

Molecular Epidemiology of *Acinetobacter baumannii* in Central Intensive Care Unit in Kosova Teaching Hospital

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Infections caused by bacteria of genus *Acinetobacter* pose a significant health care challenge worldwide. Information on molecular epidemiological investigation of outbreaks caused by *Acinetobacter* species in Kosova is lacking. The present investigation was carried out to enlight molecular epidemiology of *Acinetobacter baumannii* in the Central Intensive Care Unit (CICU) of a University hospital in Kosova using pulse field gel electrophoresis (PFGE). During March - July 2006, *A. baumannii* was isolated from 30 patients, of whom 22 were infected and 8 were colonised. Twenty patients had ventilator-associated pneumonia, one patient had meningitis, and two had coinfection with bloodstream infection and surgical site infection. The most common diagnoses upon admission to the ICU were politrauma and cerebral hemorrhage. Bacterial isolates were most frequently recovered from endotracheal aspirate (86.7%). First isolation occurred, on average, on day 8 following admission (range 1–26 days). Genotype analysis of *A. baumannii* isolates identified nine distinct PFGE patterns, with predominance of PFGE clone E represented by isolates from 9 patients. Eight strains were resistant to carbapenems. The genetic relatedness of *Acinetobacter baumannii* was high, indicating cross-transmission within the ICU setting. These results emphasize the need for measures to prevent nosocomial transmission of *A. baumannii* in ICU.

Key-Words: Genotyping, *Acinetobacter baumannii*, Kosova, intensive care unit.

Acinetobacter spp. are opportunistic pathogens that have emerged to an infectious agent of importance to hospitals worldwide [1-3]. They can be found in the natural environment, hospital surroundings and on the skin of the human body. Some strains of *Acinetobacter* can survive environmental desiccation for weeks, promoting transmission through fomite contamination in hospitals [4].

Acinetobacter spp. cause a wide range of nosocomial infections, such as ventilator-associated pneumonia, bloodstream infections, urinary tract infections, surgical site infections and meningitis, especially in immunocompromised patients staying in ICU [5]. Other risk factors for colonization and infection are recent surgery, central vascular catheterization, tracheostomy, mechanical ventilation, enteral feeding and treatment with antibiotics (third-generation cephalosporins, fluoroquinolones or carbapenems)[6,7]. Extensive use of antimicrobials within hospitals has contributed to the emergence and increase of antimicrobial resistance among *Acinetobacter* strains [8].

Numerous reports implicates *A. baumannii* as a major pathogen involved in nosocomial infections causing epidemic outbreaks or endemic occurrence with a documented high mortality rates [9-12]. An increase in the number of *A. baumannii* isolates from clinical samples has been observed in microbiology laboratory over the past few years in ICU of university hospital in Kosova. But, this was not accompanied by detailed epidemiological and clinical investigation.

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Knowledge regarding species, strains and clones of *Acinetobacter* circulating in Kosova hospitals is lacking. Published data concerning the clinical implications of *Acinetobacter spp.* infections in Kosova are scarce. A study regarding clinical samples of *Acinetobacter spp.* isolates and their susceptibility pattern undertaken during 2001-2004, showed a total of 242 *Acinetobacter spp.*, of which *A.baumannii* predominate with 81.2% [13]. The majority of samples were revealed from patients staying in ICUs (62%). Based on laboratory report between March 2005 and August 2006, *A. baumannii* was responsible for 100 of the 719 infections, which occurred in the CICU (13.9%). Other most common isolated pathogens were *P. aeruginosa* (22.1%), *S. aureus* (15.39%), and *Klebsiella pneumoniae* (12.9%).

The present study was undertaken to elucidate the molecular epidemiology of *Acinetobacter baumannii* using pulse field gel electrophoresis (PFGE). Therefore, the objectives of the present study were (i) to assess the genetic relatedness of *A. baumannii* isolates in the ICU of our university hospital; and (ii) to study the clinical features of patients from whom *A. baumannii* had been isolated.

Material and Methods

Hospital Setting and Patients

The study was conducted at the University Clinical Centre of Kosova (UCCCK), in Prishtina, the capital city of Kosova. The center has 2,100 beds with approximately 60,000 admissions per year and serves as the only referral tertiary care center for a population of around 2.1 million. The Central Intensive Care Unit is a mixed ICU with 12 beds. The bacterial isolates selected for the present study included 30 *A. baumannii* isolates from 30 patients from the ICU of UCCCK, during the period from March 2006 to July 2006. Laboratory

diagnosis of microbiological samples and susceptibility testing was done in the Department of Microbiology within the National Institute for Public Health of Kosova. The genotyping was performed in the Clinical Hospital Centre Zagreb, Department of Clinical and Molecular Microbiology in Zagreb, Croatia. Clinical specimens included cerebrospinal liquid, endotracheal aspirate, thoracic drain and tracheostoma. The following data were recorded from the medical charts of patients with *A. baumannii* infection or colonisation: age, gender, number of patient-days in hospital, underlying diseases or conditions, susceptibility pattern and clinical outcome. Nosocomial infections were classified according to standard CDC definitions, whilst *A. baumannii* was considered to be a colonising organism when it was isolated from clinical specimens, but the criteria for infection were not met [14]. Only one sample of *A. baumannii* per patient was enrolled in the study.

Microbiological Methods

A. baumannii strains were collected from clinical specimens by using standard methods, isolated in pure cultures on MacConkey agar plates. Organisms were identified by using the API system for the identification (bioMérieux, Marcy l'Etoile, France). From a fresh 18 hours plate culture of *A. baumannii*, a heavy, cloudy suspension of the organism was made in the CRYOBANK™ medium in the tube (COPAN Diagnostics Inc., CA, USA). Tube was mixed by shaking and inverting to allow the bacteria in the suspension to coat the beads. Using a sterile pipet the CRYOBANK™ medium was removed from the tube. Then, the tube was placed in a -70°C freezer to store the culture. Afterwards the samples were transported to Croatia where the bacteria were recovered removing the cap of the CRYOBANK™ tube. Using forceps one bead was rolled over the culture mediums (brain-heart infusion) and Kaufman-Müller broth. Isolates were verified in Croatia as *A. baumannii* using the Vitek 2 automatic system (bioMérieux, Marcy l'Etoile, France).

Antimicrobial Susceptibility

Antimicrobial resistance was determined by the disk diffusion method according to the Clinical and Laboratory Standards Institute criteria, former NCCLS [15]. The following antimicrobial drugs were tested: Ampicillin 10µg, Ceftriaxone 30µg, Gentamicin 10µg, Amikacin 30µg, Imipenem 10 µg, Piperacillin + tazobactam 100µg, Cefoxitin 30µg, Ceftazidime 30µg, Tobramycin 10µg, Cotrimoxazole 1.25+23.75 µg and Ciprofloxacin 5µg.

Molecular Typing by Pulsed-Field Gel Electrophoresis (PFGE) and Dendrogram Analysis

The preparation of genomic DNA of *A. baumannii* isolates was performed as described by Schwartz and Cantor with minor modifications. Macrorestriction analysis of chromosomal DNA with XbaI was carried out by PFGE following published procedures [16]. PFGE was run in a CHEF-DRIII apparatus (Bio-Rad Laboratories, CA, USA), with pulses

ranging from 5 to 50 seconds at a voltage of 6 V/cm at 10-12°C for 20 h. Products were detected after staining with ethidium bromide (50 mg/mL) and photographed with Polaroid type 667 film. A ladder of bacteriophage lambda concatemers (New England Biolabs) was used as molecular weight markers.

Clusters of possibly related isolates were identified by using the Dice coefficient of similarity and unweighted group method with arithmetic averages at 80%, which indicates four-to six fragment differences in gels. The relationships between all isolates were analysed using the *GelComparII* software package and presented as a dendrogram (Applied Maths NV, Belgium). DNA fingerprints were interpreted as recommended by Tenover et al. [17].

Results

From March 16th to July 27th 2006, a total of 30 *Acinetobacter baumannii* isolates were obtained from 30 patients (24 males, 6 females) admitted to the CICU. Their age range was from 2 to 82 years (mean age 47.5, median age 52.5 years). Based on evaluation of clinical charts, 22 patients were classified as infected and had nosocomial infections and eight of them were considered colonized with *A. baumannii*.

Isolates were most frequently recovered from endotracheal aspirate (n = 26); the other isolates were recovered from tracheostoma (n=2), thoracic drain (n=1) and cerebrospinal fluid (n = 1). Twenty patients developed nosocomial pneumoniae; one patient had a diagnosis of meningitis, and two had coinfection with bloodstream infection and surgical site infection. The most common diagnoses upon admission to the ICU were polytrauma and cerebral hemorrhage. Other pathogens were co-isolated from nine patients: *Staphylococcus aureus* from two patients, *P. aeruginosa* from 4 patients, and *Klebsiella pneumoniae* from three patients.

The clinical characteristics of patients from whom *A. baumannii* was isolated are shown in Table 1.

The length of stay in ICU ranged from 1-59 days with median time of 17 days. The median time that had elapsed between admission and isolation of *A. baumannii* was 8 days. During the ICU stay, 16 patients died (crude mortality 53.3%) and the *A. baumannii*-attributable mortality was 62.5% (10 / 16). The time-frame of admission, discharge and isolation of *A. baumannii* from ICU patients is presented in Figure 1.

The length of ICU stay for non-survivors and survivors was 14.5 and 18.5 days, respectively. The length of stay was significant in comparison between infected and colonised patients (19.5 vs. 9 days).

The length of ICU stay for epidemic strains and non-epidemic strains was 15.7 and 24.5 days, respectively.

Four patients yielded *A. baumannii* upon hospital admission and were transferred from other hospitals to ICU, while 22 patients yielded *A. baumannii* only following hospitalization. Twelve patients were transferred to ICU from other departments within the UCCK.

PFGE profiles of *A. baumannii* strains isolated from CICU is shown in the Figure 2.

Figure 1. Time frame of admission, discharge and isolation of *Acinetobacter baumannii* from ICU patients.

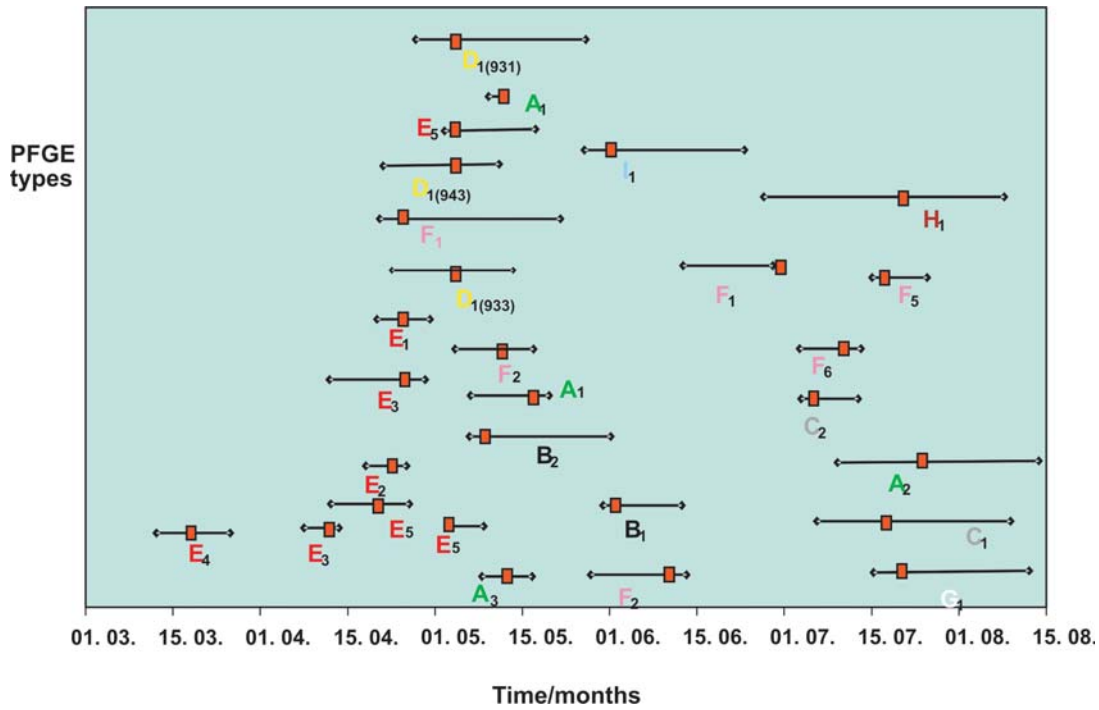


Figure 2. Dendrogram depicting 30 representative isolates of *Acinetobacter baumannii* species obtained from CICU.

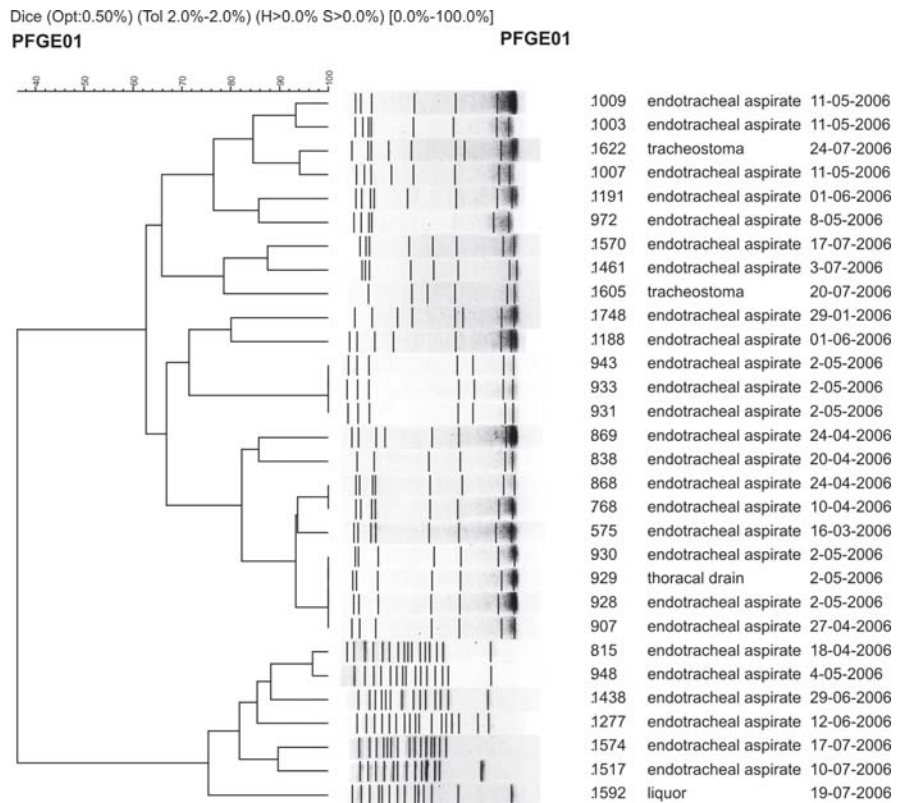


Table 1. Clinical and PFGE data of the patients with *Acinetobacter baumannii* isolates.

Nr	Gender/ age	Day of isolation	Length of ICU stay	Diagnosis	Sensitive	Outcome	Sample	Isolate	PFGE type
1	M/66	17	18	Tumor cerebri	AMI	Died	EA	1277	F4
2	M/33	4	28	Tumor cerebri	TOB, AMI, IMI	Died	CLS	1592	G1
3	M/62	3	8	Tumor cerebri	AMI, IMI, TOB, GEN	Died	EA	1007	A3
4	M/17	1	14	Diabetes mellitus	AMI, CIP	Recovered	EA	930	E5
5	M/11	12	37	Politrauma	TOB, AMI, IMI	Transferred	EA	1570	C1
6	M/62	4	6	Politrauma	AMI, IMI	Died	EA	838	E2
7	M/41	4	28	Politrauma	IMI	Recovered	EA	972	B2
8	F/33	10	14	Politrauma	AMI, IMI	Transferred	EA	868	E3
9	M/51	14	59	Cerebral infarct	TOB, AMI, CAZ	Died	TRA	1622	A2
10	M/2	1	10	Politrauma	IMI	Died	EA	1461	C2
11	M/21	8	10	Politrauma	IMI, AMI	Died	EA	1517	F6
12	M/72	3	5	Cerebral infarct	AMI, IMI	Died	EA	768	E3
13	M/43	1	13	cardiac arrest	GEN, AMI, CAZ	Recovered	EA	1191	B1
14	M/55	4	8	Politrauma	AMI, IMI	Recovered	EA	869	E1
15	M/74	18	23	Politrauma	TOB, AMI	Recovered	EA	933	D1
16	M/71	9	34	Politrauma	AMI, CIP, IMI	Recovered	EA	907	E5
17	M/57	2	8	Politrauma	GEN, TOB, AMI, IMI	Transfer	EA	1574	F5
18	M/46	14	20	Tumor cerebri	AMI, IMI	Recovered	EA	943	D1
19	F/20	2	15	Pulmonal infiltrat	AMI, IMI	Died	TC	929	E5
20	M/22	1	1	Politrauma	IMI, CAZ, AMI	Transferred	EA	1003	A1
21	M/50	5	11	Tumor cerebri	IMI	Died	EA	575	E4
22	F/24	15	30	Myocardiopathia	AMI	Died	EA	1438	F3
23	M/82	8	32	Peritonitis	AMI, IMI	Died	EA	931	D1
24	F/54	26	44	Peritonitis	TOB, AMI,	Transferred	TRA	1605	H1
25	F/72	12	34	cardiac arrest	AMI, IMI	Died	EA	1188	I1
26	M/60	10	14	Cerebral infarct	AMI	Died	EA	815	F1
27	M/74	8	17	Politrauma	IMI, CAZ, AMI	Recovered	EA	1009	A1
28	F/71	10	19	Tumor cerebri	AMI, IMI	Transferred	EA	933	D1
29	M/61	16	19	Cerebral infarct	AMI	Died	EA	907	E5
30	M/20	8	11	Politrauma	AMI, IMI	Died	EA	948	F2

EA= endotracheal aspirate; AMI=amikacin, IMI=imipenem, TOB=tobramycin, CIP=ciprofloxacin, CAZ=ceftazidime, GEN=gentamycin; TRA=tracheostoma; TC=Thoracic drain.

Genotypic analysis of *A. baumannii* isolates from ICU patients identified nine major PFGE patterns, which we named from A to I, that differed in migration of at least four DNA fragments and showed a similarity of < 80% at dendrogram analysis. Of these, PFGE pattern E predominate with isolates from nine patients. Eight isolates were resistant to carbapenems.

Discussion

Although only 5-10% of all hospitalized patients are treated in ICUs, they account for approximately 25% of all nosocomial infections [18]. The incidence of nosocomial infections in ICUs is 5-10 times higher than that observed in general hospital wards [19,20]. In developing countries the occurrence of nosocomial infections is 12-20 fold higher [21].

A. baumannii outbreaks have been reported previously, particularly in ICU wards [22-26]. Severe underlying diseases,

invasive diagnostic and therapeutic procedures used in ICUs have been demonstrated to predispose patients to severe infections with *A. baumannii* [27-29]. Our results show that nosocomial infections and colonizations by *A. baumannii* in the ICU were prolonged for several months. The impact of *A. baumannii* on ICU-acquired infections and colonization was substantial from clinical samples received in our laboratory from CICU. From March 2006 to August 2007 *Acinetobacter* spp. were the second most prevalent identified microorganism with 13.9% (100/719). Other most frequent isolates were *P. aeruginosa* (22.1%), *S. aureus* (15.3%) and *Klebsiella* spp. (12.9%). *Acinetobacter* strains (n=100) showed globally high resistance pattern to cephalosporins (76.9%). Imipenem and amikacin were the most effective drugs against *A. baumannii* with sensitivity rate of 92.4% and 85.7% respectively (unpublished data).

Previous prevalence studies in Kosova showed high rates of health care associated infections in UCCK (17.4%) and in

CICU with 68.7% of patients having nosocomial infections, with a predominance of ventilator associated pneumonia (72.7% of infections) [30,31].

There are many causes for high rate of nosocomial infections in ICU and *A. baumannii* outbreaks. Main factor remains the lack of support and implementation of prevention and control policies. The proportion of health care workers working in CICU to patients staying in ICU is only 5 HCW per 12 patients per shift. CICU is referent center for intensive care for all 6 regional hospitals and other departments within the UCCK.

Single use devices were reused due to limited budget. Suction catheters for aspiration of respiratory tract were amongst most used equipment in this group. Audit in the ward during the study period proved that these catheters were placed in a containers containing diluted chlorhexidine. The same catheters after "disinfection" were used for more than one patient carrying a significant risk for cross-infection. Some equipment used in ICU were outdated and their maintenance services were not regular.

A study of compliance with hand hygiene in CICU showed the alert rate of only 19% [32]. During the outbreak period alcoholic hand rubs were not in used in ICU. There are three washing sinks in the ward. Low number of wash sinks contributed to high rate of infection in ICU. Gloves were not changed after each contact with patients but they were used and maintained for successive patients intervention.

For many years in Kosova, the cephalosporins are the drugs of choice in empiric treatment in ICU and they have been used without any restrictions not only in ICUs but also in other hospital wards and ambulatory care. This could explain the high resistance rates of *Acinetobacter baumannii* to antimicrobials. For a decade in Kosova, all antimicrobials have been available in pharmacies without a physician's prescription.

CICU is reference center for patients from other hospital departments of CICU, from regional hospitals and also from the private hospitals. Delay of referral to this unit contributed to infections, severity of illness and poor outcome prognosis for the patients.

Delay is related to patients who are previously treated at the regional hospitals and they are not transferred on time to the CICU, which is the only ICU reference center for six regional hospitals and for 13 clinics within UCCK.

Genotypic analysis of *A. baumannii* isolates from ICU patients identified nine major PFGE patterns. The most predominant clones of *A. baumannii* (E and F) were related with more than one outbreak during the study period occurring sequentially. Case-control study was not performed in epidemiological investigation. The data were recorded from the medical charts of patients with *A. baumannii* infection or colonization. Some genetically indistinguishable *A. baumannii* isolates (931, 933 and 934) were isolated on the same day (May 2, 2006) and had similar antimicrobial susceptibility pattern, suggesting common source of infection. The median

time from admission to isolation of this bacteria revealed that it's shorter than in other publications [23-27].

In cases where the genetically same strains were not related in timely manner, the only explanation would be poor hand hygiene of health care workers (HCW). Another argument is high endemic rate of MRSA, which is 61.3% of all *S. aureus* isolates [13]. These facts suggests the horizontal transmission of the epidemic strains from one patient to another through the hospital staff.

As in other publications endotracheal aspirates were predominant clinical samples received from ICU [33,34].

The length of stay was significant in comparison between infected and colonised patients. This finding is consistent with reports of other outbreaks [6,29,33,34]. But there was no significant difference between non survivors and survivors. This can be explained with a fact that some patients spent some hospital days in regional hospitals before referral to CICU and also six patients were sent for treatment in other ICUs in neighbouring countries. As in previous studies, the respiratory tract was the most frequent site of isolation of *A. baumannii* in ICU patients [35,36]. Colonization with *A. baumannii* in not performed routinely at admission to ICU.

In conclusion, we show here that *A. baumannii* strains cause large and sustained hospital outbreak due to insufficient preventive measures. These results emphasize the need for preventive interventions in ICU.

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