TÜBİTAK

ORIGINAL ARTICLE

Turk J Med Sci
2009; 39 (5): 761-769
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E-mail: medsci@tubitak.gov.tr
doi:10.3906/sag-0804-38

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Received: April 30, 2008
Accepted: May 13, 2009

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The risk factors and spread of multidrug-resistant *Acinetobacter baumannii* in intubated patients in a medical intensive care unit

Aim: *Acinetobacter baumannii* is one of the most common causes of ventilator-associated pneumonia in intensive care units. The aim of this study was to determine the risk factors for colonization in the respiratory tract and infection with *A. baumannii* in a medical intensive care unit (MICU), and to examine the genetic link between strains and the spread of isolates.

Materials and methods: This study was conducted prospectively between 1 December 2004 and 31 January 2006 in the MICU. Patients (> 16 years old) admitted to the MICU that were mechanically ventilated and/or intubated were enrolled in the study.

Results: Ninety-eight patients were evaluated for *A. baumannii* colonization during or at the end of their intubation; 44 (45%) of these patients were colonized by *A. baumannii*. The length of intubation (OR: 1.032, P = 0.014) and diabetes mellitus (OR: 4.140, P = 0.008) were the major risk factors for colonization of the respiratory tract by *A. baumannii*. During the study period *A. baumannii* infection developed in 35 (80%) of the 44 colonized patients. The important risk factors for *A. baumannii* infection were colonization (OR: 3.962, P = 0.006) and tracheostomy (OR: 4.857, P = 0.001). Genotyping analysis was performed for 59 isolates. Overall, 3 clones (clone A, B, and C) were identified in patients; 88% were clone A (52 isolates), 7% were clone B (4 isolates), and 5% were clone C (3 isolates).

Conclusion: Length of intubation and diabetes mellitus were significant risk factors for *A. baumannii* colonization in intubated patients. Moreover, colonization and tracheostomy predisposed patients to infection. Additionally, this study documented the spread of multi-drug resistant *A. baumannii* in intubated patients in an MICU with limited resources.

Key words: *Acinetobacter*, intensive care unit, colonization, infection control

Bir dahiliye yoğun bakım ünitesinde entübe hastalarda pek çok ilaca dirençli *Acinetobacter baumannii* için risk faktörleri ve yayılımı

Amaç: Acinetobacter baumannii yoğun bakım ünitelerinde ventilatör ilişkili pnömoninin önemli etkenlerinden birini oluşturmaktadır. Kolonize hastalar çevre kontaminasyonu ve mikroorganizmanın hastadan hastaya yayılımında önemli kaynaktırlar. Bu çalışmanın amacı, bir dahiliye yoğun bakım ünitesinde (DYBÜ) *A. baumannii* ile solunum yollarının kolonizasyonu ve enfeksiyonu için risk faktörlerinin belirlenmesi ve izolatların yayılımının gösterilmesi için suşların genetik yakınlığının araştırılmasıdır.

Yöntem ve gereçler: Bu çalışma 1 Aralık 2004 ve 31 Ocak 2006 tarihleri arasında DYBÜ'sinde prospektif olarak yapıldı. DYBÜ'sine yatırılan ve mekanik ventilasyona bağlanan ve/veya entübe edilen hastalar (> 16 yaş) çalışmaya alındı. Bu hastaların endotrakeal aspiratları *A. baumannii* kolonizasyonu için entübasyonun başında (ilk 48 saat içinde), birinci haftanın sonunda ve endotrakeal tüpün çekilmesi sırasında araştırıldı. Her hasta kolonizasyon ve enfeksiyon için risk faktörleri açısından değerlendirildi.

Bulgular: Doksan sekiz hasta *A. baumannii* kolonizasyonu için entübasyon süresince veya entübasyonun sonunda değerlendirildi ve bu hastaların 44 (% 45)'ünde *A. baumannii* kolonizasyonu tespit edildi. Entübasyon süresi (OR: 1,032, P = 0,014) ve diyabetes mellitus (OR: 4,140, P = 0,008) solunum yollarının *A. baumannii* ile kolonizasyonu için önemli risk faktörleri olarak bulundu. Çalışma dönemi boyunca, 44 kolonize hastanın 35 (% 80)'inde *A. baumannii* enfeksiyonu gelişti.

Enfeksiyon gelişimi için major risk faktörleri *A. baumannii* kolonizasyonu (OR: 3,962, P = 0,006) ve trakeostomi (OR: 4,857, P = 0,001) idi. Elli dokuz izolatın genotipik analizi yapıldı. Hastalarda toplam üç klon (klon A, B, C) tespit edildi, ancak bunların % 88'i klon A'ya (52 izolat), % 7'si klon B'ye (dört izolat) ve % 5'i klon C'ye (üç izolat) aitti.

Sonuç: Entübasyon süresi ve diyabetes mellitus entübe hastalarda *A. baumannii* kolonizasyonu için önemli risk faktörleridir. Ayrıca, kolonizasyon ve trakeostomi enfeksiyon gelişimi için predispozan olarak bulundu. Aynı zamanda bu çalışma kısıtlı kaynakları olan bir yoğun bakım ünitesinde entübe hastalarda pek çok ilaca dirençli *A. baumannii*'nin yayılımını göstermektedir.

Anahtar sözcükler: *Acinetobacter*, yoğun bakım ünitesi, kolonizasyon, enfeksiyon kontrolü

Introduction

In recent years *Acinetobacter baumannii* has become the leading cause of ventilator-associated pneumonia (VAP) (1-3). The rapid development of resistance to most antibiotics has emerged as an important variable influencing mortality in patients with *A. baumannii* VAP. Moreover, *A. baumannii* has the capacity to survive on most environmental surfaces for long periods of time, which promotes environment-to-patient spread of the microorganism, especially by healthcare workers (HCWs) (4,5). Many outbreaks have been reported to be due to contamination of surfaces and devices (4), and these outbreaks were controlled by efficient environmental cleaning and appropriate hand and device disinfection (6-9). Additionally, colonization in patients plays an important role in infection and spread of the microorganism to other patients.

Erciyes University Hospital (Kayseri, Turkey) is a 1300-bed tertiary-referral teaching hospital with 10 adult intensive care units (ICUs) (103-bed capacity and 27,947 patient admissions annually). Despite efforts to control infection, nosocomial infection rates remain high (20/1000 patient days) in our ICUs. Moreover, VAP ratios are high (26.6/1000 ventilator days) and 69% of these infections are due to multidrug-resistant gram-negative pathogens (2006 hospital surveillance report). *A. baumannii* is an etiological agent in 35% of VAP cases with excess morbidity and mortality (10), and isolates are usually multidrug resistant (11). This can be due to intubated ICU patients that are colonized and easily develop pneumonia after colonization. In addition, cross-transmission can occur from colonized/infected patients to other patients because of inadequate infrastructure (12). The present study aimed to determine the risk factors for colonization of the respiratory tract and infection with *A. baumannii* in our medical ICU (MICU) and to examine the clonal relatedness of the strains in order to show the dissemination of isolates.

Methods

MICU design and infection control measures

The MICU has 9 beds and approximately 750 patients are admitted each year. Patient-based surveillance is performed actively and daily by infection control nurses. Infection control doctors visit the MICU twice each week and there are posters about infection control measures and hand hygiene on the walls. Additionally, routine infection control education programs for all health-care personnel are offered twice a year. Standard infection control measures are used for all patients. As there is no isolation room for colonized patients, HCWs try to use other contact measures (hand hygiene, gown, glove, etc.) with patients colonized or infected with multidrug-resistant organisms. The between-bed space in the MICU is only 1-1.5 m and there is an insufficient number of nurses (the 24-h nurse-to-patient ratio is 0.7). There are 3 washbasins for 9 beds and alcohol-based products are located at the bedside of each patient.

Patients

This study was conducted prospectively between 1 December 2004 and 31 January 2006 in the MICU. Patients (> 16 years old) admitted to the MICU that were mechanically ventilated and/or intubated were enrolled in the study. Endotracheal aspirate collected from these patients was screened for *A. baumannii* at the beginning of intubation (within the first 48 h), at

the end of the first week, and at the time of withdrawal of the endotracheal tube. Patients intubated for less than 48 h were not included in the study and the data on patients colonized or infected with *A. baumannii* at ICU admission were not evaluated for the risk factors.

Patient data collected for evaluating risk factors included age, sex, APACHE II score, SOFA score, underlying diseases (diabetes mellitus, hypertension, coronary artery disease, asthma, chronic obstructive lung disease, chronic renal failure, chronic heart failure, cerebrovascular disease, cirrhosis, neoplastic disease, trauma), length of stay (LOS) in hospital before MICU admission, LOS in MICU, previous antibiotic administration, length of antibiotic therapy before colonization, use of catheterization (peripheral, central venous, arterial, urinary) or nasogastric tube, total parenteral nutrition, tracheostomy, prostheses, surgery, open wounds, and decubitus ulcer.

This study was approved by the Erciyes University Ethics Committee (date: 04.05.2004; number: 132).

Microbiological studies

Organism identification

A. baumannii was preliminarily identified by the bacteriology laboratory of Erciyes University Hospital. Quantitative endotracheal aspirate cultures were performed for all patients. Lactose- and oxidase-negative isolates were subcultured and *A. baumannii* was identified using a Phoenix 100 system (Becton Dickinson, USA). Isolates were stored at –80 °C until studied.

Molecular typing by pulsed-field gel electrophoresis (PFGE)

Strains isolated from colonized and infected patients were evaluated for genetic relatedness. Genotyping analysis was performed by pulsed-field gel electrophoresis (PFGE) at the clinical microbiology laboratory of Yeditepe University, İstanbul, Turkey.

Pulsed-Field Gel Electrophoresis

Acinetobacter DNA embedded in agarose blocks was prepared, as described by Seifert et al. (13); restriction endonuclease ApaI (Promega, Madison, WI, USA) was used for digestion. The DNA macrorestriction fragments were separated in 1.1% agarose gel (Genaxis, Spechbach, Germany) by PFGE using CHEF Mapper (Bio Rad Laboratories CA, USA). l-Ladder (Sigma, Deisenhofen, Germany) was used as a molecular weight marker.

Restriction digestion and loading of the gel

The prepared plug discs were incubated in ApaI restriction buffer (provided by the manufacturer) at 25 °C for 15 min. Then, the restriction buffer was removed and replaced with 200 µL of fresh restriction buffer containing 30 U of ApaI (Promega, Madison, WI, USA). The reaction tubes were shaken gently and the discs were incubated at 25 °C for 2 h. Prior to casting the gel, the restriction mixture was removed from each tube and replaced with 200 µL of 0.5× TBE (10× TBE is 0.89 M Tris, 0.89 M boric acid, and 20 mM EDTA, pH 8.3). They were loaded into the appropriate wells, which were made using a 15-slot comb; each slot was 1.5 mm thick (Bio-Rad Laboratories Inc, CA, USA).

Electrophoresis

Electrophoresis was performed in a contour-clamped homogeneous electric field (CHEF) using a CHEF Mapper apparatus (Bio-Rad Laboratories Inc, CA, USA). The gels were covered with 2000 mL of 0.5× TBE. The running temperature was set at 14 °C. Total run time was 19 h, with switch times ranging from 5 to 20 s and linear ramping; run voltage was 6 V cm^{-1}. The gels were stained for 30 min with 300 mL of ethidium bromide solution (1 µg mL^{-1}) and destained for 45 min in distilled water with gentle shaking. The gels were observed under UV illumination and photographed using an imaging and documentation system (Quantity One SW, Bio-Rad Laboratories, CA, USA). Digital images were stored electronically.

Data analysis

Data analysis of PFGE patterns was performed visually, and the band patterns were interpreted according to the criteria suggested by Tenover et al. (14), with a difference of 6 bands or less used to define epidemiological relatedness.

Definitions

***A. baumannii* acquisition**

If an endotracheal aspirate culture was negative for *A. baumannii* at patient admission and positive for *A. baumannii* 48 h after admission, the patient was defined as *A. baumannii* colonized.

A. baumannii infection

The Centers for Disease Control and Prevention (CDC) standard criteria were used to define infection (15).

Multidrug resistance

Diminished susceptibility to 2 or more of the following 5 antibiotic classes: antipseudomonal cephalosporins, carbapenems, β-lactam-β-lactamase inhibitor combinations, antipseudomonal fluoroquinolones, and aminoglycosides (16).

Statistical analysis

Data are given as mean ± SD or median (minimum-maximum). Univariate and multiple logistic regression analyses (backward walled) were performed for the significant risk factors of *A. baumannii* acquisition and infection. All analyses were carried out using the Statistical Package for the Social Sciences (SPSS v.13.0).

Results

During the study period 408 patients were admitted to the MICU. Of those, 129 patients stayed in the MICU for less than 48 h, 118 patients were not intubated, and 9 (2%) patients were considered colonized at the time of admission. All of the colonized patients were transferred from other wards of the hospital.

Among 152 patients, endotracheal cultures were taken from 98 (64.5%) patients during or at the end of intubation and were evaluated for *A. baumannii* colonization. Median age was 62.5 years (54.83 ± 22.14 years) with a range of 16-95 years. Fifty-seven (58.2%) of the patients were male and 41 (41.8%) were female. Median LOS in the MICU was 18.38 days (range: 3-111 days) and median LOS in the hospital was 27.55 days (range: 7-113 days). Fifty-six (57%) of 98 patients were transferred from other parts of the hospital to the MICU. Demographic characteristics of the patients are shown in Table 1.

Endotracheal cultures were taken from 86 patients at the end of first week and 39 (45%) of these patients were observed to be colonized with *A. baumannii*. Respiratory secretions at the end of extubation were obtained from 23 patients. Eleven of these patients were extubated in the first week and only 1 (9%) patient was colonized with *A. baumannii*, whereas 12 patients were extubated after the first week and 4 (33%) were colonized with *A. baumannii*. Overall, 44 (45%) of 98 patients were colonized with *A. baumannii*. Mean time for *A. baumannii* acquisition was 7.46 ± 3.74 days (range: 2-16 days).

Table 1. Patient demographic characteristics (n = 98).

Characteristics		
Age (mean ± SD)	54.83 ± 22.14	
APACHE II score (mean ± SD)	24.51 ± 7.64	
SOFA score (me an ± SD)	7.78 ± 3.41	
Underlying diseases and interventions	No. of patients (%)	
Diabetes mellitus	20	(20)
Hypertension	30	(31)
Coronary heart disease	20	(20)
Chronic obstructive lung disease	20	(20)
Chronic renal failure	19	(19)
Cirrhosis	5	(5)
Cerebrovascular disease	22	(22)
Neoplastic disease	14	(14)
Trauma	11	(11)
Previous antibiotic use	91	(93)
Peripheral catheter	63	(64)
Central venous catheter	70	(71)
Arterial catheter	13	(13)
Urinary catheter	92	(94)
Tracheostomy	31	(32)
Prosthesis	5	(5)
Operation	15	(15)
Open wound	13	(13)
Decubitus ulcer	14	(14)

During the study period *A. baumannii* infection developed in 35 (80%) of 44 colonized patients. *A. baumannii* infection developed in 8 (19%) patients, unless colonization was determined. The majority of *A. baumannii* infections were VAP (81%) and the others were bacteremia (18.6%). Mean time for the development of *A. baumannii* infection was 9.33 ± 5.84 days after admission (range: 4-33 days).

Isolate resistance rates are shown in Table 2. All the isolates were multi-drug resistant.

Table 2. Resistance rates of the *A. baumannii* isolates.

Antibiotics	Resistant isolates/n (%)	
Ampicillin/sulbactam	18/26	(69)
Ceftazidime	50/50	(100)
Cefepime	48/54	(89)
Ciprofloxacin	60/62	(97)
Levofloxacin	44/52	(85)
Piperacillin/tazobactam	33/35	(94)
Tobramycin	18/39	(46)
Gentamycin	38/51	(75)
Amikacin	57/59	(97)
Imipenem	30/41	(73)
Meropenem	46/58	(79)

Risk factors for *A. baumannii* colonization

According to univariate logistic regression analysis, APACHE II score, age, length of intubation, diabetes mellitus, coronary artery disease, and prostheses were significant risk factors (Table 3). However, according to multiple logistic regression analysis only length of intubation (OR: 1.032, P = 0.014) and diabetes mellitus (OR: 4.140, P = 0.008) were significant risk factors.

Risk factors for *A. baumannii* infection

According to univariate logistic regression analysis, LOS in the MICU, LOS in the hospital, tracheostomy, diabetes mellitus, and *A. baumannii* colonization were significant risk factors (Table 4).

Table 3. Risk factors for *A. baumannii* colonization based on univariate logistic regression analysis.

Risk factors	OR	95% CI	P
Age	1.02	1.00-1.05	0.04
Sex	1.77	0.74-4.25	0.2
APACHE II	1.07	1.00-1.13	0.04
SOFA	1.035	0.91-1.18	0.6
Diabetes mellitus	4.07	1.46-11.37	0.007
Hypertension	1.61	0.64-4.03	0.3
Coronary artery disease	3.11	1.12-8.59	0.03
Chronic obstructive lung diseae	1.37	0.48-3.89	0.5
Chronic renal failure	2.65	0.95-7.46	0.06
Chronic heart failure	1.49	0.57-3.91	0.42
Cerebrovascular disease	1.94	0.72-5.22	0.2
Cirrhosis	0.58	0.062-5.43	0.6
Neoplastic disease	0.35	0.07-1.68	0.2
Trauma	2.19	0.61-7.84	0.2
LOS-MICU	1.02	0.10-1.04	0.07
LOS-hospital	1.02	0.10-1.04	0.08
Previous antibiotic use	2.667	0.31-23.20	0.3
Length of antibiotic therapy	0.98	0.88-1.09	0.7
Peripheral venous catheter	0.87	0.35-2.14	0.7
Central venous catheter	2.40	0.93-5.71	0.07
Arterial catheter	0.94	0.26-3.33	0.9
Urinary catheter	2.19	0.24-19.59	0.5
Nasogastric tube	0.97	0.40-2.35	0.9
TPN	4.2	0.51-34.79	0.2
Length of intubation	1.03	1.01-1.06	0.02
Tracheostomy	2.30	0.93-5.71	0.07
Prosthesis	10.88	1.16-102.07	0.04
Operation	2.43	0.79-7.48	0.1
Open wound	2.31	0.70-7.60	0.17
Decubitis ulcer	0.94	0.27-3.30	0.9

Table 4. Risk factors for *A. baumannii* infection based on univariate logistic regression analysis.

Risk factors	OR	95% CI	P
Age	1.01	0.99-1.03	0.3
Sex	1.98	0.87-4.49	0.1
APACHE II	1.01	0.95-1.06	0.9
SOFA	0.95	0.84-1.07	0.4
Diabetes mellitus	2.97	1.06-8.29	0.04
Hypertension	2.11	0.88-5.05	0.09
Coronary artery disease	1.36	0.51-3.65	0.5
Chronic obstructive lung diseae	0.8	0.30-2.23	0.7
Chronic renal failure	1.55	0.57-4.23	0.4
Chronic heart failure	1.25	0.50-3.11	0.6
Cerebrovascular disease	1.09	0.42-2.82	0.9
Cirrhosis	1.99	0.32-12.46	0.46
Neoplastic disease	0.67	0.21-2.17	0.5
Trauma	0.4	0.11-1.77	0.3
LOS-MICU	1.07	1.02-1.12	0.004
LOS-hospital	1.02	1.00-1.04	0.04
Previous antibiotic use	5.14	0.60-44.45	5.1
Length of antibiotic therapy	0.90	0.81-1.01	0.07
Peripheral venous catheter	1.07	0.46-2.45	0.9
Central venous catheter	3.18	1.20-8.43	0.02
Arterial catheter	0.53	0.15-1.84	0.3
Urinary catheter	4.2	0.47-37.37	0.2
Nasogastric tube	1.0	0.45-2.24	1.0
TPN	3.49	0.70-17.37	0.1
Length of intubation	1.02	0.99-1.048	0.09
Tracheostomy	5.35	2.11-13.59	0.00
Prosthesis	1.98	0.32-12.46	0.46
Operation	1.14	0.38-3.44	0.8
Open wound	0.77	0.23-2.56	0.7
Decubitis ulcer	0.67	0.21-2.18	0.5
A. baumannii colonization	4.44	1.75-11.29	0.002

However, according to multiple logistic regression analysis only *A. baumannii* colonization (OR: 3.962, P = 0.006) and tracheostomy (OR: 4.857, P = 0.001) were significant risk factors for infection.

Genetic relatedness

Genotyping analysis was performed for 59 isolates (9 isolates from patients colonized at admission, 44 isolates from patients colonized in the MICU, and 6 isolates from infected patients in the MICU). Overall, 3 clones (clone A, B, and C) were indentified in the patients; 88% belonged to clone A (52 isolates), 7% to clone B (4 isolates), and 5% to clone C (3 isolates). Fourteen clone A isolates (10 A-1 and 4 A-2) had minor variations. Clones B and C were isolated from patients colonized in the MICU.

Discussion

*Staphylococcus aureu*s and nonfermentative gram-negative bacilli are reported to be the leading causes of VAP (17). *A. baumannii* is one of the most frequent nonfermentative gram-negative pathogens that cause VAP in the series and causes alarm in ICUs due to high drug resistance and to high mortality rates.

Despite efforts directed at infection control, the incidence of multidrug-resistant *A. baumannii* has continued to rise. *A. baumannii* can survive on surfaces for long periods of time and develops resistance to many classes of antibiotics. Moreover, all of the isolates in the present study were multidrug resistant. In many infections and outbreaks of this pathogen, medical devices and hospital staff hands are the essential reservoirs of these isolates (14).

In our ICUs *A. baumannii* infections constitute the most common cause of hospital-acquired infections with a high rate of mortality (10,11). In a recent study we evaluated *A. baumannii* bacteremia and 87.8% of *A. baumannii* bloodstream infections occurred in ICUs. The respiratory tract was the most common source (39%) of bacteremia (11). Colonization of respiratory secretions plays an important role in the pathogenesis of pneumonia. Sedated patients on mechanical ventilation usually aspirate the bacteria around the cuff of the endotracheal tube, and due to their underlying comorbid conditions they develop pneumonia (16,18,19). Additionally, *A. baumannii* adherence to human bronchial epithelial cells was observed in vitro (20). Identification of specific risk factors for airway colonization by specific microorganisms will help focus infection control measures.

On the other hand, endotracheal tubes predispose to VAP by producing mucosal damage in the trachea, and implanting exogenous and endogenous bacteria, leading to accumulation of secretions by influencing cough reflexes and the formation of biofilm (17). Tracheostomy is another important risk factor for infection. Tracheostomy tubes disrupt swallowing and predispose to aspiration (21). Ibrahim et al. (22) reported that the presence of a tracheostomy was strongly associated with the development of pneumonia. Moreover, tracheostomy was an important risk factor for *A. baumannii* infection in the present study.

A. baumannii usually grows in moist environments. Respiratory devices (ventilators, ventilator circuits, resuscitation bags, ventilator spirometers, etc.) are the most common sources for *A. baumannii* colonization and outbreaks. *A. baumannii* can rapidly colonize intubated patients in ICUs, in which it is endemic. Furthermore, patients' underlying diseases play a role in overcoming the pulmonary defense mechanisms and lead to colonization (23,24). In our ICUs 45% of intubated patients were colonized with *A. baumannii*. The length of intubation and diabetes mellitus were the most important risk factors for colonization. Colonization usually occurred during the first week (88.6%) (mean colonization day was 7.3), as indicated in the literature (25). In most studies prior use of antibiotics was identified as an important risk factor for acquisition of *A. baumannii* (26,27); however, it was not an important risk factor in the present study. The high proportion of antibiotic use in our ICUs might explain this result and overuse of antibiotics may explain the high rate of resistance observed in the present study.

Environmental contamination from colonized patients and its correlation with patient colonization and infection have been reported. Environmental cleanliness is a key factor in the control of outbreaks (6-9,28,29); however, in the present study colonization pressure was not determined, and hand and environmental sampling was not carried out. Colonized patients are the major reservoir for *A. baumannii*, and patient-to-patient and environment-to-patient transmission usually occurs via HCWs' hands. The genetic relatedness of the isolates (88% of the isolates belonged to clone A) shows the dissemination of the microorganism. The major mode of transmission from patient to patient is the contaminated hands of HCWs; therefore improving hand hygiene compliance among HCWs and standard precautions may be adequate to control multidrug-resistant pathogens in endemic settings. In many studies hand hygiene compliance is poor (< 50%) and many factors influence compliance. In developing countries understaffing, high workload, poorly accessible sinks, and expensive alcohol-based hand disinfectants are the major barriers for hand hygiene compliance, and adequate compliance is practically impossible (30). Nonetheless, in our ICUs alcohol-based products placed bedside, 3 washbasins for 9 beds, understaffing (the 24-h nurse-to-patient ratio is 0.7), and heavy workload are important reasons for low compliance and healthcare-associated infections. In a recent study Hugonnet et al. (31) reported that 26.7% of all infections acquired in the ICU could be

prevented, providing that the 24-h nurse-to-patient ratio is maintained at > 2.2. We also showed dissemination of multidrug-resistant pathogens in our other studies (11,32). After these studies and surveillance reports by the infection control team that showed high infection rates and cross-transmission, a new MICU was built in the hospital. In this new MICU, the bed-to-bed space is > 2.5 m, washbasins are provided for every 2 beds, and 3 isolation rooms were built. Nevertheless, the nurse-patient ratio remains low, and we are implementing an infection control program and surveillance of health-care-associated infections.

It has been suggested that colonization represents the submerged part of an iceberg and that infection reflects the tip of an iceberg. Colonization may be the first step of an infection (33). Moreover, 80% of our colonized patients manifested infection and infection occurred in 8 patients, unless colonization was determined; however, we screened only respiratory secretions and only during the first week and extubation. Colonization could have occurred at other body sites and/or during the intervals between screenings.

In conclusion, these results indicate the spread of multidrug-resistant *A. baumannii* in intubated patients. Colonized patients and environmental contamination might act as the major reservoirs for the bacteria and infection. Inadequate infrastructure in ICUs (lack of staff, lack of isolation rooms, high workload, etc.) in developing countries is the main determinant of multidrug-resistant pathogen dissemination. To control these pathogens, determination of the ideal number of staff to reduce workload, and contact isolation measures in combination with vigorous environmental decontamination, hand hygiene, and appropriate equipment decontamination is required.

Acknowledgements

We thank Ahmet Öztürk for the statistical analyses. This study was supported by the Erciyes University Research Foundation and The Scientific and Technological Research Council of Turkey (TÜBİTAK project no: 105S505).

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