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Original Article

Contribution of Contaminated Environmental Surfaces to the Transmission of Multidrug-resistant *Acinetobacter baumannii* in Special Care Units of a Hospital in Tehran, Iran

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Introduction: *Acinetobacter baumannii* is one of the major causes of nosocomial infections. We investigated the presence of *A. baumannii* among patients and the environment in special care units of a hospital in Tehran, Iran. **Methods:** Sixty-nine non-duplicate clinical and environmental samples were collected from ICU, Post-ICU, and CCU in EbneSina Hospital (Tehran, Iran) from June 2015 to April 2016. The isolates were identified using standard biochemical tests, and their identity was confirmed by detection of bla_{oxa51} gene. Susceptibility to 14 antibiotics was determined by the disc diffusion method, and genetic fingerprinting of the isolates was performed by random amplified polymorphic DNA (RAPD-PCR). **Results:** We recovered 66 *A. baumannii* isolates, 41 from patients, and 25 from the environment. All isolates from patients were resistant to all tested antibiotics except colistin. Environmental isolates were resistant to piperacillin (100%), tetracycline, piperacillin/tazobactam, beta-lactams and quinolones (86%), and amikacin (81%) but sensitive to colistin. The RAPD-PCR results revealed 35 clusters with 80% similarity. Despite the heterogeneity among the RAPD-PCR profiles, similar patterns were observed among 11 clusters comprising both clinical and environmental isolates. **Conclusion:** The results of this research suggest that the presence of *A. baumannii* on hospital surfaces could have played an essential role in causing infection in the hospitalized patients. *J Med Microbiol Infec Dis, 2018, 6 (2-3): 37-42.*

Keywords: *Acinetobacter baumannii*, RAPD-PCR, Nosocomial Infections, Genetic Fingerprinting, ICU.

INTRODUCTION

Acinetobacter baumannii is an important opportunistic pathogen and responsible for a wide range of nosocomial infections including pneumonia, bacteremia, meningitis, urinary tract, burn and wound infections. These infections often occur in critically ill patients and usually after a relatively extended hospitalization period in intensive care units (ICUs) [1-3]. Previous studies in some hospitals highlighted the critical role of environmental contaminated inanimate objects and surfaces such as supply carts, floors, infusion pumps and ventilator touch pads by *A. baumannii* in the transmission of the infection to patients [4-6]. The extraordinary ability of *A. baumannii* to adapt to variable conditions like tolerance to desiccation and its remarkable ability to develop resistance to various antibiotics has led to the emergence of multidrug-resistant (MDR) *A. baumannii* strains which contribute to the persistence of these organisms in the hospital environment for extended periods of times [7-9]. Hospital outbreaks caused by *A. baumannii* are known to be associated with contaminated environments as well as healthcare personnel [6, 8-10]. An estimated 20% to 40% of nosocomial infections were attributed to cross-contamination via the hands of healthcare personnel, as a result of direct contact with patients or indirectly by contaminated inanimate surfaces [6]. Here, we investigated the potential cross-contamination of *A. baumannii* between the patients and their environment in EbneSina Hospital, Tehran, Iran. We also compared the antibiotic resistance patterns and the genetic fingerprints of the isolates obtained from various surfaces as well as the *A. baumannii*-infected patients.

MATERIAL AND METHODS

Bacterial Isolates. Forty-one non-duplicate specimens were collected from sputum, wounds, urine, and catheters of patients hospitalized in ICU, Post-ICU, and CCU of EbneSina Hospital, Tehran, Iran from June 2015 to April 2016. Also, 28 environmental samples were randomly collected from various inanimate objects and surfaces of all three wards (patients' beds, bedrails, bedside tables, patients' file covers, ventilators, supply carts, nursing station and wash basins). The patients' specimens were swabbed and streaked on MacConkey agar plates (Merck, Germany) and incubated at 37°C overnight. For the collection of environmental samples, moistened cotton

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swabs were used to swab the surfaces, transferred individually to tubes containing 5 ml Brain Heart Infusion broth (BHI, Merck, Germany) and incubated at 37°C overnight [11]. The swabs were streaked on MacConkey agar plates followed by incubation of plates at 37°C overnight. The isolates were then identified using standard biochemical tests including motility, catalase, oxidase, arginine dehydrogenase, sugar fermentation and H_2S production on triple sugar iron agar (TSI), methyl red (MR), Voges–Proskauer (VP) tests, oxidation/fermentation of glucose (O/F test), growth on MacConkey and Simmons Citrate agar, and growth at 42°C. Detection of the β-lactamase bla_{oxa-51}, an intrinsic gene in *A. baumannii*, was used for molecular identification of the isolates.

Antibacterial susceptibility. Susceptibility to 14 antibiotics was determined by the disc diffusion method according to the CLSI 2017 guidelines [12]. The antibiotics (Mast, UK) included cefepime (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), piperacillin (100 µg), piperacillin/tazobactam (110 µg), imipenem (10 µg), meropenem (10 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), amikacin (30 µg), gentamicin (10 µg), tetracycline (30 µg) and colistin (10 µg). *Pseudomonas aeruginosa* ATCC 27853 and *E. coli* ATCC 25922 were used as susceptible controls.

DNA extraction and PCR amplification. DNA was extracted by the boiling method as described before [13]. Detection of bla_{oxa-51} gene (353 bp) was performed by the primers: Forward; 5'-TAA TGC TTT GAT CGG CCT TG-3', and Reverse; 5'-TGG ATT GCA CTT CAT CTT GG-3' previously used by others [13]. PCR reaction mixtures (25 µL) contained 1 µL of DNA template, 1.4 mM $MgCl_2$, 0.2 mM dNTPs, 0.4 µM of each primer, and 0.6 U of *Taq* DNA polymerase in the buffer provided by the manufacturer (CinnaGen, Tehran, Iran). The amplifications were performed in a Peltier thermocycler (MG25$^+$, Long Gene Scientific Instruments, China) programmed for an initial denaturation at 94°C for 5 min, 30 cycles of 94°C for 25 s, 52°C for 40 s and 72°C for 50 s, followed by a final elongation step at 72°C for 6 min. PCR products were run on 1% agarose gels and visualized after staining with RedSafe (iNtRON Biotechnology, Korea) using an image analysis system (UVLtec; St John's Innovation Centre, UK).

RAPD-PCR. The 25 µL PCR reactions contained 4 µL of template DNA, 0.5 mM dNTPs, 4 mM $MgCl_2$, 1.5 U of *Taq* DNA polymerase, and 3 µM M13 primer (5′-CGGCAGCGCC-3′) in the buffer provided by the manufacturer (CinnaGen, Tehran, Iran) [14]. Amplifications were performed for denaturation at 94°C for 5 min followed by 35 cycles of 94°C for 45 s, 45°C for 1 min and 72°C for 2 min, with a final extension period of 10 min at 72°C. The PCR products were run on 1.5% agarose gels, stained, and visualized as described above. Analyses of RAPD-PCR fingerprints and generation of dendrograms were carried out using GelCompare II software (Applied Maths, Belgium).

RESULTS

Identification of the *A. baumannii* isolates. Identification of *A. baumannii* isolates was confirmed by biochemical tests as well as the presence of bla_{oxa-51} gene. Overall, 41 isolates were obtained from patients' specimens among which, 30 (73%) were from sputum (21 from ICU and nine from PICU patients). Nine isolates (22%) were recovered from wound infections (six from ICU and three from CCU). One isolate was obtained from urine (PICU) and one from a central venous pressure line in ICU. The majority of the isolates were from ICU (68%) followed by PICU (27%) and CCU (5%). Out of the 28 environmental samples, 25 were identified as *A. baumannii* among which, 8 (36%) were from patients' beds, six (24%) from ventilators, three (12%) from trolleys, two (8%) from the nursing station, two (8%) from the metal covers of patients' files, one from a bedside table (4%), one from an infants' bed warmer (4%) and one (4%) from a mop in ICU. The distribution of *A. baumannii* isolates from the patients' and environmental surface isolates are shown in Figure 1.

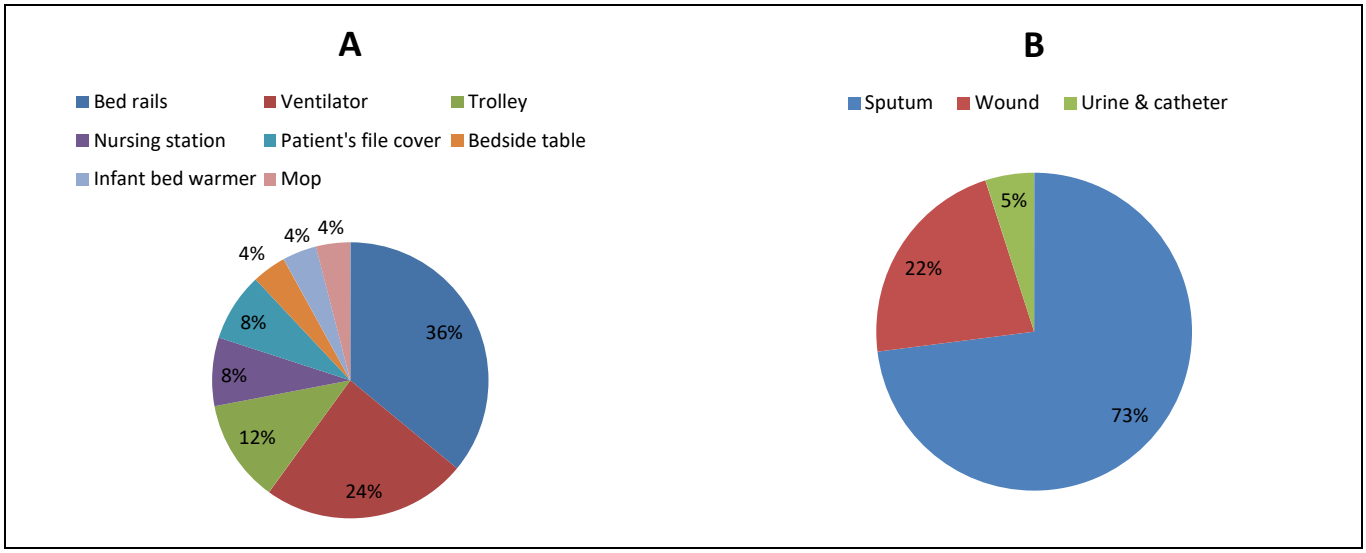
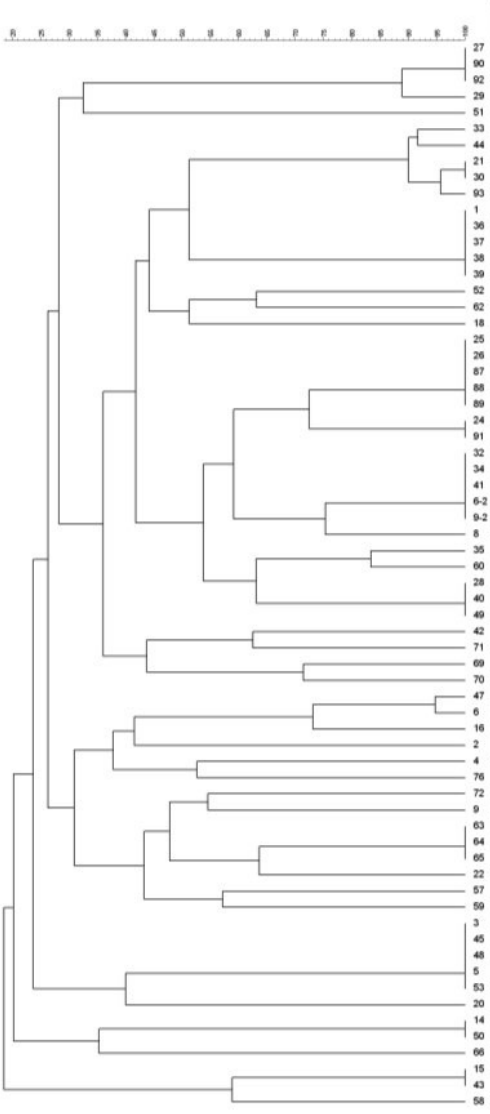


Fig. 1. Distribution and details of environmental (A) and clinical (B) isolates of *Acinetobacter baumannii* obtained from ICU, post-ICU, and CCU in EbneSina Hospital, Tehran, Iran

Antibiotics susceptibility test. All clinical isolates were resistant to all tested antibiotics except colistin (100% susceptibility). Intermediate resistance was not observed. Among the environmental isolates, 25 (100%) showed resistance to piperacillin, 24 (86%) to tetracycline, piperacillin/tazobactam, all tested β-lactams and quinolones, and 23 (81%) to amikacin. No resistance was detected to colistin.

RAPC-PCR analyses. RAPD-PCR analyses showed 35 clusters with 80% similarities (Figure 2). Despite the overall heterogeneity, similar fingerprints were observed among 11 clusters comprising 34 isolates including both clinical and environmental (Figure 2, clusters A-K) suggesting bacterial dissemination between patients and the environment. As seen in Figure 2, cluster A is comprised of five isolates, four from patients (designated A-D) and one from the ventilator of the room of the patient A. From five isolates observed in the cluster B, three belonged to the patients (designated E-G), and two were environmental (bed and ventilator of patient E). Cluster C included two isolates, one from a patient (designated H) and one from the nursing station in ICU. Cluster D comprised five isolates, three from patients (designated I-K) and two from beds of the patients K and J. Out of the three isolates in cluster E, one was from the sputum of a patient (designated L), and the other two were from the bed and the infant bed warmer of the same patient. Three isolates from different patients were grouped in the cluster F (designated M-Q). Cluster G included five isolates, three from patients (designated P-R) and two from the bed rails of patients R and Q. Cluster H had two isolates, one from a patient (designated S) and one from the ventilator of the same patient. Cluster I also had two isolates, one from a patient (designated T) and one from the bed of the same patient. Cluster J contained three identical isolates, one from a patient (designated U) and two were environmental isolates (trolley and nursing station). Finally, two isolates were observed in the cluster K, one from a patient (designated V) and the other from the bed rail of the same patient (Figure 2).



RAPD-PCR Cluster	Patient/specimen	Specimen source	Isolation site	Isolation date
J	-	nursing station	CCU	04/07/2015
J	Patient U	wound	CCU	07/08/2015
J	-	trolley	CCU	04/07/2015
-	-	-	-	-
-	-	-	-	-
-	-	-	-	-
-	-	-	-	-
K	Patient V	bed rail	P.ICU	24/04/2016
K	Patient V	sputum	P.ICU	14/04/2016
-	-	-	-	-
A	Patient A	ventilator	ICU	14/03/2016
A	Patient A	sputum	ICU	10/03/2016
A	Patient B	wound	ICU	26/02/2016
A	Patient C	wound	ICU	19/02/2016
A	Patient D	wound	ICU	22/02/2016
-	-	-	-	-
-	-	-	-	-
-	-	-	-	-
B	Patient E	bed	CCU	04/07/2015
B	Patient E	ventilator	CCU	04/07/2015
B	Patient E	wound	CCU	03/07/2015
B	Patient F	sputum	CCU	04/07/2015
B	Patient G	wound	CCU	27/06/2015
C	-	nursing station	ICU	01/10/2015
C	Patient H	wound	ICU	01/07/2015
D	Patient I	sputum	P.ICU	27/02/2016
D	Patient J	sputum	P.ICU	08/03/2016
D	Patient K	sputum	ICU	10/03/2016
D	Patient K	bed	ICU	14/03/2016
D	Patient J	bed	P.ICU	10/03/2016
-	-	-	-	-
-	-	-	-	-
-	-	-	-	-
E	Patient L	bed	ICU	09/10/2015
E	-	infant bed	ICU	01/10/2015
E	Patient L	sputum	ICU	09/10/2015
-	-	-	-	-
-	-	-	-	-
-	-	-	-	-
-	-	-	-	-
-	-	-	-	-
-	-	-	-	-
-	-	-	-	-
-	-	-	-	-
-	-	-	-	-
-	-	-	-	-
-	-	-	-	-
F	Patient M	sputum	P.ICU	03/04/2016
F	Patient N	sputum	P.ICU	02/04/2016
F	Patient O	sputum	ICU	26/02/2016
-	-	-	-	-
-	-	-	-	-
-	-	-	-	-
G	Patient P	bed rail	ICU	14/03/2016
G	Patient Q	sputum	ICU	11/04/2016
G	Patient P	sputum	ICU	11/04/2016
G	Patient Q	ventilator	ICU	14/03/2016
G	Patient R	wound	ICU	17/03/2016
-	-	-	-	-
H	Patient S	ventilator	ICU	13/04/2016
H	Patient S	sputum	ICU	01/04/2016
-	-	-	-	-
I	Patient T	bed	ICU	13/04/2016
I	Patient T	sputum	ICU	03/04/2016
-	-	-	-	-

Fig. 2. Analysis of *Acinetobacter baumannii* clinical and environmental isolates based on random amplified polymorphic DNA typing using primer M13. The dendrogram was generated using GelCompare II software

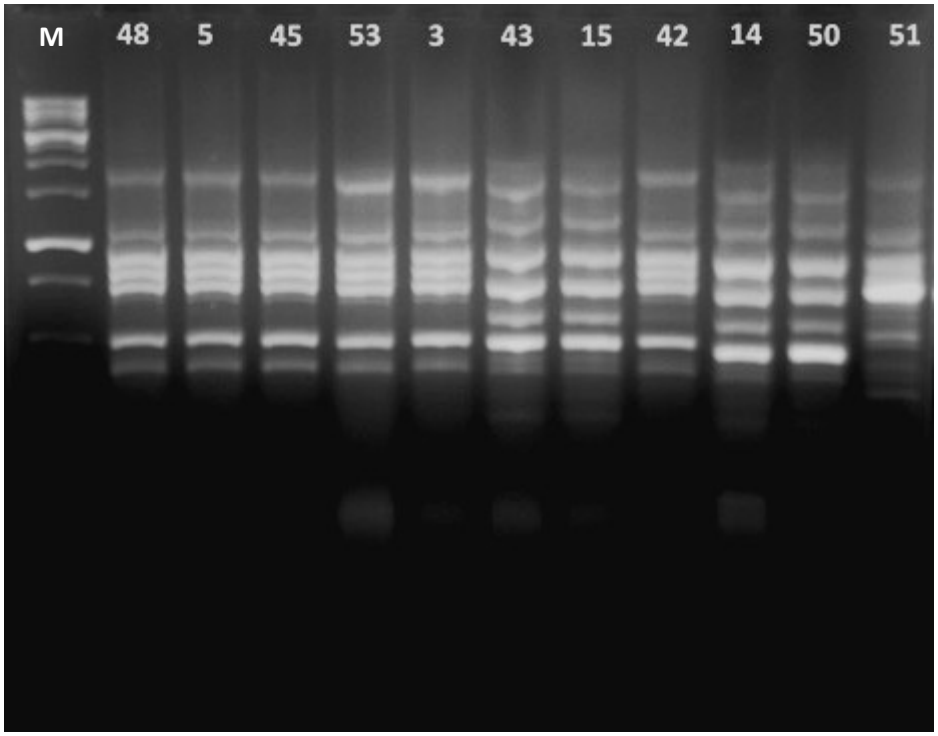


Fig. 3. RAPD-PCR analysis of clinical and environmental isolates of *Acinetobacter baumannii*. Lane M; 100 bp DNA ladder (Cinnagen, Tehran, Iran). RAPD fingerprints of cluster G: lanes 3, 5, 45, 48, 53; cluster H: lanes 14, 50; cluster I: lanes 15 and 43, two clinical isolates with unique fingerprints: lanes 42, 51.

All 34 isolates distributed in the 11 clusters were resistant to tested antibiotics and 100% sensitive to colistin. The majority of the isolates (22/34) were recovered from ICU including all isolates in clusters A, C, E, G, H and I, as well as two isolates from the cluster D and one from cluster F. Clusters B and J isolates were obtained from CCU and cluster K and two isolates of cluster F were from PICU. The remaining 32 isolates had unique RAPD-PCR fingerprints. The fingerprint profiles of clusters G, H, I and two patient isolates with unique fingerprints are reflected in Figure 3.

DISCUSSION

Environmental contamination of inanimate objects by multidrug-resistant (MDR) *A. baumannii* in hospital settings contributes to the dissemination of the organism and outbreaks of infection in intensive care units. In an outbreak by MDR *A. baumannii* in a burn unit, pulse field gel electrophoresis (PFGE) profiles of the isolates from some patients and the environment were identical [15]. Another study using PFGE revealed that 85% of the environmental isolates from supply carts, floors, infusion pumps, and ventilator touch pads were genetically related to the patients' isolates [4]. Study of an outbreak caused by MDR *Acinetobacter calcoaceticus-A. baumannii* complex in a surgical intensive care unit showed that patients, healthcare workers as well as medical equipments carried the predominant strain responsible for the outbreak [5]. Various molecular methods like enterobacterial repetitive intergenic consensus (ERIC)-PCR, PFGE and multilocus sequence typing (MLST)-PCR have demonstrated the genetic similarity of *A. baumannii* isolates from patients and their immediate environments [10]. Royer and colleagues (2015) used PFGE for fingerprinting of the *A. baumannii* isolates obtained from ventilators of pneumonia patients and the environment in an adult ICU and showed that cross contamination occurred by two predominant isolates [9]. In an outbreak in ICU of a hospital in Riyadh, Saudi Arabia, most of the clinical and environmental *A. baumannii* isolates (bed rails, curtain, stethoscope, computer mouse and telephone) belonged to two REP (repetitive element palindromic)-PCR clusters [16]. Also, a recent study identified MDR *A. baumannii* from clinical isolates and environmental samples using 16SRNA typing [8].

There are a few reports from Iran on the presence of MDR *A. baumannii* isolates in ICU environments, but there is no report on genetic similarity of the environmental and clinical isolates. Tajeddin and colleagues (2016) demonstrated contamination of healthcare workers' hands and the environmental surfaces of the ICU (oxygen masks, ventilators, and bed linens) with *A. baumannii* [17]. During a six months study, in the city of Qom, Iran, the environmental surfaces in the ICUs of four hospitals and NICU of one hospital exhibited higher contamination of *Acinetobacter* spp. compared to other Gram-negative bacteria [18]. Another recent study detected antibiotic-resistant *A. baumannii* in the air, water, and inanimate surfaces in different wards of four hospitals in Isfahan, Iran [19].

All clinical and environmental isolates of this study were multidrug-resistant. Our RAPD-PCR fingerprints

analyses of both clinical and environmental isolates revealed that 34 isolates distributed among 11 clusters had identical profiles, strongly suggesting that the source of infection might have been the environmental contamination. The origin of the majority of the isolates (64.7%) was the ICU, while 23.5% were from CCU and 11.8% from the PICU. These results indicate the highest rate of contamination in the ICU. We chose the RAPD-PCR analyses for the comparison of the isolates. Previously, this method had revealed reproducible profiles for *A. baumannii* clinical isolates [13]. Savov and colleagues (2012) also employed RAPD-PCR for comparison between clinical and environmental isolates from a hospital ICU. They found a single predominant RAPD fingerprint among both groups of the isolates and suggested that an epidemic strain was responsible for infecting the patients in the ICU [20].

According to our knowledge, this is the first report from Iran revealing the genetic similarity between clinical and environmental isolates of *A. baumannii* in a health center. Presence of environmental clones within the ICU allows dissemination of the organism via healthcare workers and medical equipment. Our experiments were performed in a relatively short period, *i.e*., 10 months. The dates of the isolates collection (Figure 2) suggested that cross-contamination between environmental strains and the patients' isolates occurred as early as one month up to three months into the study. Our results also showed that cross-contamination between patients and the hospital environment could readily lead to major outbreaks. Hence, implementation of control measures is required to prevent the spread of *A. baumannii* in hospitals. For this reason, regularly updated cleaning and disinfection strategies should be employed to reduce the number of pathogens in the hospital environments.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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