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Original articles

Carbapenem resistance in *Acinetobacter baumannii*: where is it heading?

Resistenza ai carbapenemi in Acinetobacter baumannii: where is it heading?

Özcam Deveci[1], Tuba Dal[2], Recep Tekin[1], Fatma Bozkurt[1], Alicem Tekin[2], Saim Dayan[1]

[1]Faculty of Medicine, Department of Infectious Diseases and Clinical Microbiology, Dicle University, Diyarbakir, Turkey;
[2]Faculty of Medicine, Department of Microbiology and Clinical Microbiology, Dicle University, Diyarbakir, Turkey

INTRODUCTION

Healthcare-associated infections (HAIs) are a major cause of morbidity and mortality in hospitalized patients. HAIs are encountered in intensive care unit (ICU) patients 5-10 times more frequently than in patients from other services [1]. Antimicrobial drug therapy is an important part of medical treatment in ICUs. These infections cause a prolonged hospital stay, as well as impaired quality of life and increase the cost of hospitalization [2, 3]. Increased resistance to commonly used antimicrobial drugs lead to treatment difficulties of infections with these pathogens [4].
Non-fermentative microorganisms such as Pseudomonas and Acinetobacter species are the most common causes of HAIs in ICU patients [5]. Acinetobacter species are important causes of nosocomial infections such as bloodstream and urinary tract infections, ventilator-associated pneumonia (VAP), wound infection, meningitis, endocarditis, and a variety of other infections [6]. *Acinetobacter baumannii* has been the most frequently encountered causative agent in nosocomial infections among Acinetobacter species in the last 15 years. Acinetobacter species are the most frequently isolated Gram negative microorganisms from the skin of hospital personnel [7].

Corresponding author
Tuba Dal
E-mail: tuba_dal@yahoo.com

Carbapenems are frequently used antibiotics in the treatment of Acinetobacter infections. However, with the increase in multi-drug resistant (MDR) strains defined as non-susceptible to carbapenems and two or more additional classes, treatment options have decreased [7, 8].
In this study, our twofold aim was to investigate susceptibility pattern changes of carbapenems and aminoglycosides for treating *A. baumannii* strains isolated from HAIs over a four-year study period, and evaluate treatment strategies.

MATERIALS AND METHODS

A total of 127 *Acinetobacter calcoaceticus-baumannii complex* strains isolated from various clinical specimens of patients diagnosed with HAIs in our hospital between 2007 and 2010 were included in this study. Patients with *A. baumannii* colonization were excluded. Colonization, according to CDC guidelines, was defined as the presence of microorganisms on skin, on mucous membranes, in open wounds, or in excretions or secretions but without causing adverse clinical signs or symptoms [9].
In the study the above *A. baumannii* strains were referred to as *Acinetobacter calcoaceticus-baumannii complex* strains. This study was planned as an active, patient-based and laboratory-based prospective case-control surveillance study. During the study period, among HAIs, 53 (42%) were from ICU and 74 (58%) from non-ICU. Diagnosis of hospital-acquired

infection was determined on the basis of diagnostic criteria of Centres for Disease Control and Prevention (CDC) for hospital-acquired infection. According to these criteria, hospital-acquired infection is defined as "a localized or systemic condition resulting from an adverse reaction to the presence of an infectious agent(s) or its toxin(s) that was not present on admission to the care facility" [9].

Mid-stream urine samples of patients with suspected urinary tract infection were inoculated on 5% sheep blood agar and Eosin-Methylene Blue (EMB) agar (Oxoid Ltd., Basingstoke, United Kingdom), quantitatively. Media were incubated at 35 ± 2°C for 20-24 hours. After incubation, urine samples with one type of aerobic bacterial growth on the surface of 5% sheep blood agar media and with a colony count $\geq 10^5$ CFU/ml were analysed. Blood samples taken from patients with suspected bacteraemia and/or sepsis were inoculated into blood culture bottles and incubated in BACTEC 9120 and 9240 (Becton Dickinson, MD, USA) blood culture systems for 7-10 days at 37°C. Blood samples in which bacterial growth was detected were inoculated on 5% sheep blood agar, EMB agar, and chocolate agar media. These media were incubated at 35 ± 2 °C for 20-24 hours. Wound swabs and other clinical specimens were directly inoculated to the 5% sheep blood agar, EMB agar, and chocolate agar and incubated at 35 ± 2° C for 20-24 hours. All of the strains isolated from clinical specimens were identified by conventional methods and BD Phoenix™ 100 (Becton Dickinson, MD, USA). Antimicrobial susceptibility testing of *A. baumannii* isolates was performed by the Kirby-Bauer disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) recommendations [10]. In the disk diffusion method imipenem (10 µg), meropenem (10 µg), gentamicin (10 µg), and amikacin (30 µg) discs (Oxoid Ltd., Basingstoke, UK) and Mueller-Hinton agar (Oxoid Ltd., Basingstoke, UK) plates were used. The direct colony suspensions prepared with 0.5 McFarland turbidity density equivalents were inoculated on Mueller-Hinton agar plate and incubated at 35 ± 2°C for 20-24 h, aerobically. After incubation, inhibition zone diameters around the disks were measured in mm and the results were recorded as susceptible, intermediate and resistant. In our study for the convenience of evaluation, intermediate values were considered as resistant. For quality control of fully automated microbiology testing system and antimicrobial susceptibility, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as standard strains.

RESULTS

In our hospital, a total of 127 *A. baumannii* strains were isolated from HAI patients in a four-year period. Taking them by years, 26 strains were isolated in 2007, 31 in 2008, 35 in 2009 and 35 in 2010. The total number of strains isolated from ICU was 53 (42%) (Figure 1). It was observed that susceptibility to imipenem

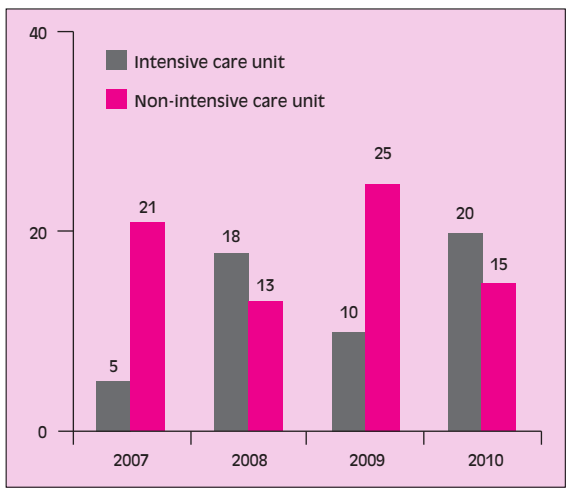

Figure 1 - Distribution of *A. baumannii* isolates from ICU and non-ICU, between 2007 and 2010.

Table 1 - Annual distribution of antibiotic susceptibility profile of Acinetobacter baumannii isolates (%).

Year	*Imipenem*	*Meropenem*	*Gentamicin*	*Amikacin*
2007	50	55	11	38
2008	23	29	10	26
2009	22	25	11	40
2010	20	12	22	48

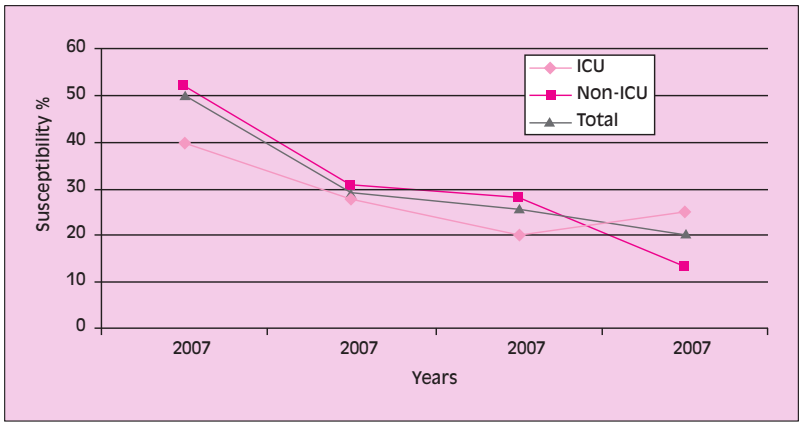

Figure 2 - Changes in imipenem susceptibility in ICU and non-ICU *A. baumannii* isolates between 2007 and 2010.

and meropenem decreased over the studied years. Susceptibility rates between 2007 and 2010 (Table 1) decreased for imipenem from 50% to 20% and for meropenem from 55% to 12%. On the other hand, a susceptibility increase was noted for gentamicin (from 11 to 22%) and amikacin (from 38 to 48%). Susceptibility ratios for carbapenems decreased in parallel in ICU and non-ICU isolates (Figure 2). The majority of *A. baumannii isolates* were recovered from wound infections and blood samples, respectively.

DISCUSSION

Turkey is a large country serving as a bridge between Asia and Europe. Due to the country's geographical diversity, infectious agents and antibiotic susceptibility profiles vary from region to region. Ours is a tertiary hospital located in Diyarbakir and serves a large portion of the population in Turkey's South East Anatolia Region. This region covers a large area with inhabitants of several different nationalities. For this reason, it is important to obtain resistance profile data of microorganisms from the region. To our knowledge, there is no large-scale study available examining carbapenem resistance of *A. baumannii* isolates in the region over a long period. It is known that *Acinetobacter baumannii* is the most frequently isolated Acinetobacter species isolated from HAIs. Broad-spectrum antimicrobial drug usage is an important risk factor for HAIs.

Hospitalization in ICU, weakness of the general condition of the patient and poor immune status represent risks for development of antibiotic-resistant *A. baumannii* infection. *A. baumannii* can easily colonize the surfaces of mechanical equipment used in ICUs, body surfaces of patients and hospital staff. In addition, these microorganisms may be isolated from ICU patients due to the use of broad-spectrum antimicrobial drugs. In a study, it was reported that 34% of *A. baumannii* isolated from hospitalized patients came from ICU patients. In our study, most *A. baumannii* isolates were from non-ICU patients. However, it may be stated that isolation of *A. baumannii* in ICU patients had a substantial level.

A. baumannii strains have become resistant to many antimicrobials, including carbapenems as of late. Environmental factors also play a major role in acquiring resistance. Rates of susceptibility to carbapenem of *A. baumannii* isolates have been shown to vary from region to region and even from hospital to hospital [5, 10-14]. Variations were observed in studies conducted in different locations of Turkey. In a study performed in 2008, the susceptibility rate for imipenem was found to be 38% [5]. In a 4-year study (2000-2004) by Gazi et al. susceptibility to imipenem was shown to decrease from 71.7% to 37.8% [10]. In 2006, Ozer et al. reported that imipenem susceptibility was 56% and in the same year in another study it was found to be 36% [11, 12]. According to the report of Turkey's National Nosocomial Surveillance System, the weighted average rate of carbapenem resistance of *A. baumannii* increased from 47% to 62.8% between 2008-2009. According to this report, the resistance rate increased to 69.6% in 2010 [15]. In our study, imipenem susceptibility decreased from 50% to 19% during the four-year period. The number of antibiotic-resistant *A. baumannii* strains has increased worldwide, as in Turkey. Although the antibiotic susceptibility testing methods vary from one hospital to another, similar results have

been observed in *A. baumannii* strains isolated from ICU all over Turkey. In a Turkish ICU study, 58.8% of *A. baumannii* isolates were determined as imipenem-resistant [16]. In another Turkish study the rate of imipenem resistance was 42% [17]. In a five-year Spanish study, imipenem resistance increased from 1.3% to 80% [18]. In a large-scale United States study, it was reported that carbapenem resistance increased 3.7-fold (95% confidence interval [CI] 3.4-4.3) from 20.6% in 2002 to 49.2% in 2008 [19]. In a Chinese study imipenem resistance increased rapidly from 58.5% in 2006 to 88.4% in 2009 [20]. On comparing studies, it may be stated that carbapenem resistance of *A. baumannii* increased in our hospital as in other countries. We suggest that serious infection control measures and infection management policies are also necessary for our hospital.

In different studies, the most frequently isolated *A. baumannii* clinical specimens were reported as respiratory samples and blood cultures [16, 17]. In one study, *A. baumannii* was frequently isolated from tracheal aspirates and wound samples followed by blood and urine samples [5]. In our study, the great majority of *A. baumannii* strains were isolated from wound infections.

In conclusion, *A. baumannii* is an important pathogen in ICU and non-ICU patients. Over the years, susceptibility rates for imipenem have decreased in *A. baumannii* infections. Treatment of infections with *A. baumannii* has become difficult. For the prevention and control of antibiotic resistance among Acinetobacter species, antibiotic usage restrictions and infection control precautions should be applied worldwide.

Keywords: *Acinetobacter baumannii*, antimicrobial susceptibility testing, health care associated infections, carbapenem.

SUMMARY

In the context of the substantial increase in antibiotic resistance rates of *Acinetobacter baumannii*, we aimed to evaluate the susceptibility rate changes of *A. baumannii* strains for carbapenems. *A. baumannii* strains isolated from patients diagnosed with healthcare-associated infections between 2007-2010 were included. A total of 127 *A. baumannii* strains [53 (42%) from the intensive care unit and 74 (58%) from the non-intensive care unit] were isolated. Conventional methods and an automated microbiology system were used for identification. Susceptibility testing was studied by Kirby-Bauer disk diffusion method. In 2007, five of 26 strains, in 2008, 18 of 31, in 2009, 10 of 35, in 2010, and 20 of 35 were obtained from intensive care unit patients. The susceptibility rate for imipenem was 50% in 2007 but 20% in 2010, while for meropenem it was 55% in 2007 but 12% in 2010. Prevention and control of antibiotic resistance among Acinetobacter species needs antibiotic usage restrictions and infection control precautions.

RIASSUNTO

Le percentuali di resistenza agli antibiotici in Acinetobacter baumannii *è in progressive aumento. Il presente studio è stato condotto al fine di valutare l'andamento del profilo di sensibilità ai carbapenemi in isolati di* A. baumannii. *Nello studio sono stati considerati 127 isolati di* A. baumannii *[53 (42%) da Unità di Terapia Intensiva (UTI) e 74 (58%) da altri reparti] raccolti nel periodo 2007-2010 da pazienti con diagnosi di healthcare-associated infection (HAI). L'identificazione batterica è stata effettuata con metodi tradizionali e con sistema automatico. I test di sensibilità agli antibiotici sono stati effettuati con metodo di diffusione con dischetto* (Kirby-Bauer). *Il numero di isolati di* A. baumannii *riscontrati in pazienti ricoverati in UTI è stato di 5/26 nel 2007, di 18/31 nel 2008, di 10/35 nel 2009 e di 20/35 nel 2010. È stata osservata una notevole riduzione della sensibilità di* A. baumannii *a imipenem, dal 50% nel 2007 al 20% nel 2010. Altrettanto notevole è stata la riduzione della sensibilità a meropenem, passata dal 55% del 2007 al 12% nel 2010.*

La prevenzione e il controllo della resistenza agli antibiotici in Acinetobacter necessita di una attenta politica di restrizione dell'uso di antibiotici e di strette misure di controllo delle infezioni.

REFERENCES

[1] Weber D.J., Baasch R., Rutala W.A. Nosocomial infections in the ICU. The growing importance of antibiotic resistant pathogens. *Chest.* 115, 34-41, 1999.

[2] Çetin E.S., Kaya S., Pakbaþ Ý., Demirci M. Yoðun Bakým Ünitelerinde Yatan Hastalardan Ýzole Edilen Mikroorganizmalar ve Antibiyotik Duyarlýlýklarý. *Ýnönü. Üniv. Týp Fak. Derg.* 14, 69-73, 2007.

[3] Paterson D.L. Restrictive antibiotic policies are appropriate in intensive care units. *Crit. Care Med.* 31, 25-28, 2003.

[4] Flournoy D.J., Reinert R.L., Bell-Dixon C., Gentry C.A. Increasing antimicrobial resistance in gram-negative bacilli isolated from patients in intensive care units. *Am. J. Infect. Control.* 28, 244-250, 2000.

[5] Mansur A., Kuzucu Ç., Ersoy Y., Yetkin F. Ýnönü Üniversitesi Turgut Özal Týp Merkezinde 2008 Yýlýnda Yatan Hastalardan Ýzole edilen Acinetobacter Suþlarýnýn Antibiotik Duyarlýlýklarý. *ANKEM Derg.* 23, 177-181, 2009.

[6] Maragakis L.L., Perl T.M. Epidemiology, antimicrobial resistance, and treatment options. *Clin. Infect. Dis.* 46, 1254-1263, 2008.

[7] Saltoðlu N. *Acinetobacter baumannii* infeksiyonlarý ve tedavisi. XIII. Türk Klinik Mikrobiyoloji ve Ýnfeksiyon Hastalýklarý Kongresi, Antalya 2007, 204-207. KLÝMÝK Derneði, Ýstanbul.

[8] Pachon Ibanez M.E., Jimenez Mejias M.E., Pichardo C., Llanos A.C., Pachon J. Activity of tigecyline (GAR-936) against *Acinetobacter baumannii* strains, including those resistant to imipenem. *Antimicrob. Agent Chemother.* 48, 4479-4481, 2004.

[9] http://health.utah.gov/epi/diseases/legionella/plan/cdcdefsnosocomial%20infection.pdf

[10] Gazi H., Sürücüoðlu S., Kurutepe S., Ýnmez E., Dinç G., Özbakkaloðlu B. Yoðun bakým ünitesinde ve diðer ünitelerde yatan hastalardan izole edilen *Acinetobacter baumannii* suþlarýnda in-vitro antibiyotik direnci. *ANKEM Derg.* 19, 115-118, 2005.

[11] Özer M., Tatman Otkun M., Memiþ D., Otkun M. Yoðun bakým ünitesinde hastane infeksiyonu etkenleri, antibiyotik duyarlýlýklarý ve antibiyotik kullanýmý. *Ýnfeksiyon Derg.* 20, 165-170, 2006.

[12] Bayram A., Balcý Ý. Patterns of antimicrobial resistance in a surgical intensive care unit of a university hospital in Turkey. *BMC Infect. Dis.* 6, 155, 2006.

[13] Appleman M.D., Belzberg H., Citron D.M., et al. In vitro activities of nontraditional antimicrobials against multiresistant *Acinetobacter baumannii* strains isolated in an intensive care unit outbreak. *Antimicrob. Agents Chemother.* 44, 1035-1040, 2000.

[14] Bergogne-Bérézin E., Towner K.J. Acinetobacter spp. as nosocomial pathogens: Microbiological, clinical, and epidemiological features. *Clin. Microbiol. Rev.* 2, 148-165, 196.

[15] Arman D. Gram Negatif Bakteri Ýnfeksiyonlarýnda Direnç Sorunu ve Tedavi Önerileri. Hastane enfeksiyonlarý kongre kitabý. 54-57, 2010.

[16] Eser Ö.K., Kocagöz S., Ergin A., Altun B., Hasçelik G. Yoðun Bakým Ünitelerinde Enfeksiyon Etkeni Olan Gram Negatif Basillerin Deðerlendirilmesi. *Ýnfeksiyon Derg.* 19, 75-80, 2005.

[17] Çolpan A., Güngör Þ., Baykam N., Dokuzoðuz B. Yoðun bakým ünitelerinden izole edilen Acinetobacter suþlarýnýn antibiyotik direnç durumlarýnýn araþtýrýlmasý. *Ýnfeksiyon Derg.* 16, 55-58, 2002.

[18] Ruiz J., Nunez M.L., Perez J., Simarro E., Martinez-Campos L., Gomez J. Evolution of resistance among clinical isolates of Acinetobacter over a 6-year period. *Eur. J. Clin. Microb. Infect. Dis.* 18, 292-295, 1999.

[19] Mera R.M., Miller L.A., Amrine-Madsen H., Sahm D.F. *Acinetobacter baumannii* 2002-2008: increase of carbapenem-associated multiclass resistance in the United States. *Microb. Drug Resist.* 16, 209-215, 2010.

[20] He C., Xie Y., Zhang L., et al. Increasing imipenem resistance and dissemination of the ISAba1-associated blaOXA-23 gene among *Acinetobacter baumannii* isolates in an intensive care unit. *Med. Microbiol.* 60, 337-341, 2011.