TÜBİTAK

Turkish Journal of Medical Sciences

http://journals.tubitak.gov.tr/medical/

Research Article

Turk J Med Sci
(2014) 44: 643-648
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doi:10.3906/sag-1304-126

Clonal comparison of *Acinetobacter* strains isolated from intensive care patients and the intensive care unit environment

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Received: 26.04.2013 • **Accepted:** 19.08.2013 • **Published Online:** 27.05.2014 • **Printed:** 26.06.2014

Background/aim: *Acinetobacter baumannii* is one of the most commonly encountered microorganisms in nosocomial infections. It is thought that strains found in the environment can be a source for contamination of patients by *Acinetobacter* strains that are resistant to environmental conditions. This study was carried out to compare *Acinetobacter* strains isolated from a variety of nonviable environments and from patients in intensive care units (ICUs), and to explore whether environmental areas may be a source for bacterial contamination.

Materials and methods: We studied *A. baumannii* strains isolated from ICU patients. When *A. baumannii* was isolated from the clinical sample of a patient, swab samples were collected from various areas in the ICU. VITEK 2 (bioMérieux, France) was used for the identification of *A. baumannii* strains and for antibiotic sensitivity tests. DNA fingerprinting analysis was performed with the repetitive sequence-based polymerase chain reaction (rep-PCR) method using a DiversiLab device (bioMérieux).

Results: During the study, a total of 100 *A. baumannii* strains including 92 samples from 61 patients and 8 samples from the environment were isolated. All of the isolated strains were found to have multiresistance to antibiotics. DNA fingerprinting results showed that 7 of the 8 strains isolated from the environment were identical to many strains isolated from the patients. The greatest similarity between samples was found for 1 *A. baumannii* strain isolated from a computer keyboard, which was identical to the bacterium isolated from 17 other samples.

Conclusion: A high level of similarity was found between strains isolated from the environment and patients, suggesting deficiencies in implementation of infection control measures.

Key words: *Acinetobacter baumannii*, source, rep-PCR, nosocomial infection

1. Introduction

Acinetobacter is one of the major causative agents of nosocomial infections, which are resistant to multiple drugs and are difficult to treat. In fact, *Acinetobacter* species are bacteria that have low virulence. However, they are identified as important opportunistic pathogens among patients, particularly in those with impaired functioning of normal host defense mechanisms (1). Specifically, these are the most prevalent infectious agents encountered in intensive care units (ICUs) all over the world (2,3). The most important factors that lead to frequent identification of *Acinetobacter* spp. as causative agents in nosocomial infections include their resistance to external conditions, long-term viability in environmental areas, and their spread among patients (4). *A. baumannii* spp. often colonize the skin and lower respiratory tracts of hospitalized patients, and they are isolated from sputum, blood, urine, and fecal samples of the patients (5). While *Acinetobacter* species are commonly found in water and soil, they can also reside in frozen foods, clothes, patient beds, and ventilator circuits and on many other materials and surfaces in the hospital environment (6,7). As part of infection control measures, close-contact isolation is recommended when a multiresistant *Acinetobacter* strain is isolated from a patient. This is a preemptive procedure to prevent transmission from one patient to another. Implementation of appropriate disinfection practices in the surrounding areas is the most important protective method. However, the failure to prevent *Acinetobacter* infections, the occurrence of outbreaks in many centers, and the fact that *Acinetobacter* strains continue to be a major cause of mortality and morbidity among ICU patients suggest that there are still some deficiencies in implementation.

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This study was performed with the aim of investigating the DNA-clonal relatedness of the *Acinetobacter* strains isolated from various inanimate areas in the ICU and strains isolated from ICU patients hospitalized in the authors' hospital and of evaluating the extent to which environmental areas represent a source for bacterial contamination.

2. Materials and methods

This study was carried out on *A. baumannii* strains isolated from various clinical samples of patients hospitalized in the internal ICU of the Gaziantep University Faculty of Medicine Hospital from January 2010 to June 2011. Strains included in the study were isolated from tracheal aspirate, blood, catheter, urine, and cerebrospinal fluid (CSF) samples. Care was taken to ensure that transportation of the samples to the laboratory occurred inside suitable containers and/or transportation media. Samples from blood cultures were delivered to the laboratory after being collecting in BacT-Alert (bioMérieux, France) automated blood culture bottles. Apart from blood cultures, suitable samples were inoculated into 5% sheep blood agar and eosin methylene blue agar and were incubated for 24–48 h at 35 °C under aerobic conditions. Subcultures were obtained in the aforementioned growth media after observing growth signals in blood culture samples. Isolated bacteria were identified at the species level using conventional methods and the VITEK 2 automated identification system (bioMérieux). Surface swab samples were collected from inanimate surfaces in the ICU (e.g., patient beds, patient blankets, aspiration devices, cabinets, lavatory, faucets, ventilator circuits, air conditioners, nurses' desks, curtains, and door handles) when *A. baumannii* was isolated from a sample obtained from an ICU patient. Cotton swabs moistened with sterile saline were used when collecting samples from surrounding areas in order to eliminate the inhibitory effect of fatty acids from the cotton swabs. Since environmental samples were obtained after observing the growth of *A. baumannii* in a sample, environmental samples were obtained after a mean of 24 h. The same procedure used for clinical samples was followed for growth and identification of these samples.

Antibiotics susceptibility testing for the isolated bacteria was performed using VITEK 2.

Isolates were stored at –80° C until the time of DNA sequence analysis.

2.1. Rep-PCR

The similarity of DNA patterns of isolates was determined using a DiversiLab device (bioMérieux), which utilized an automated repetitive sequence-based polymerase chain reaction (rep-PCR) method.

2.1.1. PCR Steps

1) DNA extraction was manually performed from pure *A. baumannii* cultures.
2) DNA amplification with rep-PCR: rep-PCR was performed using a commercial master mix, a PCR buffer, a primer mix, and Taq polymerase. Amplification was completed according to the recommended procedure for 35 cycles. Steps for each cycle are shown in Table 1.
3) Automated microfluidic electrophoresis was performed using a bioanalyzer.
4) Web-based interpretation and evaluation:

Analyses were performed using DiversiLab software (version 2.1.66), and Pearson's correlation coefficient was used to determine the distance between the matrices. The unweighted pair group method with arithmetic mean (UPGMA) was used to generate the dendrograms.

A dendrogram showing the automatic reading of computer-generated DNA fingerprints, a similarity matrix that displays and color codes similar DNA sequences, and a dot chart that plots the similarity between strains were used to interpret the data.

In the similarity matrix, red indicates 100%–95% similarity, orange 95%–90% similarity, blue 90%–80% similarity, pink 80%–70% similarity, and gray 70%–0% similarity. When 100%–95% similarity was found between strains, those strains were considered identical.

This study was conducted after obtaining approval from the local ethics committee and was supported by the Gaziantep University Scientific Research Projects Unit.

3. Results

During the study period, 100 *A. baumannii* strains were isolated, including 92 from patients and 8 from the ICU environment. Clinical samples belonged to 61 patients. A total of 54 strains were isolated from different clinical samples of 23 patients. In the patient cohort, 21 (34%) patients were female and 40 (66%) were male. The age range of the patients was 16–86 years (58.0 ± 17.7).

The breakdown of strains isolated from patients and sampling sites is shown in Table 2.

Table 1. DNA amplification cycle.

Step	Temp (°C)	Time (s)
Initial denaturation	94	120
Denaturation	94	30
Annealing	45	30
Extension	70	90
Final extension	70	90
Hold	4	-

Table 2. The distribution of *A. baumannii* isolates according to the isolated clinical samples.

Sample type	Sample number	Percent (%)
Tracheal aspirate	48	52
Blood	26	28
Urine	9	10
Catheter	6	7
Probe tap	2	2
CSF	1	1
Total	92	100

Table 3. The distribution of *A. baumannii* strains isolated from environmental samples.

Area	Isolates
Bed	1
Ventilator screen	1
In front of door curtain in patient room	1
Outer cover of dustbin	1
Staff uniform	2
Faucet	1
Computer keyboard	1
Total	8

A total of 400 swab samples were obtained from inanimate surfaces of the patients' environment. *A. baumannii* was isolated from 8 of these samples. Although this was a prospective study, environmental samples were obtained approximately 24 h after the identification of the bacteria that had grown in the patients' clinical samples, since there was no way to predict which patients would show growth of *A. baumannii*. The breakdown of strains isolated from inanimate areas is shown in Table 3.

Results of antibiotic susceptibility for study strains are shown in Table 4.

Colistin, which showed efficacy against all strains except one, was found to be the most effective antimicrobial for all strains tested. Other effective antimicrobials were tobramycin (94%) and tigecycline (79%). A high level of resistance was found against carbapenems (98% for imipenem, 99% for meropenem). The effectiveness of beta-lactam antibiotics was found to be very low, and while only one isolate (1%) was susceptible to cefepime, none of the isolates showed susceptibility to ceftriaxone, ceftazidime, or piperacillin. Moreover, there were not any isolates showing susceptibility to antimicrobial combinations containing beta-lactamase inhibitors (e.g., piperacillin/tazobactam and ampicillin/sulbactam). All strains isolated from environmental areas, except 1, showed resistance to most of the antibiotics tested, and 1 strain (isolated from a faucet) was found to be susceptible to all antibiotics. The antibiotic susceptibility of environmental isolates was as follows: colistin, 100% (8 isolates); tobramycin, 100% (8 isolates); tigecycline, 75% (6 isolates); amikacin, 50% (4 isolates); gentamicin, 25% (2 isolates); and ceftriaxone, ciprofloxacin, imipenem, trimethoprim/sulfamethoxazole, ampicillin/sulbactam, and cefoperazone/sulbactam, 12.5% (1 isolate each).

DNA fingerprinting analyses of the isolates showed that 7 of the 8 strains isolated from the environment were identical to many of the strains isolated from the patients.

The greatest sample similarity was found for one *A. baumannii* strain isolated from a computer keyboard, which was identical to the bacterium isolated from another 17 samples. The similarity matrix comparing strains isolated from patients and strains isolated from

Table 4. Antimicrobial susceptibility results of *A. baumannii* strains.

Antibiotics	Ceftriaxone	Ciprofloxacin	Colistin	Amikacin	Imipenem	SXT[1]	Meropenem	Levofloxacin	SAM[2]	Cefepime	SFP[3]	Ceftazidime	TZP[4]	Tigecycline	Tobramycin	Gentamicin
Susceptible number (%)	-	1 (1)	91 (99)	37 (40)	2 (2)	1 (1)	1 (1)	1 (1)	-	1 (1)	9 (10)	-	-	72 (78)	86 (94)	20 (22)
Resistant number (%)	92 (100)	91 (99)	1 (1)	55 (60)	90 (98)	91 (99)	91 (99)	91 (99)	92 (100)	91 (99)	83 (90)	92 (100)	92 (100)	20 (22)	6 (6)	72 (78)

SXT[1]: Trimethoprim/sulfamethoxazole, SAM[2]: ampicillin/sulbactam, SFP[3]: cefoperazone/sulbactam, TZP[4]: piperacillin/tazobactam.

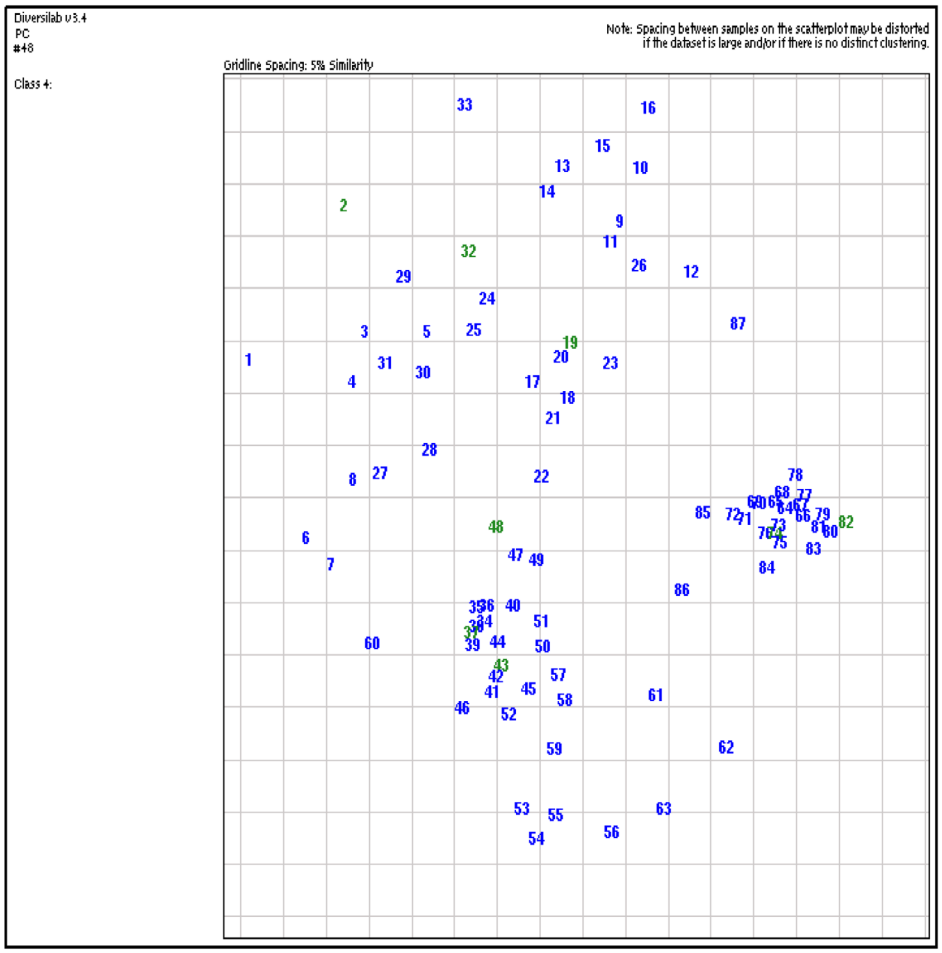

Figure. The similarity matrix of *A. baumannii* strains in hospital and environment samples.

surrounding areas is shown in the Figure (strains isolated from environmental sources are shown in green, and strains isolated from clinical samples are shown in blue).

The similarity between isolated strains is shown in Table 5.

As a result of the comparison of 54 samples obtained from 23 patients, bands with >95% similarity were observed in only one patient (blood culture and tracheal aspiration samples); similarity in other samples was not at the level to prove same clonal origin.

Table 5. The similarity between strains.

Environment sample	Similarity
2 (Faucet)	No similarity was determined
19 (Staff uniform)	17, 18, 20, 21
32 (Staff uniform)	24, 25
37 (Ventilator screen)	34, 35, 36, 38, 39, 40, 41, 42, 43, 44
43 (Curtain)	34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 46
48 (Dustbin-outer cover)	47, 49
74 (Keyboard)	64, 65, 66, 67, 68, 69, 70, 73, 75, 76, 77, 78, 79, 80, 81, 82, 83
82 (Bed)	64, 65, 66, 67, 68, 69, 70, 73, 74, 75, 76, 77, 79, 80, 81, 83

When the similarity between sample groups was investigated, 3 clones were detected in the blood cultures. Of those, 2 samples in clone 1, 3 samples in clone 2, and 6 samples in clone 3 were found to be identical. Of the tracheal aspirate samples, similarity was found only between 2 samples. All other samples were found to be different from each other.

4. Discussion

Acinetobacter strains are opportunistic pathogens that are commonly found in soil and water, and their role in hospital-acquired infections as the causative agent has been increasing day by day (8). Multiresistant *Acinetobacter* species emerge in hospitals, especially in ICUs, and occasionally cause outbreaks. The widespread use of antimicrobials in ICUs and the increased need for invasive diagnostic and therapeutic methods lead to the development of infections by resistant bacteria, primarily *Acinetobacter* species (9,10). According to data from the National Nosocomial Infections Surveillance System in the United States, the incidence of nosocomial infections caused by *Acinetobacter* spp. has increased substantially, and while 1.4% of gram-negative nosocomial infections were caused by *Acinetobacter* species in 1975, this rate had increased to 6.2% in 2003 (11).

A. baumannii has been reported to be the most common *Acinetobacter* species isolated from patients and the hospital environment. Its tolerance of dryness and its multidrug resistance contribute to the ability of *A. baumannii* to cause hospital outbreaks (1). *Acinetobacter* species can survive in humid environments, such as ventilator devices at hospitals, and also in dry conditions for extended periods. Additionally, they frequently colonize the skin of both patients and hospital staff. These areas are also important reservoirs for cross-contamination of patients (11–15).

In this study, the DNA similarities of the strains isolated from the environmental areas in the ICU and the strains from clinical samples of patients were compared. The isolates were not evaluated on the basis of the causative agent or colonization. A single isolate from each patient was included in the study as long as the clinical sample was identical; when the strain was isolated from a different clinical sample, that clinical sample was also included in the study. During the study, 400 swab samples were collected, and *A. baumannii* was isolated from 8 of them. In their study, Ayan et al. (14) collected 154 samples from the environmental areas of an ICU and did not isolate *A. baumannii* from any of these samples. Since the level of adherence to the disinfection procedures varies in each hospital, these results were not surprising. However, during the sampling procedure in our study, cotton swabs used to collect the samples were soaked in sterile saline to eliminate the inhibitory effect of fatty acids. This was thought to increase the chance of successful isolation.

Strains isolated in our study were found to be multidrug resistant. Additionally, all strains but one (a strain isolated from a faucet) isolated from environmental samples were multidrug resistant.

Carbapenem resistance was found in 98% of strains isolated in this study. In previous studies conducted in the authors' hospital, Karsligil et al. (16) found carbapenem resistance at a rate of 9.6% in 2004, whereas it was 53.5% in Özgür Akın et al.'s 2009 study (17); the significant increase in the resistance rate is remarkable. It was reported that carbapenem-resistant *A. baumannii* infections commonly occur in many hospitals (4,18–20). In fact, variable rates of antibiotic resistance in different hospitals are not surprising. However, a striking increase was observed in antimicrobial resistance as in the case for carbapenems.

Colistin was found to be the most effective antimicrobial for all strains tested, and while resistance was observed in only one of the strains, the others were susceptible. Susceptibility rates to tobramycin and tigecycline were 94% and 79%, respectively.

Biotyping, antibiotyping, ribobiotyping, PCR-based fingerprinting, and pulsed-field gel electrophoresis (PFGE) of genomic DNA were used for exploring the similarity between bacteria (14). PFGE is the gold standard for bacterial discrimination. However, PCR-based fingerprinting methods have been increasingly used in recent years. In this study, a similar test was used, and the results were obtained by accessing a large database through web-based software.

The fact that strains isolated from the environment were identical to many of the patient samples suggested that environmental areas in the ICU of the authors' hospital could be a significant source for contamination in the ICU. In fact, when a multidrug-resistant *A. baumannii* strain is isolated from a patient, procedures for close-contact isolation are implemented in our ICU as part of infection control practices. However, the similarity between the strains isolated from the areas with no direct contact with patients and strains isolated from the patients is surprising. For instance, the strain isolated from a computer keyboard on a nurse's desk was similar to 17 other samples, and the strain isolated from a curtain between patient beds was similar to 12 clinical samples.

Another result that was surprising for us was that, among 54 different clinical strains isolated from 23 patients, similarity was found between tracheal aspirate and blood culture samples isolated from 1 patient. Unlike the other 7 strains, an *A. baumannii* strain isolated from a wash basin had a susceptible antibiotic profile, and its DNA sequence did not show any similarity to other strains. This suggested that this strain could be an *Acinetobacter* species found in water.

Lowering infection-related mortality and morbidity rates in ICUs is a multidisciplinary task. Active surveillance practices should be implemented in ICUs in accordance with infection control measures, and procedures for antibiotic use should be tailored to individual resistance rates observed in the units and areas monitored periodically that could be a potential source for bacteria (the skin, nose, and throat of healthcare professionals, environmental areas).

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