Molecular epidemiology of *Acinetobacter baumannii-Acinetobacter calcoaceticus* complex isolated from clinical specimens at an intensive care unit

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The *Acinetobacter calcoaceticus-baumannii* (*A. calcoaceticus-A. baumannii*) complex is an important cause of nosocomial infections, increasing -mortality and morbidity in hospitals, especially in intensive care units (ICUs).[1] The aim of this study was to determine susceptibility patterns and clonal relationships of 76 *A. calcoaceticus-A. baumannii* complex isolates collected between March 2006 and July 2007 in the Anesthesia ICU of Karadeniz Technical University Hospital, Trabzon, Turkey.

The study was performed over a 17-month period in the 8-bed Anesthesia ICU of the Karadeniz Technical University Farabi Hospital, Trabzon, Turkey Local ethical committee approval was obtained. All isolates of *A. calcoaceticus-A. baumannii* complex were recovered from routine clinical specimens (respiratory tract, blood, urine, skin, and wound samples) from the Anesthesia ICU, identified using standard techniques, identification being confirmed using the Phoenix identification/antimicrobial susceptibility Testing (ID/AST) system (Becton Dickinson Diagnostic Instrument Systems, Sparks, MD, USA). Susceptibility of the *A. calcoaceticus-A. baumannii* complex isolates was investigated using the standardized Kirby-Bauer disk diffusion method and BD Phoenix NMIC/ID-55 panels (Becton Dickinson Diagnostic Instrument Systems, Sparks, MD., USA). The antimicrobial agents tested were by disk diffusion as follows: Amikacin (30 µg), ampicillin-sulbactam (10/10 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), piperacillin-tazobactam (100/10 µg), tetracycline (30 µg), gentamicin (10 µg), and trimethoprim-sulfamethoxazole (1.25/23.75 µg). Susceptibility test results were categorized as susceptible, intermediate, or resistant according to criteria recommended by the Clinical and Laboratory Standards Institute (M100-S16).[2] Isolates of intermediate sensitivity were categorized as resistant. For Pulse-field gel electrophoresis (PFGE) analysis, bacterial DNA was prepared as previously described with some modifications.[1] The DNA restriction was carried out with SmaI enzyme. Electrophoresis was performed in a contour-clamped homogeneous electric field (CHEF) DRIII apparatus (Bio-Rad Laboratories, Hercules, CA., USA) for 19 hours at 14°C, with an electric field of 6 V/cm and pulse angle of 120°, the pulse time being increased from 5-20 seconds. The lambda phage concatemers were run simultaneously as a size marker. The gels were observed under UV light and photographed using an imaging system. The DNA restriction patterns were interpreted using well-established criteria. Isolates with identical patterns were considered genetically indistinguishable, while those that differed by 1-3 bands were defined as subtypes of the same clone, 4-6 bands as possibly related, ≥7 bands as epidemiological relatedness.[1]

Seventy-six patients (59 male, 17 female) admitted to the Anesthesia ICU who developed Acinetobacter infection with an age range from 7-65 (median age, 37 years) were enrolled. The 76 Acinetobacter strains were isolated from 76 clinical specimens, including 41 (54%) tracheal aspirates, 20 (26%) blood, 7 (9%) wound, 5 (7%) catheter, and 3 (4%) urine. The most frequent infection type was ventilator-associated pneumonia, followed by bloodstream infections. The *A. calcoaceticus-A. baumannii* complex isolates were resistant to most of the antimicrobial drugs, but carbapenems were the most active agents and all 76 strains collected during the study period were sensitive to imipenem and meropenem. All isolates were resistant to piperacillin, cefepime, cefotaxime, and ceftazidime. Sensitivity to levofloxacin was determined in 48.7% of the isolated tested, ciprofloxacin in 34.2%, amikacin in 23.7%, and gentamicin was determined in 2.6%. Of the bacterial isolates, 15 antibiotypes were defined using the results of antibiotics susceptibility testing of *A. calcoaceticus-A. baumannii* complex isolates and designated as I to XV (**Table 1**). Antibiotypes I-V and VII were common among the isolates tested, and this antibiotic susceptibility pattern showed 64 (84.2%) Acinetobacter strains. Seventy-six Acinetobacter isolates were analyzed by PFGE during the study. In our study, the first isolates *A. calcoaceticus-A. baumannii* identified from each of the patients were subjected to PFGE studies. We determined 7 different main clones (designated as A to G) and 2 subtypes to clone A (subtypes A1 and A2), 2 subtypes to clone B (subtypes B1 and B2) and 2 subtypes to clone D (subtypes D1 and D2). The most common PFGE patterns were found as A1 (n=13; 17.1%), A2 (n=11; 14.5%), D1 (n=15; 19.7%), D2 (n=15; 19.7%), and C (n=10; 13.2%). The remaining PFGE types were involved: B1 (n=3; 3.9%), B2 (n=6; 7.9%) and just one isolate to each of clones E, F, and G. There was no clonal difference among clinical samples such as blood, tracheal aspirate, wound, and urine.

Acinetobacter spp. are opportunist pathogens that are widely distributed in soil and fresh-water sources, their levels increasing every day as a cause of nosocomial

Table 1 - Relevant characteristics of the 76 *Acinetobacter calcoaceticus-baumannii* complex isolates.

Antibiotypes (resistance patterns)	Genotypes										Total
	A1	A2	B1	B2	C	D1	D2	E	F	G	
I (Gm Ak Pi Cax Cpe Cft Caz SxT Te Cp)	7	1						1			**9**
II (Gm Ak Pi PTc Cax Cpe Cft Caz SxT Te Cp Lvx)	1	3	1			4	2				**11**
III (Gm Pi PTc Cax Cpe Cft Caz SxT Te Cp Lvx)	1	1		2	3						**7**
IV (Gm Ak A/S Pi PTc Cax Cpe Cft Caz SxT Te Cp Lvx)	1	2		4	5	2	4				**18**
V (Gm Ak A/S Pi PTc Cax Cpe Cft Caz SxT Te)	1			1	1	2	7			1	**13**
Others	2	4	2	1	2	4	2		1		**18**

Gm - gentamicin, Ak - amikacin, A/S - ampicillin/sulbactam, Pi - piperacillin, PTc - piperacillin-tazobactam, Cax - ceftriaxone, Cpe - cefepime, Cft - cefotaxime, Caz - ceftazidime, SxT - trimethoprim-sulfamethoxazole, Te - tetracycline, Cp - ciprofloxacin, Lvx - levofloxacin

infections.[3] Due to their ability to acquire multi-drug resistance, surviving on dry surfaces for a long time and at various temperatures and pH conditions, they become as important as *Pseudomonas species* in nosocomial infections.[1,3,4] In the last decade, these infections emerged as an important health care problem, especially in ICUs where advanced invasive diagnostic and therapeutic procedures are carried out and antibiotics are used extensively and indiscriminately.[3,5] In our study, antibiotypes I-V and VII that appeared in 64 isolates (84.2%) were most common antibiotic susceptibility patterns. Although antibiotyping is a phenotyping method, its discriminatory power is as low as those of other phenotyping methods. Antibiotyping is of limited value because isolates that are not genetically and epidemiologically related may have the same susceptibility by acquisition of the same plasmid; simultaneously, isolates genetically and epidemiologically related may have different susceptibility patterns during infection.[6] In this research, antibiotic susceptibility patterns were less helpful regarding the epidemiological study of Acinetobacter strains. Minor variations were frequently observed among outbreak isolates, as our period of study was a long one and antibiotypes were difficult to interpret without the help of PFGE. In addition, strains exhibiting the same susceptibility types were placed into different PFGE patterns. It has been concluded that antibiotypes may be suitable as screening methods in epidemiological investigations, but require confirmation of results by complementary techniques such as PFGE. Although many molecular techniques are available, PFGE is regarded as the genotyping "gold standard."[1] The PFGE patterns of the outbreak strains were easily separated from epidemiologically unrelated strains because they exhibited distinctly different band patterns. Seventy-six isolates were observed, 31.6% belonging to clone A, 11.8% to clone B, 13.2% to clone

C, 39.4% to clone D, and just one isolate to each of clones E, F, and G. Although it is possible to identify the mode of spread, source, and vectors of infections using molecular typing methods, the application of PFGE is highly demanding, difficult and time-consuming and also requires skilled staff, let alone more scientific considerations like unavailability of standardization for each microorganism. Furthermore, it is still an expensive technique, although it helps cut down hospital expenses in preventing hospital-acquired infections. Therefore, it is recommended by the authorities that the practice of PFGE should be reserved for university hospitals or for central laboratories.

Seventy-three *Acinetobacter calcoaceticus-baumannii* complex isolates (96%) were involved in 4 main clones, causing several outbreaks over the 17 months. The PFGE analysis of *A. calcoaceticus-A. baumannii* complex isolates led to the identification of 4 major clones represented by 96% of isolates, which supports cross-transmission of Acinetobacter among patients. This finding emphasizes that the implementation of aggressive and efficient infection control measures could successfully reduce the incidence of infection with Acinetobacter in the ICU. In previous studies,[4] it has been reported that *Acinetobacter spp.* nosocomial infections in the ICU can be prevented at a level of approximately 66.7%, although our study suggests this can be improved still further. However, the question of how the transmission between the patients had occurred could not be ascertained since environmental and/or finger samples were not available. One another important limitation of this study was that no comparison could be made between services since it was conducted only in the anesthesia ICU.

In conclusion, most *Acinetobacter spp.* infections that arise in patients admitted to ICUs are preventable cross-infections, and the use of such molecular techniques as PFGE in surveillance can bring on a

reduction in infection levels with the taking of the requisite precautionary measures. We suggest that a larger number of sample from patients, hospital staff members, and the environment should be obtained, and studied to establish more accurately the nature of transmission route between patients.

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