Molecular Epidemiology of Nosocomial *Acinetobacter baumannii* Isolates

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Abstract: In view of the high rate and rapid spread of multidrug-resistant (MDR) *Acinetobacter baumannii* causing nosocomial infections especially in ICUs, this study was conducted to elucidate the antimicrobial susceptibility and the molecular epidemiology of the *A. baumannii* isolated from nosocomial infections in 3 ICUs in the El-Zaitoun Specialized Hospital, Cairo. During a 7-month study period, a total of 20 *A. baumannii* were isolated from nosocomial infections and environmental sources in the 3 ICUs. Susceptibility of the isolates to different antimicrobials was determined by the disk diffusion method. Molecular typing of all isolates was performed using the random amplified polymorphic DNA (RAPD)-PCR assay. *A. baumannii* were most frequently isolated from endotracheal aspirates (14), 4 strains from post-operative wound infection and 2 from environmental samples. All isolates were MDR and were totally resistant to imipenem, ampicillin/sulbactam, ceftazidime, ciprofloxacin, piperacillin/tazobactam and ceftriaxone. A high resistance rate was observed to amikacin and trimethoprim/sulfamethoxazole (90% each) gentamicin (85%) and doxycycline (75%). Molecular typing revealed circulation of 8 RAPD-fingerprints, of which fingerprint A accounted for 50% of *A. baumannii* strains including the 2 environmental isolates. Fingerprint B comprised 20% while the other isolates showed different RAPD-fingerprints. In conclusion, there was an increase in the rate of MDR *A. baumannii* in the ICUs which necessitates the implementation of an appropriate antibiotic policy. The intrahospital spread of especially one RAPD fingerprint of *A. baumannii* and its isolation from environmental sources emphasize the need of strict adherence to infection control measures in hospitals as well as the value of molecular typing to investigate spread of infection.
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1. Introduction

Acinetobacter species, especially *A. baumannii,* have been increasingly reported as significant microorganisms involved in various nosocomial infections and several hospital outbreaks especially in intensive care units (ICUs) (Seifert et al., 2005, Wilks et al., 2006, Munoz-Price and Weinstein, 2008). Apart from being intrinsically resistant to certain classes of antibiotics, *A. baumannii* strains can acquire resistance easily to a wide variety of antibacterial agents (Friedland et al., 2003, Wroblewska et al., 2007). Extensive use of antimicrobial chemotherapy within hospitals has contributed to the emergence and increase in the number of *A. baumannii* strains that are resistant to a wide range of antibiotics, including broad-spectrum beta-lactams, aminoglycosides, and fluoroquinolones (Salazar de Vegas et al., 2007, Cetin et al. 2009).

Besides resistance to antibiotics, difficulty to control *A. baumannii* nosocomial infections and outbreaks is also attributed to the ability of these bacteria to survive in the hospital environment. *A. baumannii* does not have fastidious growth requirements and is able to grow at various temperatures and pH conditions and to persist in either moist or dry conditions in the hospital setting (Wendt et al., 1997). These properties favor the transmission of *A. baumannii* between patients, either via human reservoirs or via inanimate materials (Zarrilli et al., 2007).

Understanding the epidemiology of nosocomial *A. baumannii* infections is essential to develop effective strategies to control their spread. The use of modern molecular techniques, such as pulsed-field gel electrophoresis (PFGE) and polymerase chain reaction-based typing, has shown to be suitable for the investigation of hospital outbreaks (Prashanth and Badrinath, 2005).

PFGE has been validated as a useful epidemiologic tool to study *A. baumannii* outbreak and is considered as the gold standard of epidemiological typing (Prashanth and Badrinath, 2005, Seifert et al., 2005). However, this method is cumbersome, time consuming and expensive. Random Amplified Polymorphic DNA **(**RAPD)-PCR is a rapid and simple method with a similar sensitivity and specificity as PFGE. RAPD-PCR has a particular significance in the epidemiological tracing because of the nature of RAPD profiling that generates fingerprints as well as it can be applied to detect polymorphism in a wide variety of organisms (Menichetti et al., 2000, Uma Karthika et al., 2009,

Trajkovska-Dokic et al., 2011). In RAPD-PCR, random primer sequences may be used in organisms where a specific genome sequence is not known. Random parts of the organism genome are produced, which are expected to be identical among related species, and so similar banding patterns should be produced in gel electrophoresis (Seifert et al., 1994).

The objective of the present study was to elucidate the antimicrobial susceptibility and molecular epidemiology of *A baumannii* isolated from nosocomial infections t in 3 ICUs at El-Zaitoun Specialized Hospital, Cairo.

2. Materials and Methods
Bacterial Isolates

This study was conducted in 3 different ICUs in El-Zaitoun Specialized Hospital, Cairo, during the period from June to December, 2011. A total of 20 strains of *A. baumannii* were isolated from different clinical samples collected from patients who developed nosocomial infections as well as from medical devices, patient-associated objects and room equipment from the patients' environment. Nosocomial infections were defined by standard Centers for Diseases Control and Prevention definition (Garner et al., 1996).

Bacterial Culture and Antimicrobial Susceptibility

All the specimens from patients and environment were cultured on blood agar media under aerobic conditions at 37°C for 24 hours. Isolates were identified as members of the genus Acinetobacter by Gram staining and biochemical analyses and were confirmed as *A.baumannii* by Analytical Profile index 20 NE (API 20 NE) (BioMérieux, France). All *A. baumannii* strains were stored at -20°C in nutrient broth (Oxoid, England) containing 20% glycerol, until performing the genotyping analysis.

Susceptibility of the isolates to different antimicrobial agents was determined by the disk diffusion method following the Clinical Laboratory Standards Institute guidelines (CLSI, 2010). The antimicrobial agents used were: ampicillin/ sulbactam, ceftazidime, ciprofloxacin, imipenem, gentamicin, amikacin, piperacillin/tazobactam, ceftriaxone, doxycycline, trimethoprim/sulfamethoxazole (Oxoid, England). *A. baumannii* isolates were defined as MDR if they were resistant to representative antibiotics of at least three different classes of antimicrobial agents (Falagas et al., 2006).

Molecular Typing

The genetic relatedness of *A. baumannii* isolates was determined by Random amplified polymorphic DNA (RAPD)-PCR analysis (Figure 1). Total DNA was extracted using MagNA pure compact nucleic acid isolation kit (Roche, Germany) according to manufacturer's protocol.

The RAPD-PCR fingerprinting was performed according to Chang et al., (2009) with 0.1 ng of *A. baumannii* DNA, 0.1 mM of primer p1281 (5'-AACGCGCAAC-3') and p1283 (5'-GCGATCCCCA-3'), and standard PCR reagents (Roche, Germany). PCR amplification was performed using a thermal cycler (Hybaid Omnigene, UK) and the cycling program was as follows: initial denaturation cycle at 94° C for 10 minutes; 36 cycles at 94°C for 1 minutes, 45°C for 1 minute, and 72°C for 1 minute 30 seconds; and final cycle at 72°C for 10 minutes. The RAPD products were separated by electrophoresis in 2% agarose gel then visualized by UV illumination. A molecular size standard DNA marker was included on gel. The RAPD profiles were analyzed using the Gel-Pro Analyzer software (Media Cybernetics, USA). If the isolates had the same RAPD profiles, the second primer was used to confirm their identical genetic backgrounds.
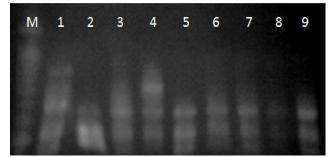

Figure 1. RAPD Fingerprints of *9 A. baumannii* Isolates   M: DNA marker

Of the 20 *A.baumannii* isolates, 14(70%) were isolated from endotracheal aspirates (ETA) of patients suffering from ventilator-associated pneumonia, 4(20%) from post-operative wound infection, while 2(10%) were from environmental sources; ventilator humidifier and the surface of a bedside table.

The antimicrobial susceptibility patterns showed that all the *A. baumannii* isolates were MDR. All isolates were imipenem resistant and were also totally resistant to ampicillin/sulbactam, ceftazidime, ceftriaxone, ciprofloxacin, and piperacillin/tazobactam. A high resistance rate was also observed to amikacin and

trimethoprim/sulfamethoxazole (90% each) gentamicin (85%) and doxycycline (75%) (Table 1).

Various antimicrobial drug susceptibility patterns were detected as shown in table 1. The first pattern (I) consisted of 13(65%) out of the 20 *A.baumannii* which were resistant to all the antibiotics. The 2 environmental isolates belong to pattern I. Two (10%) of the *A.baumannii* isolates were resistant to all antibiotics except doxycyline (pattern II) while another 2(10%) strains were susceptible only to doxycline and gentamicin (pattern III). The rest of the isolates (3/20; 15%) showed different susceptibility patterns to gentamicin, amikacin, doxycycline and trimethoprim/ sulfamethoxazole.

RAPD-PCR fingerprinting yielded 8 RAPD profiles among the 20 *A. baumannii* isolates designated from A to H; these are listed in table (2) together with the antibiogram data. Fingerprint A predominated, collectively accounting for 10(50%) of the strains (8 from patients and 2 from environment) isolated from the 3 ICUs. Four strains (20%) showed a different RAPD-fingerprint designated as fingerprint B. On the other hand 6(30%) *A. baumannii* isolates presented different RAPD patterns. No correlation was found between the typing results and the time of sampling or the origin of the strains.

All isolates belonging to fingerprint A were resistant to all the antibiotics tested (pattern I), while fingerprint B included 4 strains sensitive to doxycycline ± gentamycin (patterns II, III). Fingerprints C, D and E comprised isolates resistant to all antibiotics (pattern I), whereas the remaining showed different patterns of antibiotic susceptibility (table 2).

Table 1. Antimicrobial Susceptibility and Antibiotypes of the 20 *A. baumannii* Isolates

Antibiotic	I n.13*	II n.2	III n.2	IV n.1	V n.1	VI n.1	Total no. (%)	
							S	R
Ampicillin/ sulbactam	R	R	R	R	R	R	0(0)	20 (100)
Ceftazidime	R	R	R	R	R	R	0(0)	20 (100)
Ciprofloxacin	R	R	R	R	R	R	0(0)	20 (100)
Imipenem	R	R	R	R	R	R	0(0)	20 (100)
Gentamycin	R	R	S	S	R	R	3(15)	17(85)
Amikacin	R	R	R	S	R	S	2(10)	18(90)
Piperacillin/ tazobactam	R	R	R	R	R	R	0(0)	20 (100)
Ceftriaxone	R	R	R	R	R	R	0(0)	20 (100)
Doxvcycline	R	S	S	R	R	S	5(25)	15 (75)
Trimethoprim/sulfamethoxazole	R	R	R	R	S	S	2(10)	18(90)

*including environmental samples; S-sensitive; R-resistant

Table 2. RAPD Fingerprints of all *A. baumannii* Isolates, their Source and Antimicrobial Susceptibility Pattern

Fingerprint no.(antibiotype)	Sample (no.)	Antimicrobial susceptibility pattern	
		Sensitive	Resistant
A 10(I)	ETA(6),wound (2) environment (2)	-	All
B 2(II) 2(III)	ETA wound, ETA	DO DO, CN	SAM, CAZ, CIP, IMP,CN, AK, TZP, CRO, SXT SAM, CAZ, CIP, IMP, AK, TZP, CRO, SXT
C 1(I)	ETA	-	All
D 1(I)	wound	-	All
E 1(I)	ETA	-	All
F 1(IV)	ETA	CN, AK	SAM, CAZ, CIP, IMP, TZP, CRO, DO, SXT
G 1(V)	ETA	SXT	SAM, CAZ, CIP, IMP, CN, AK,TZP,CRO, DO
H 1(VI)	ETA	AK,DO, SXT	SAM, CAZ, CIP, IMP, CN , TZP, CRO

ETA-endotracheal aspirate; SAM-Ampicillin/ sulbactam; Ceftazidime-CAZ; CIP -Ciprofloxacin; IMP- Imipenem; CN-Gentamicin; AK- Amikacin; TZP-Piperacillin-Tazobactam;CRO -Ceftriaxone; DO-doxycycline; SXT - Trimethoprim/sulfamethoxazole

4. Discussion

A. baumannii has become a well-recognized pathogen responsible for nosocomial infections and outbreaks especially in ICU patients (Munoz-Price and Weinstein, 2008). In the present study, 18 *A. baumannii* strains were isolated from ICU patients who developed nosocomial infections, while 2 were isolated from environmental samples.

Analysis of antimicrobial susceptibility patterns of the 20 *A. baumannii* isolates showed that they were all MDR and were totally resistant to imipenem, ampicillin/sulbactam, amikacin ciprofloxacin, piperacillin/tazobactam and cephalosporins. In agreement with these findings, other studies reported that all the *A. baumannii* isolated from nosocomial infections in ICUs and hospital wards were MDR (Alp et al., 2006, Chang et al., 2009, Frickmann et al., 2010, Trajkovska-Dokic et al. 2011). In the study of Uma Karthika et al.(2009) all the isolates were resistant to imipenem, while 80%, 72%, 42 % and 36 % of isolates were resistant to amikacin, and ciprofloxacin, ceftriaxone ceftazidime, respectively. On the other hand, Frickmann et al. (2010) reported that among 24 tested antibiotics, the isolates were only susceptible to colistin. A high rate of *A.baumannii* resistance was also demonstrated by Trajkovska-Dokic et al. (2011), where all the 20 *A. baumannii* isolated from patients in pediatric ICU and wards revealed 100% resistance to at least 11 antibiotics. Similar to our observations, they reported that all the 20 *A.baumannii* isolates were totally resistant to imipenem, ceftriaxone, ciprofloxacin, piperacillin-tazobactam, however, 90% were susceptible to trimethoprim/sulfamethoxazole.

The increase in the rate of MDR among the nosocomial *A. baumannii* isolates even to carbapenems, the drugs of choice for nosocomial Acinetobacter infections (Uma Karthika et al., 2009, Yang et al., 2010), reflects the extensive use of antibiotics in hospitals, the huge ability for Acinetobacter to acquire resistance genes and has created a challenge for appropriate therapy (Dijkshoorn et al., 2007, Salazar de Vegas et al., 2007, Trajkovska-Dokic et al. 2011).

Molecular typing in the present study revealed the circulation of 2 main RAPD fingerprints; A which accounted for 50% of the *A.baumannii* isolates and B for 20%. This high genetic relationship among the typed strains indicates high dissemination rate of *A. baumannii* strains among the ICU patients causing nosocomial infections.

Intrahospital spread of *A.baumannii* clones has also been well documented in several studies.

Abbo et al. (2005) demonstrated that 50% of the *A. baumannii* isolated from patients in ICUs and hospital wards belonged to 2 dominant clones. In the study of Alp et al. (2006), detection of 3 major clones among the ICU patients with nosocomial blood stream infection made them assume cross-transmission as a major source of infections. Cetin et al. (2009) also reported the circulation of 2 main PFGE types which accounted for 44% of the isolates examined and coexisted with epidemiologically unrelated sporadic strains.

All the isolates belonging to the RAPD fingerprint A had a similar antibiogram of being totally resistant to all the antibiotics, suggesting a common source of infection (Raka et al., 2009). The simultaneous occurrence of resistance to antibiotics in this predominant RAPD fingerprint might have been responsible for the high rate of infection caused by this strain during the study period. On the other hand, strains belonging to RAPD fingerprint B showed minor variations in the antibiograms (sensitivity to doxycycline ± gentamicin). This result is also parallel with previous reports stating that clonally related strains of Acinetobacter that differ in susceptibility patterns may coexist within a single hospital, dependent on the selective pressure related to antibiotic exposure (Gallego and Towner 2001, Falagas and Kopterides, 2006, Cetin et al., 2009).

The presence of antimicrobial susceptibility pattern variation within clones and similarities between clones, as was revealed in this study, indicate that antimicrobial susceptibility pattern is not a useful marker for clonality (Abbo et al., 2005). It might be suitable as a screening method in epidemiological investigations, but requires confirmation by more precise and complementary techniques (Trajkovska-Dokic et al. 2011).

Based on RAPD-PCR analysis, all the *A. baumannii* isolates belonging to fingerprint A were isolated throughout the study period from the 3 ICUs. In the study of Hammami et al. (2007) the major RAPD profile A was found in 5 patients and 3 times in materials in 3 ICUs. They attributed this to the circulation of the ICU doctors between the 3 units that shared a common medical staff. The high epidemiological relatedness among these strains suggests that one and a unique strain of *A. baumannii* has circulated for this period of time causing nosocomial infections in the ICUs, as a result of their transmission between these places. This strain could be an endemic strain of *A. baumannii* in this setting.

In agreement with Raka et al., (2009), *A. baummannii* isolates were most frequently recovered

from ETA (14; 70%). Six of the isolates from ETA had the same RAPD fingerprint A as that of the isolate from the ventilator humidifier. This confirms that procedures associated with mechanical ventilation might have been the mode of *A baumannii* patient-to-patient transmission in ICUs (Cetin et al., 2009) and highlights the importance of proper aseptic technique concerning mechanical ventilation. Notably, the other environmental isolate also belongs to RAPD-fingerprint A and has the same resistance pattern as well as another 2 nosocomial strains isolated from post-operative wound infection. Overall, these data suggest that the contaminated environment is the likely source of nosocomial infection in the present study.

The ability of *A. baumannii* to survive on different surfaces and objects facilitated nosocomial spread. Numerous outbreaks have been reported originating from environmental contamination or hand carriage, and were consequently controlled by basic infection control measures such as hand hygiene, isolation, aseptic preparation of parenteral nutrition solutions and elaborated disinfection protocols specifically addressing the patients' environment (Menichetti et al., 2000, Wang et al., 2003, Herruzo et al., 2004, Frickman et al., 2010). Moreover, a study by Wagenvoort et al. (2002) showed that health care workers (HCWs) attending *A. baumannii* positive patients may become nasal and skin carriers of the outbreak strain, thereby contributing to the continued spread. Therefore, strict adherence to infection control measures is necessary to control the spread of nosocomial pathogens including intensification and modification of cleaning procedures for contaminated equipment, hand hygiene and cohorting the patients infected with MDR *A.baummannii.*

In conclusion, the increase in the rate of MDR *A. baumannii* isolates in this study necessitates continuous antimicrobial susceptibility testing, strict limitations of antibiotics use in hospitals and investigations for the usage of other antibiotics for the treatment of infections caused by these strains. The isolation of *A. baumannii,* especially of one RAPD fingerprint, from nosocomial infections and from environmental samples during the study period suggested that the intrahospital spread was related to cross-transmission via medical equipments, hands of HCWs and patients and implicate the environment as the source. This emphasize the need of strict adherence to infection control measures for preventing nosocomial infections in hospitals as well as the value of molecular typing to investigate spread of infection.

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