacterial isolation time following admission was 9 days (range, 4-45 days). The most common predisposing factor was cerebrovascular disease (n = 29), especially for patients in the ICU, neurosurgery, and neurology wards. Other underlying diseases were pneumonia (n = 10), especially for the patients in respiratory medicine service, trauma (n = 8), and urinary infection (n = 2) wards. All patients had undergone one or more invasive procedures, and all had received antibiotics before the isolation of *A baumannii*. Catheterization (urinary or intravenous; n = 42), mechanical ventilation (n = 37), and surgery (n = 17) were the main risk factors identified in these patients.

PFGE types of *A baumannii* strains

PFGE typing of the 66 *A baumannii* strains yielded 36 PFGE patterns (Fig 1). Twelve of these patterns (A, A1, A2, A3, E, I, J, K, K2, N, P, S) were indistinguishable including 42 strains (grouping rate, 63.6%), 6 (E1, K1, N1, S1, V, V1) were closely related (9.1%), and 5 (J1, J2, O, O1, P1) were possibly related (7.6%). PFGE types A and K were the predominate types including 12 and 17 clonally related strains, respectively. A total of 53 (80.3%) genotyped strains showed clonal relationship. On the other hand, 13 PFGE patterns (19.7%) were different; 8 of these sporadic PFGE types were isolated from January 2006 to July 2006, whereas 5 were isolated from August 2006 to the end of February 2007 (Table 2).

Susceptibilities to antimicrobial agents and antibiotyping

Most of *A baumannii* isolates were multidrug resistant. All isolates were resistant to third-generation cephalosporins, aztreonam, and tetracyclin. Only 1 of the strains presented resistance to tigecycline (PFGE type B). Resistance to minocyclin was detected in only 1 (1.5%) strain; of the remaining 65 strains, 12

Fig 1. A denrogram showing clonal relationship of the 66 *A baumannii* strains. The strains having identical PFGE profile were marked with the same capital letters, the strains having 2 to 6 band differences from the ancestor clone were showed with digits at the right side of the letters.

(18.2%) were recorded as of intermediate sensitivity, whereas 53 (80.3%) were fully susceptible to minocyclin. Resistance to colistin was detected in 8 (12.1%) of the strains tested, intermediate sensitivity was detected in 17 (25.8%) of the strains, and 41(62.1%) strains were susceptible to colistin. Resistance rates to other antibiotics tested are listed in Table 3.

Thirteen antibiotypes named as I to XIII were observed among the isolates tested. Antibioypes I, V, VII, and VIII included 38 (57.6%) of the 66 strains. Antibiotype I (16 strains) was the most frequently determined antibiotype. The common characteristic of these strains was their resistance to most of the antibiotics tested except carbapenems, netilmicin, and tobramycin. The other most frequently determined antibiotypes were antibiotype VII (8 strains) and VIII (7 strains). All strains in these antibiotypes were resistant to carbapenems, carboxi-ureidopenicillins, broad-spectrum cephalosporins, monobactams, and amikacin, and they were susceptible to other aminoglycosides tested (netilmicin, tobramycin, and gentamicin). In addition, antibiotype VIII was resistant to all other antibiotics tested except minocyclin, tigecyclin, and colistin, and antibiotype VII was susceptible to ampicillin-sulbactam, trimethoprim-sulfamethoxazole, and cefoperazone-sulbactam. Antibiotype V was detected among 7 strains, which were susceptible to ampicillin-sulbactam, trimethoprim-sulfamethoxazole, and cefoperazone-sulbactam and aminoglycosides except amikacin. Characteristics of the other antibiotypes can be seen in Table 3.

Comparison of 2 typing results with each other and epidemiologic data

Using 2 typing methods together decreased the number of the strains in groups and increased the number of types. Two common PFGE types (type K and type A) were subdivided by antibiotyping. Four distinct antibiotyping groups (VI, VII, VIII, and IX) were determined in the PFGE type K including 17 strains. Six strains were classified as group VII, 5 were group VI, 4 were group IX, and 2 were group VIII. Among the 12 strains in the PFGE type A, 3 antibiotypes (groups I, II, and III) were determined. Antibiotype group I included 7 strains, group II included 4 strains, and group III included 1 strain.

No correlation was found between typing results and the origin of the strains. Moreover, neither clonally related strains nor the strains in the same antibiotype were restricted in a specific time period. Of the 17 strains in the most common PFGE type K, 13 strains were isolated in March (2), April (6), and May (5), and the remaining 4 strains were isolated in November (2) and December (2). The second most frequently determined PFGE type was A, consisting of 12 strains. Six of these strains were isolated in the first half and the remaining 6 were isolated in the second half of 2006. Similarly, the 16 strains in the most frequent antibiotype (I) were detected almost on every month of the year and from every ward of the hospital (Table 2).

DISCUSSION

A baumannii causes a threat to hospitalized patients, and many outbreaks because of this bacterium have been reported in different countries.[9,22,23] During recent years, it has become clear that most clinical isolates of *A baumannii* belong to groups of closely related strains, referred to as clones, that spread geographically at the national or international level.[12] Although certain clones present with widespread dissemination, isolates of *A baumannii* from hospitals in the same country, or even from within a single hospital, may show significant genetic diversity.[24,25] In the present study, molecular typing revealed the circulation of 36 different PFGE types, of which type A (12) and K (17) accounted for 44% of the isolates examined. High clonal relationship (80.3%) among the typed strains supported high dissemination rate of *A baumannii* strains among the patients in our hospital. The epidemiologic data obtained in this study suggested that the increase in the number of *A baumannii* infections in our hospital was caused by the interhospital spread of especially 2 epidemic clones (PFGE types K and A), which coexisted with epidemiologically unrelated sporadic strains.

We determined that clonally related strains can survive for a long time in our hospital and cause nosocomial infections at various times. For example, PFGE type A was first isolated from a patient in the ICU in January 2006, and other strains in this clone were isolated from the ICU and other wards of the hospital during the following months throughout that year. Although clonal relatedness among the strains continued for a long period, their drug susceptibility profiles were changeable. Some of the strains in the same clone were determined as susceptible to carbapenems, netilmicin, and tobramicin during the first half of 2006, but the other clonally related strains isolated during the second half of 2006 were resistant to tobramycin or netilmicin, and they were only susceptible to carbapenems, minocyclin, and tigecyclin. Although most of the strains that were detected to be clonally related with PFGE and that were isolated on close dates presented similar antibiotic susceptibility patterns, the genotype or antibiotype of the strains isolated from the same ward was not always the same. This is in agreement with other previous data showing that sequential

Table 2. Characterization of *A baumannii* isolates according to PFGE type, antibiotypes, clinics, and date of isolation

PFGE types (n)	Antibiotypes (n)	Clinics	Date of isolation (no. of strains isolated)
A (3)	I	İntensive care unit	Feb 06
	II i (2)	Neurosurgery, respiratory medicine	Dec 06, Jan 07
A1 (3)	I (3)	Neurosurgery, intensive care unit, other surgery departments	Jun 06
			Jan (2) 06
A2 (4)	I (3)	Internal medicine, orthopedics, other surgery departments	Jun, Feb, Oct 06
	III	Internal medicine	Sept 06
A3 (2)	II i(2)	Internal medicine, intensive care unit	Oct 06, Feb 07
B	IV	İntensive care unit	Jan 07
C	V	Neurology	Jun 06
D	I	Neurosurgery	Feb 06
E (3)	V (3)	Internal medicine (2)	Jun, May 06
		Neurology	Feb 06
E1 (1)	V	Internal medicine	Jul 06
F	I	Respiratory medicine	Jun 06
G	III	Orthopedics	Jan 07
H	I	Neurosurgery	Oct 06
I (2)	I (2)	Neurosurgery, neurology	May, Nov 06
J (2)	I (2)	Neurosurgery, intensive care unit	Jan (2) 07
J1 (1)	I i	İntensive care unit	Jul 06
J_2 (1)	I i	İntensive care unit	May 06
K (11)	VI (3)	Respiratory medicine, internal medicine, orthopedics	May, Apr (2) 06
	VII (3)	Internal medicine, neurology (2)	Mar (2), Apr 06
	VIII (2)	İntensive care unit (2)	Apr, May 06
	IX (3)	Orthopedics, intensive care unit, OS	Dec (2), Nov 06
K_1 (1)	IX	Orthopedics	Nov 06
K_2 (5)	VI (2)	Neurosurgery, intensive care unit	Apr (2) 06
	VII (3)	Internal medicine, intensive care unit (2)	May (3) 06
L	X	Respiratory medicine	Jan 06
M	X	Neurosurgery	Jan 06
N (2)	VIII i	İntensive care unit	Mar 06
	XI i	Neurosurgery	Jan 07
N_1 (1)	VIII i	Internal medicine	Apr 06
O (1)	V	Orthopedics	Aug 06
O1 (1)	V	İntensive care unit	Jun 06
P (2)	VIII (2)	Respiratory medicine, intensive care unit	May, Apr 06
P1 (1)	VIII	Respiratory medicine	Jul 06
R	XII	İntensive care unit	Jan 06
S (3)	XIII (3)	Respiratory medicine	Feb 07
		İntensive care unit (2)	Jun, Dec 06
S1 (1)	XIII	İntensive care unit	Feb 07
T	XII i	Orthopedics	Dec 06
U	XII i	İntensive care unit	Dec 06
V (1),	VII	İntensive care unit	May 06
V1 (1)	VII	İntensive care unit	Jul 06
Y	VI	İntensive care unit	Feb 06
Z	X	Neurosurgery	Jan 06

NOTE. Each of A1, A2, and A3 PFGE types was clusters, and they were also possible related with PFGE type A clone; PFGE type E1 was closely related with E clone; each of J1 and J2 PFGE types was possibly related with J clone; PFGE type K1 was closely related with K clone, and K2 clone with 5 strains was possible related with K clone; PFGE type N1 was closely related with N clone; PFGE type O and O1 were possible related; PFGE type P1 was possible related with P clone; PFGE type S1 was closely related with S clone; PFGE types V and V1 were closely related.

i, isolates showing intermediate resistance to minocyclin.

A baumannii epidemics in the same ward were caused by different clones, one replacing the other in a well-defined temporal order.[26]

It has been previously shown that *A baumannii* infections can be selected because of the broad antibiotic resistance exhibited by this organism.[27-29] We therefore evaluated whether the spread of the epidemic PFGE clones A and K in our hospital would have been sustained by a particular multidrug-resistant phenotype. Twelve of the 17 strains in the PFGE type K were highly resistant. In particular, all of these strains were resistant to carbapenems and amikacin. The simultaneous occurrence of resistance to amikacin and carbapenems in this epidemic clone might have been

Table 3. Antimicrobial resistance and antibiotypes of the 66 *A baumannii* isolates

	Number of resistant strains in antibiotypes													
Antibiotics	**I** n = 16	**II** n = 4	**III** n = 2	**IV** n = 1	**V** n = 7	**VI** n = 6	**VII** n = 8	**VIII** n = 7	**IX** n = 4	**X** n = 3	**XI** n = 1	**XII** n = 3	**XIII** n = 4	**Total no. of resistant strains (%)**
Imipenem	0	0	0	0	0	0	8	7	4	3	0	3	4	29 (44)
Meropenem	0	0	0	1	0	0	8	7	4	3	1	3	4	31 (47)
Gentamicin	16	4	2	1	0	0	0	0	0	0	1	3	4	31 (47)
Amikacin	16	4	2	1	7	6	8	7	4	0	1	0	0	56 (84.8)
Tobramycin	0	4	2	0	0	0	0	0	0	0	1	3	4	14 (21.2)
Netilmicin	0	0	2	0	0	0	0	0	0	0	0	0	0	2 (3)
Ampicillin-sulbactam	16	4	2	1	0	0	0	7	4	3	1	3	0	41 (62.1)
Trimethoprim-sulfamethoxazole	16	4	2	1	0	0	0	7	0	0	1	3	4	38 (57.6)
Piperacillin-tazobactam	16	4	2	1	7	6	8	7	0	3	1	3	4	62 (94)
Cefoperazone-sulbactam	16	4	2	1	0	0	0	7	4	3	1	3	0	41 (62.1)
Ciprofloxacin	16	4	0	1	7	6	8	7	4	3	0	3	4	63 (95.5)
Levofloxacin	16	4	0	1	7	6	8	7	4	3	0	3	4	63 (95.5)
Minocyclin	0	0	0	1	0	0	0	0	0	0	0	0	0	1 (1.5)
Colistin	0	4	2	1	0	0	0	0	0	0	1	0	0	8 (12.1)
Tigecycline	0	0	0	1	0	0	0	0	0	0	0	0	0	1 (1.5)

responsible for the high rate of infection caused by this strain during the study period. On the other hand, 5 of 17 strains with genotype K showed an important difference about their antibiotic susceptibility pattern that these strains were susceptible to carboxi-ureidopenicillins in addition to carbapenems, ampicillin-sulbactam, trimethoprim-sulfamethoxazole, and cefoperazone-sulbactam. All of the 12 strains in the second epidemic PFGE clone (type A) were resistant to carboxi-ureidopenicillins, broad-spectrum cephalosporins, monobactams, and amikacin but susceptible to carbapenems. This result is also parallel with the previous reports stating that clonally related strains of *Acinetobacter* that differ in susceptibility patterns may coexist within a single hospital, dependent on the selective pressure related to antibiotic exposure.[30,31]

The use of antibiotics can contribute to the persistence and spread of the outbreaks caused by multidrug-resistant *A baumannii*.[27,28,32] In our hospital, the first choice of therapy for *A baumannii* infections was carbapenems in combination with aminoglycosides, especially amikacin and gentamicin in previous years. Piperacillin-tazobactam was also among the selected antibiotics. After our published data[33] indicating relatively high resistance rates to amikacin (48.8%) and gentamicin (47%), the physicians in our hospital selected tobramicin as a choice for the combination. As a result of this practice, in a 1-year period, resistance to imipenem, amikacin, and tobramycin increased from 32.3% to 44%, 48.8% to 84.8%, and 5.4% to 21.2%, respectively. This finding showed concordance with the previous reports indicating the emergence and spread of resistance to amikacin or carbapenems during hospital outbreaks of multidrug-resistant *A baumannii*.[28,29,34] Although carbapenems are still widely used for treatment of infections caused by *A baumannii*, resistance to these antibiotics is reported increasingly worldwide, and this constitutes a major therapeutic problem.[35] We found that 31 of 66 (47%) *A baumannii* strains were resistant to at least one of the carbapenems. These carbapenem-resistant strains were not restricted in a specific PFGE type; however, 12 (39%) of these strains belonged to the epidemic PFGE clone K. This finding is in agreement with previous data showing that the spread of carbapenem resistance in *A baumannii* strains isolated from different hospitals was due to the acquisition of new epidemic clones.[34,35]

The increasing resistance rate prompted investigations into the usage of other antibiotics such as colistin or tigecyclin for the treatment of infections caused by these panresistant *A baumannii* strains.[36,37] Therefore, we investigated the resistance profile of our strains for colistin and tigecyclin and detected that tigecyclin was the most effective antibiotic against *A baumannii* strains tested. The second most effective antibiotic was minocyclin, to which resistance was detected in only 1 (1.5%) strain. The effectivity of colistin was also noteworthy with a resistance rate of 12.1%. The susceptibility rate of colistin was lower than it has been reported in other studies.[16,36] This difference may be due to the evaluation criteria that we have taken into consideration (≥17 mm for susceptibility to colistin). We have also detected that resistance to quinolones and piperacillin-tazobactam has also raised to an unacceptable level, probably because of the higher frequency of use of these antibiotics in our hospital in the past.

Potential risk factors for infection of hospitalized patients with multidrug-resistant *Acinetobacter* strains include length of ICU stay, underlying diseases or conditions, exposure to carbapenems or third-generation cephalosporins, hospital size (>500 beds), and urinary catheterization.[9] In agreement with other studies, we found that *Acinetobacter* infections were commonly seen in intensive care and surgical units. In the present study, the most frequently identified predisposing factors were cerebrovascular diseases, especially for patients in the ICU, and all of these patients had undergone one or more invasive procedures such as mechanical ventilation or catheterization. This showed concordence with the data suggesting that invasive diagnostic and therapeutic procedures used in hospital ICUs predispose subjects to severe infections with *A baumannii*.[3,22] We can suggest that procedures associated with mechanical ventilation might have been the mode of *A baumannii* patient-to-patient transmission for the patients in the same ward in this study. In partial support of this hypothesis, the respiratory tract was the most frequent site of isolation for the patients with cerebrovascular disease.

CONCLUSION

In conclusion, *A baumannii* infections were mainly observed among the patients having predisposing factors and hospitalized in the ICU and surgical wards. Most of these infections were caused by 2 epidemic clones, which were probably selected because of their resistance to the majority of antimicrobial agents. Survival of the clonally related *A baumannii* strains for more than 1 year emphasizes the importance of more strict prevention programs. The increased incidence of multidrug-resistant *A baumannii* nosocomial infections necessitates creation of an intensive program to enact antimicrobial susceptibility testing and molecular typing of the hospital isolates.

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