Analysis of Cross-Transmission and Antimicrobial Resistance of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* Isolates Causing Nosocomial Infection in an Intensive Care Unit

Bir Yoğun Bakım Ünitesinde Hastane Enfeksiyonuna Neden Olan *Pseudomonas aeruginosa* ve *Acinetobacter baumannii* İzolatlarının Çapraz Taşınımı ve Antimikrobiyal Direncinin Analizi

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ABSTRACT Objective: *Pseudomonas aeruginosa* and *Acinetobacter baumannii* cause nosocomial infections in intensive care units. We investigated the antimicrobial susceptibility and cross-transmission of these bacteria amongst patients in an intensive care unit. **Material and Methods:** Thirty-three *P. aeruginosa* (from 26 patients) and 48 *A. baumannii* isolates (from 41 patients) responsible for nosocomial infections were isolated from patients between October 2009 and June 2010. Pulsed field gel electrophoresis was used to investigate clonal relationship among isolates. Susceptibility to amikacin, ceftazidime, gentamycin, imipenem, cefepime, piperacillin/tazobactam, aztreonam, and meropenem was examined using the disk diffusion method. **Results:** *P. aeruginosa* isolates formed 18 pulsotypes; five of these were clusters including 2 or more strains having indistinguishable PFGE patterns and the remaining 13 were unique. After excluding the repeated samples of the same patients, the clustering rate was estimated as 38.5%. The 48 *A. baumannii* isolates formed 13 pulsotypes; eight pulsotypes were clusters including totally 41 strains of which five were from repeated samples of five patients. The clustering rate was 87.8% for the isolates obtained from 41 different patients. The antimicrobial resistance rates of *P. aeruginosa* ranged from 27-39%, but were 45.5-91% for *A. baumannii* isolates. **Conclusion:** Despite an implemented infection control program, *P. aeruginosa* and *A. baumannii* isolates showed cross-transmission among patients, and the antimicrobial resistance rate of *A. baumannii* isolates was very high. These findings indicate that the current infection control programs should be reassessed and modifications should be made according to the specific hospital and staffing conditions.

Key Words: *Acinetobacter baumannii; pseudomonas aeruginosa*

ÖZET Amaç: *Pseudomonas aeruginosa* and *Acinetobacter baumannii* ciddi hastane enfeksiyonlarına neden olur. Bu çalışmada, Üçüncü basamak bir hastanenin, yoğun bakım ünitesinde bu bakterilerin hastalar arasında çapraz geçişini ve antibiyotik duyarlılığını araştırdık. **Gereç ve Yöntemler:** Ekim 2009 ve Haziran 2010 arasında, hastane enfeksiyonundan sorumlu 33 *P. aeruginosa* (26 hastadan) ve 48 *A. baumannii* (41 hastadan) izolatı, hastalardan izole edildi. İzolatlar arası klonal bağlantıları araştırmak için Pulsed field gel electrophoresis kullanıldı. Amikasin, seftazidim, gentamisin, imipenem, sefepim, piperasillin/tazobaktam, aztreonam ve meropenem duyarlılıkları disk diffüzyon yöntemi kullanılarak incelendi. **Bulgular:** *P. aeruginosa* izolatları onsekiz pulsotip, iki veya daha fazla suş içeren beş küme oluşturdu. On üç izolat herhangi bir kümeye dahil değildi. Aynı hastaların tekrarlayan örnekleri çıkarıldıktan sonra kümeleşme oranı %38,5 olarak hesaplandı. Kırk sekiz *A. baumannii* izolatı 13 pulsotip oluşturdu. Sekiz pulsotip, beş hastanın tekrarlayan beş örneğini de kapsayan 41 suşu içeren kümeler oluşturdu. Kırk bir farklı hastadan elde edilen izolatlar için kümeleşme oranı %87,8 bulundu. *P. aeruginosa* suşlarının antibiyotiklere direnç oranları %27–39 aralığındayken *A. baumannii* suşlarının %45,5-91 aralığındaydı. **Sonuç:** Hastanede uygulanan bir enfeksiyon kontrol programına rağmen, yoğun bakım hastalarında, *P. aeruginosa, A. baumannii* izolatları çapraz geçişler gösterdi. *A. baumannii* suşlarının antimikrobiyal dirençleri çok yüksekti. Bu bulgular göstermiştir ki; mevcut kontrol programları yeniden değerlendirilmeli, hastane ve personel durumuna özel değişiklikler yapılmalıdır.

Anahtar Kelimeler *Acinetobacter baumani; Pseudomonas aeruginosa*

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Nosocomial infections are a problem worldwide and are reported in 7-8% of in patients. *Pseudomonas aeruginosa* and *Acinetobacter baumannii* often colonize intensive care units (ICUs), and are responsible for 6-25% of nosocomial infections. Treatment of infections caused by these bacteria is very difficult because of their notable virulence characteristics and frequent antibiotic resistance. The widespread use of broad-spectrum antibiotics in ICUs is an important risk factor for resistance development.[1-5]

Epidemiological analysis of antibiotic-resistant *P. aeruginosa* and *A. baumannii* is important for control of hospital infections. In particular, tracking the clonal dissemination of multidrug-resistant bacterial species can help in the development of strategies to control cross-transmission of microorganisms and infection outbreaks in ICUs.[1,6,7]

In this study, we examined the antibiotic resistance and cross-transmission of *P. aeruginosa* and *A. baumannii* isolates associated with infections in intensive care patients over a 9-month period.

MATERIAL AND METHODS

This study was performed at Kırıkkale University Faculty of Medicine, Turkey. This 150-bed hospital had recently been restored, and contains an ICU with six beds. An infection control program and controlled drug-use strategies were already in place in the ICU.

The 81 samples were collected from 58 patients with nosocomial infections in the ICU from October 2009 to June 2010. The isolates were identified by conventional methods (the appearance of bacterial colonies, Gram staining, microscopy, triple sugar iron agar and oxidase tests) and results were confirmed using an API 20NE identification kit (Biomerieux, France).

Susceptibility of the strains to amikacin (30 μg) (AK), ceftazidim (30 μg) (CAZ), gentamicin (10 μg) (GN), imipenem (10 μg) (IMP), cefepime (30 μg) (FEB), piperacillin/tazobactam (100/10 μg) (PTZ), aztreonam (30 μg) (AZT), and meropenem (10 μg) (MER) was examined using the disk diffusion method according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI).[8]

Clonal relationship among isolates were determined using pulsed-field gel electrophoresis as described by Durmaz et al. (2009).[9] Briefly, the bacterial cell plugs were lysed with lysozyme and proteinase K. The bacterial DNA from the plugs was then digested with 30 U of *Apa*I (Promega, Maddison, WI, USA). Fragmented DNA was electrophoresed in 1% pulsed-field-certified agarose gels (Bio-Rad, Hercules, CA, USA) using a CHEF-DR II system (Bio-Rad). The electrophoresis conditions were 14°C at 6 V/cm^2 for 20 h. The initial and final switch times were 5 s and 30 s, respectively. The DNA band profiles were analyzed using GelCompar software (version 6.0; Applied Maths, Sint-Martens-Latem, Belgium). A 1% band tolerance was used for comparison of DNA profiles. Strains were evaluated as indistinguishable, closely-related, possibly-related, or different according to the criteria of Tenover et al. (1995).[10]

RESULTS

The 33 *P. aeruginosa* isolates were collected from 26 patients with nosocomial infection. The majority of these isolates (33%) came from urine samples, followed by wound (27.3%), sputum (21.2%), and blood (9%) samples. In total, there were 18 *P. aeruginosa* pulsotypes (PPT) and five pulsotypes were clusters including 2 or more strains showing indistinguishable PFGE patterns and the remaining 13 were not identical. Five clusters included 19 strains, of which six were isolated from the repeated samples of three patients. The clustering rate was estimated as 38.5% for the 26 isolates of 26 patients. Eight isolates characterized as PPT3 were isolated from eight different samples collected from six patients. This pulsotype is sensitive to all of antimicrobials except PTZ. The characteristics of *P. aeruginosa* isolates are shown in Figure 1.

In total, 24% of *P. aeruginosa* isolates were sensitive to all of the tested antimicrobials; however, the 40% showed multi-drug resistance (drug resistance for more than two). Resistance rates for *P. aeruginosa* to each of the antibiotics ranged from 27-39%. All of *P. aeruginosa* strains isolated from

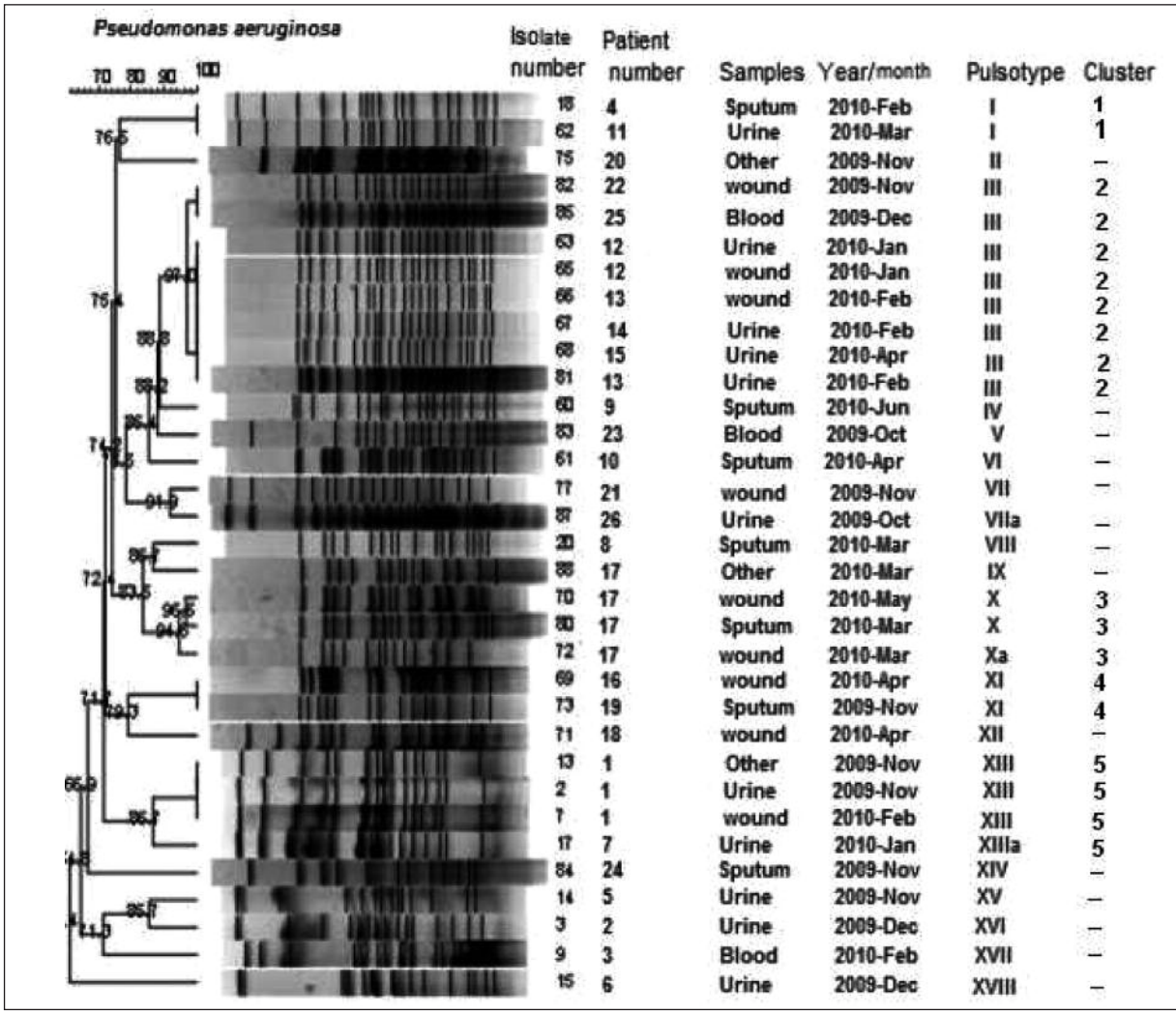

FIGURE 1: Epidemiological findings and PFGE patterns of 33 *P. aeruginosa* isolates. The numbers on branches indicate similarity between pulsotypes * a: indicates sub-pulsotype which differ from the original one with 2 or 3 bands, * (-) indicates unique PFGE pattern.

same patients have different antimicrobial resistance profile.

Sensitivity screening results are given in Table 1.

The 48 *A. baumannii* isolates were collected from 41 patients. The majority of isolates (45.8%) were identified from sputum samples, followed by blood (21%), wound (12.5%), cerebral vascular fluid (10.4%), and urine (8.3%) samples.

PFGE typing of the 48 isolates yielded 13 pulsotypes; eight pulsotypes were clusters including totally 41 strains of which five were from repeated samples of five patients. The clustering rate was

TABLE 1: Antimicrobial susceptibility of *P. aeruginosa* and *A. baumannii* isolates.

Drugs	*P. aeruginosa*		*A. baumannii*	
	I%	R%	I%	R%
PTZ	45.5	30.3	4.5	90.9
AK	0	30.3	6.8	72.7
GN	3	27.3	9,1	45.5
FEB	15.2	30.3	2.3	81.8
IMP	3	30.3	0	70.5
MER	0	33.3	0	70.5
CAZ	3	36.4	4.5	84.1
AZT	0	39.4	0	90.9

S: Sensitive; I: Intermediate; R: Resistant; PTZ: Piperacillin-tazobactam; CAZ: Ceftazidime; FEB: Cefepime; IMP: Imipenem; MER: Meropenem; GN: Gentamicin; AK: Amikacin; AZT: Aztreonam.

87.8% for the isolates obtained from 41 different patients. Analysis showed that 43 isolates (89.5%) were epidemiologically linked. *A. baumannii* pulsotype 11 (APT11) caused the largest outbreak. It peaked with five patients in June 2010, and 10 patients were affected over a 5-month period. Isolates belonging to APT5 and APT8 infected total 10 patients over 4 months, while APT10 isolates caused serious infections in five patients in December 2009 and January 2010. APT13 included four isolates from different patients, and isolates characterized as APT1, APT3, or APT4 were isolated from seven patients. The characteristics of *A. baumannii* isolates are shown in Figure 2.

Isolates belonging to APT5 showed varying degrees of resistance to aminoglycosides. Amongst the APT5 isolates, isolate 35 (I35) was sensitive to AK and GN, while isolate 30 was intermediate to AK but sensitive to GN. Isolates 21, and 24 were only sensitive to GN. Isolate 96 was resistant to AK and intermediate to GN, and isolate 48 was resistant to both tested aminoglycosides. In addition, similar varying resistance profiles for aminoglycosides were observed for isolates belonging to APT11 and PPT13.

The sensitivity rates include the only one of isolate from each patient. Therefore, the resistance rates of *A. baumannii* strains were evaluated as

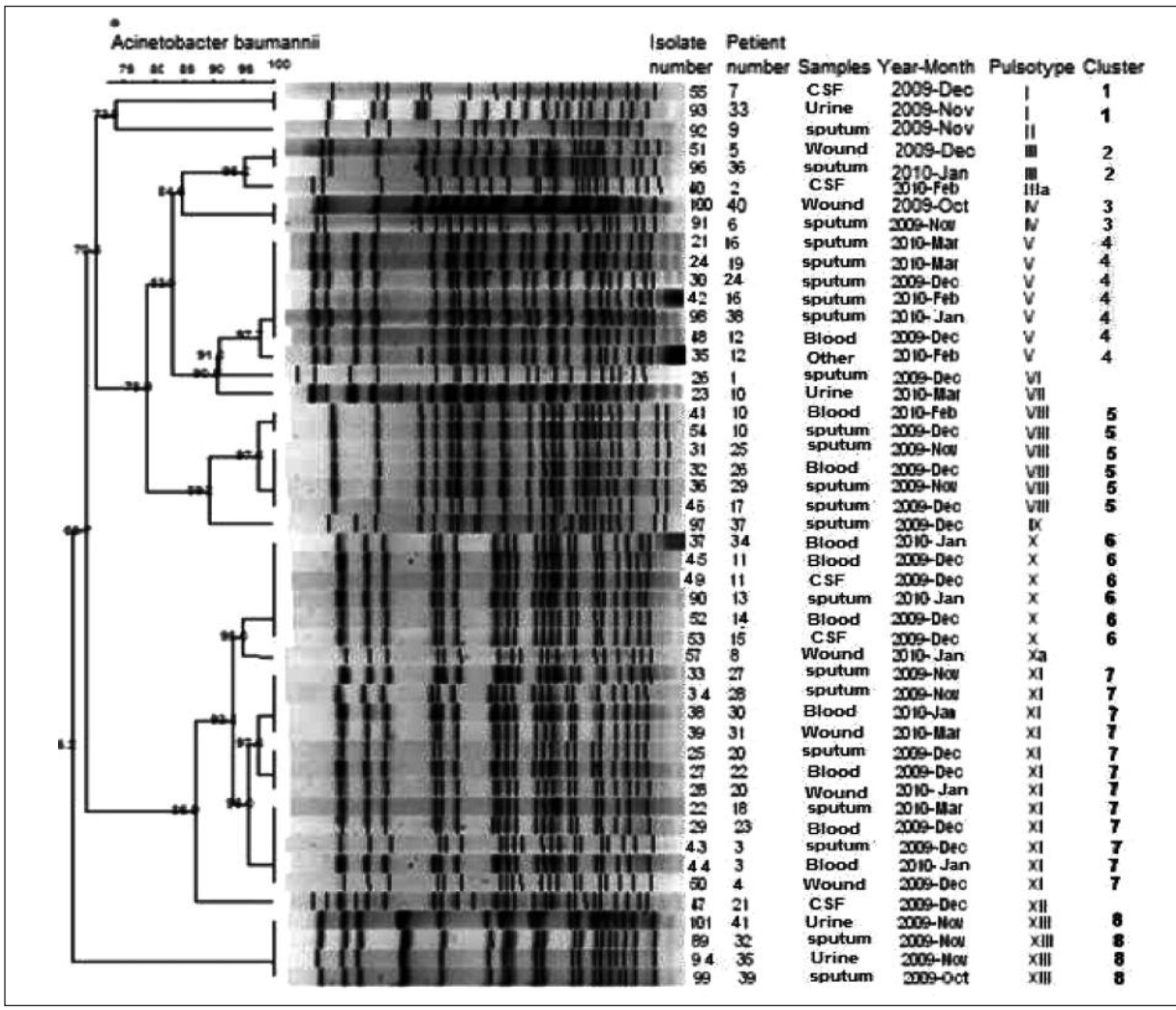

FIGURE 2: Epidemiological findings and PFGE patterns of 48 *A. baumannii* isolates. The numbers on branches indicate similarity between pulsotypes * a: indicates sub-pulsotype which differ from the original one with 2 or 3 bands.

total 44 isolates. In total, 91% of *A. baumannii* isolates showed multi-drug resistance. In addition, 16 isolates were resistant to all antibiotics tested, and a further 10 isolates were resistant to all antibiotics except gentamicin. Overall, antibiotic resistance rates of *A. baumannii* isolates were very high (70-91%), except for gentamicin (45.5%) (Table 1).

DISCUSSION

P. aeruginosa and *A. baumannii* are important opportunistic pathogens, causing repeated nosocomial infections and outbreaks, especially in ICUs. *P. aeruginosa* is frequently isolated from urine, tracheal aspirate, and wound samples, while *A. baumannii* is the most frequent cause of ventilator-associated pneumonia and catheter-associated bloodstream infections.[5,1,11,12] In the current study, *P. aeruginosa* strains were most frequently isolated from urine samples (33.3%), and *A. baumanni* isolates were most often associated with sputum samples (45.8%).

In the investigated ICU, these two bacterial species were responsible for five outbreaks of disease during the study period. *P. aeruginosa* pulsotype 3 was responsible for one of the outbreaks, while the other four outbreaks were caused by *A. baumannii* pulsotypes 5, 8, 10, and 11. The outbreak isolates of *A. baumannii* were resistant to most of the antimicrobials examined in our study.

Antibiotic resistance of nosocomial infection agents may occur because of frequent use of broad-spectrum antibiotics in ICUs. Recent studies have reported resistance rates for *P. aeruginosa* of 55-82% for FEB, 36-88% for AZT, 35-96% for CAZ, 35-53% for MER, 36-100% for GN, 37-63% for IMP, 24-77% for PTZ, and 18-82% for AK in ICUs.[13-20] In the current study, drug resistance rates for the *P. aeruginosa* isolates (27-39%) were lower than these previously reported rates; however, the overall resistance rates of the *A. baumanni* isolates (45.5-91%) were very high. Previous studies from ICUs have reported antibiotic resistance rates for *A. baumannii* of 64-100% for FEB, 73-100% for AZT, 64-92% for CAZ, 45-90% for MER, 45-100% for GN, 55–90% for İMP, 38-93% for TZP, and 27-93% for AK.[15,19,21,22] The antimicrobial resistance rates for the *A. baumannii* isolates from the current study were therefore within the upper limits of these previous findings, except gentamicin.

Bacterial resistance rates are affected by cross-transmission of strains among patients in ICUs. The patient isolates are usually derived from a few common ancestors, who exists in ICU, and they have similar pulsotype and resistance profile. In this study, clusters had similar antibiotic resistance profiles. Antimicrobial resistance can develop in persistent bacteria in ICUs because of heavy drug use.[23] Interestingly, in this study, varying levels of resistance to gentamicin and amikacin were observed in some pulsotypes of both *A. baumanni* and *P. aeruginosa* (AP5, AP11, and PP13). It is likely that these pulsotypes have been present in our hospital for an extended period, and have acquired resistance to these drugs while present in the ICU. If we interpret according to antimicrobials susceptibility table, PTZ and FEB may be the most commonly used drugs for treatment of *P. aeruginosa* and gentamicin may be the least used antimicrobial in this unit. But in this study, the frequency of use of antimicrobials were not investigated.

CONCLUSION

Five outbreaks occurred during the 9-month study period, with 67 patients affected by nosocomial *A. baumanni* and *P. aeruginosa* infections. Nosocomial infections and the development of antimicrobial resistance can be prevented with infection control programs, which should be prepared according to the conditions of the specific hospital, and by controlled drug use. Despite infection control measures being in place at our hospital, the findings of the current study suggest that these protocols should be reviewed. These findings also indicate that an infection control program and controlled drug use may not be sufficient for control of nosocomial infections. Epidemiological surveillance and subsequent revision of control programs is essential for individual hospitals.

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