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Original article

Antimicrobial susceptibility, plasmid profiles, and RAPD-PCR typing of *Acinetobacter* bacteria

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Background: Multiple-drug resistant *Acinetobacter* have widely spread in the last decades imposing a serious nosocomial source of infection. Nevertheless, little knowledge was gaimed on tracing the development of antibiotic resistance in *Acinetobacter* species.

Objectives: Explore *Acinetobacter spp.* via antimicrobial susceptibility, plasmid profiles, and random amplified polymorphism DNA polymerase chain reaction (RAPD-PCR) typing.

Methods: One hundred twelve *Acinetobacter* isolates (including 66 *A. baumannii* and 46 non-*Acinetobacter baumannii* strains) were obtained from three university hospitals. The source of infection of these isolates included blood, urine, wound, and respiratory tract. Their susceptibilities to 17 antibiotics were tested and then all *Acinetobacter* isolates were typed by plasmid analysis and RAPD-PCR method.

Results: *A. baumannii* isolates revealed nine different patterns of antibiotic resistance. Of those, non-*A. baumannii*, were associated with plasmid and RAPD-PCR typings ($p < 0.05$). *A. baumannii* was more resistant to multiple antibiotics than non-*A. baumannii* ($p < 0.05$). Seven different plasmid profiles were observed among 112 *Acinetobacter* isolates. Plasmids were found in 107 (95.5%) of the 112 isolates. Unlike in RAPD-PCR typing, there was no difference between the type of *Acinetobacter, A.* or non-*A. baumannii* strains and plasmid profiles ($p > 0.05$). By RAPD-PCR, six profiles were found for each *A.* and non-*A. baumannii* strains. The pattern 6 was the most common pattern among the isolates. Both plasmid and RAPD-PCR typing showed no association between plasmid profiling and site of infection ($p > 0.05$).

Conclusion: There is a wide spread of multi-drug resistant *Acinetobacter spp.*, particularly *A. baumannii*, in the Middle East region that can be traced efficiently by plasmid and genotyping typing of *Acinetobacter.* More care should be taken for tracing the development of antimicrobial resistance of *Acinetobacter* using precise molecular typing techniques.

Keywords: *Acinetobacter baumannii*, antimicrobial susceptibility, molecular typing, plasmid profiles RAPD-PCR

Bacteria of the genus *Acinetobacter* are usually of non-motile, non-fermetative, aerobic, and gram-negative coccobacilli [1]. The organisms are usually commensal. However, *Acinetobacter species* are emerging as important opportunistic pathogens because they rapidly evolve toward multi-drug resistance. They are involved in various nosocomial infections such as bacteremia, urinary tract infection,

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secondary meningitis, surgical site infection, and nosocomial and ventilator-associated pneumonia, especially in patients admitted to intensive care unit [2, 3].

Studies on DNA-DNA relationships within the genus *Acinetobacter* have resulted in the description of 21 DNA homology groups [4]. *A. baumannii* is the species most frequently isolated from patients and hospital environment [5-7]. Several outbreaks of its infection or colonization in surgical, neonatal, and burn intensive care units have been reported. The epidemiology of these infections remains unclear, because it is ubiquitous and infections may occur on either a sporadic or an epidemic basis [8-10].

Extensive use of antimicrobial chemotherapy within hospitals has contributed to the emergence and increase in the number of *A. baumannii* strains resistant to a wide range of antibiotics, including broad-spectrum beta-lactams, aminoglycosides, and fluoroquinolones [11-13]. Due to the multiple antibiotic resistance, nosocomial infections caused by *A. baumannii*, this bacteria was found difficult to treat. These therapeutic difficulties are associated with the fact that these bacteria have a significant ability for survival in the hospital environment, thus favoring the transmission between patients, either via human reservoirs or via inanimate materials [5, 6, 11, 12].

Many traditional and molecular typing methods have been employed for the epidemiological investigation of outbreaks caused by *Acinetobacter spp.* [2]. Random amplified polymorphism DNA polymerase chain reaction (RAPD-PCR) fingerprinting and plasmid typing require little knowledge of the biochemistry or molecular biology of the species being studied [14, 15]. Hence, RAPD-PCR and plasmid typing methods can be applied to detect polymorphism in a wide variety of organisms. These methods are very rapid and simple to generate fingerprints.

In this study, we isolated *Acinetobacter* strains, conduct biochemical identification of these isolates, and determined their antimicrobial susceptibility and typing profile by both RAPD-PCR and plasmid patterns. In addition, plasmid and RAPD-PCR typing profiles of the isolated *Acinetobacter* strains were compared to the antibiotic resistance profiles and the source of infection as an attempt to recognize and trace the pathogenic and genotypic background correlated with multiple-drug resistance phenomenon of *Acinetobacter* strains in the region of the Middle East.

Materials and methods

One hundred and twelve *Acinetobacter spp.* isolates were obtained from three teaching hospitals (Imam Khomayni, Shariati, and Sina) in Tehran between April 2003 and July 2009. The location of infections included was blood, urine, wound, and respiratory tract.

The specimens were cultured on blood agar and McConkey agar. Isolates were identified as members of the genus *Acinetobacter* by Gram negative, coccoid, oxidase negative, non-motile, and non-fermenting bacteria. They were identified as *A. baumannii* by the simplified identification scheme including growth at 37°C, 41°C, 44°C; production of acid from glucose under oxidative condition; gelatin hydrolysis; and assimilation of different carbon sources [6].

Antimicrobial susceptibility

Bacterial susceptibilities to 17 different antimicrobial agents were determined by the agar disk diffusion method (Kirby-Bauer) [1, 16]. The following antimicrobial agents (Hi Media Laboratories, Mumbai, India) at the indicated concentrations were tested: ticarcillin (Ti 75 μg), ceftriaxone (Ci 30 μg) ,cefixime (Cfx 5 μg), meropenem (Mr 10 μg), aztreonam (Ao 30 μg), cephotaxime (Ce 30 μg), ticarcillin/clavulanic acid (Tc 75/10 μg), ceftazidime (Ca 30 μg), cefoprazone (Cs 75 μg), ceftizoxime (Ck 30 μg), colistin (C 110 μg), polymixinB (Pb 300 μg), amikacin (Ak 30 μg), piperacillin/tazobactam (Pt 100/10 μg), carbenicillin (Cb 10 μg), tobramycin (Tb 10 μg), and netillmicin (Nt 30 μg).

DNA extraction

Bacterial strains were cultured aerobically in Luria broth (LB) at 37°C. Two milliliters of overnight culture were centrifuged at 4,000 rmp for 20 minutes. The pellet was re-suspended in 620 mL of lysis buffer (10 mM Tris-HCl, 50 mM EDTA,100 mM NaCl, pH 8) from Merck (Germany) containing 1% Sodium lauryl sulfate (SDS) and 0.4 mg/mL of proteinase K (Sigma, USA). The mixture was incubated for one hour at 56°C and then for one hour at 100°C. An equal volume of phenol/chloroform/isoamylalcohol (BDH, UK) was added to the mixture and centrifuged at 10,000 rpm for 10 minutes. The supernatant was added to an equal volume of chloroform. After being centrifuged at 10,000 rpm, the top layer was collected, and DNA was precipitated with two volumes of cold isopropanol (Merck, Darmstadt, Germany) at -20°C for 10

minutes. The pellet was obtained by centrifugation for 20 minutes and washed with 1.5 mL of 70% cold ethanol (Merck, Darmstadt , Germany). Finally, the pellet was re-suspended in 100 mL of TE 1 X buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8) from Promega, USA and 1 µL was used for RAPD- PCR mixture [17].

Plasmid typing

Plasmid DNA was prepared as described previously [2, 5, 6] with minor modifications. Briefly, isolates were grown on LB agar plates for 24 hours at 37°C. Cells from half of the plate were suspended in 1.5 mL of a solution containing 2.5 M NaCl, 10mM EDTA (pH 8.0), 250 µL of 0.5% alkyltrimethylammonium bromide (ATAB), 250 µL of 1% Triton X-100, and 200 µL of lysozyme (10 mg/mL) from Sigma, USA. After incubation in water bath for 15 minutes at 56°C, protein was extracted with phenol-chloroform-isoamyl alcohol (25:24:1) (Merck, Darmstadt, Germany), and plasmid DNA was precipitated with ice-cold isopropanol (Merck, Darmstadt, Germany). The precipitate was collected by centrifugation and dissolved in 80 µL of TE buffer (pH 8.0). After addition of 1 µL of RNase (500 µL/mL) (Promega, Sunnyvale, USA), the solutions were incubated for 30 minutes at 37°C. Electrophoresis was performed on horizontal gel containing 0.8% agarose in TAE buffer (0.04 M Tris acetate, 0.001M EDTA [pH 8.2]). The sample (20 µL) and 5 µL of running dye were loaded on to the gel and run for two hours at 130 v. Agarose gels were stained with ethidium bromide and photographed by gel documentation instrument.

RAPD-PCR

The RAPD-PCR fingerprinting was carried out using AP-ARB11 primer (5'-CTAGGACCGC-3') and AP- PG05 (5'-AGCCCAGCTATGAAC-3') synthesized by Research Genetics (Huntsville, USA) [18]. DNA templates were amplified in a total reaction volume of 50 µL containing 2.5 U of AmpliTaq Gold thermostable polymerase (Roche, Switzerland), 50 pmol of each primer, 200 µM of each deoxynucleotide, 1.5 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.3), and 50 mM KCl from Promega, USA. Amplification was carried out with denaturation at 94°C for five minutes, followed by 40 cycles according to the following program: 94°C for 30 seconds, 40°C for one minute, and 72°C for one minute, plus a final extension of 10 minutes at 72°C to complete partial polymerizations. The PCR product was run and visualized in 2% agarose gels stained with ethidium bromide (Sigma, USA) [19].

Statistical analysis

Data of the current study was analyzed using SPSS version 12.0.0.1 and MS Excel 2007. McNemar test was used for dichotomous data with Yates' correction. P-values less than 0.05 were considered significant.

Results

During the period between April 2003 and July 2009, 112 *Acinetobacter spp.* isolates including 66 *A. baumannii* and 46 non-*A. baumannii* were obtained from three hospitals in Tehran as a representative for the region of the Middle East. Biochemical identification showed similar patterns for all *A. baumannii* isolates. **Table 1** shows the distribution of the isolates in terms of the sample site. Most strains were isolated from urine samples followed by blood samples. In addition, there was no significant association between the infection site and the type of *Acinetobacter* whether *A bouymani* or non-*A bounmani* ($p > 0.05$).

Table1. Distribution of the isolates in terms of the sample site.

Isolates	*Acinetobacter spp.*		Total
Source	*A. baumannii* Number (%)	*Non- A. baumannii* Number (%)	Number (%)
Blood	18 (38)	8 (17)	26 (23.2)
Urine	32 (48)	19 (42)	51 (45.5)
Wound	9 (9)	8 (17)	17 (15.2)
Respiratory tract	7 (5)	11 (24)	18 (16.1)
Total	66 (100)	46 (100)	112 (100)

Antimicrobial susceptibility

Analysis of antibiotic resistance patterns showed that all *Acintobacter spp.* were multi-drug resistant isolates. This study showed that All *A. baumannii* isolates had 100 % resistance to at least 11 used antibiotics as shown in **Table 2**.

All *A. baumannii* isolates were resistant to cefoprazone, ceftazidime, ticarcillin/ clavulanic acid, aztreonam, meropenem, ceftizoxime, carbenicillin, cefixime, ceftriaxone, ticarcillin, and cephotaxime. Despite this, all of them were sensitive to colistin. Susceptibility of *A. baumannii* isolates to the other tested antibiotics revealed nine different patterns designated arbitrarily from A to I.

Pattern **A** accounted for six of *A. baumannii* isolates that were resistant to most antibiotics but sensitive to tobramycin, polymixinB, piperacillin/ tazobactam, and colistin. Pattern **B** accounted for nine of *A. baumannii* isolates that were resistant to all antibiotics but sensitive to tobramycin, netilmicin, polymixinB, piperacillin/tazobactam, amikacin and colistin. Ten *A. baumannii* isolates showed pattern **C**, being resistant to all antibiotics but sensitive to amikacin, tobramycin, polymixinB, piperacillin/ tazobactam and colistin. Pattern **D** accounted for three *A. baumannii* isolates that were resistant to all antibiotics except intermediate resistant to polymixin B, but susceptible to amikacin, and colistin. Twenty-three isolates showed pattern **E**, being resistant to all of antibiotics but sensitive to polymixin B, piperacillin/ tazobactam and colistin. Four isolates belonged to pattern **F**, being resistant to all of antibiotics but susceptible to tobramycin, polymixin B and colistin. Four isolates showed pattern **G**, being resistant to all of antibiotics except intermediate resistant to polymixin B but sensitive just to colistin. Four isolates showed pattern **H** as resistant to all of antibiotics but sensitive to amikacin, polymixin B, piperacillin/tazobactam and colistin. Finally, three isolates showed pattern I as resistant to all of antibiotics except intermediate resistant to polymixin B and piperacillin/tazobactam, but sensitive to tobramycin, netilmicin, amikacin, and colistin.

Table 2. Antimicrobial susceptibility of 112 *Acinetobacter spp.* strains.

Antibiotic	*Acinetobacter spp.*					
	***A. baumannii* isolates**			***Non- A. baumannii* isolates**		
	R Number (%)	**I Number (%)**	**S Number (%)**	**R Number (%)**	**I Number (%)**	**S Number (%)**
Cefoprazone	66 (100)	0	0	27 (58.7)	9 (19.6)	10 (21.7)
Ceftazidime	66 (100)	0	0	15 (32.6)	10 (21.7)	21 (45.7)
Ticarcillin/ Clavulanic acid	66 (100)	0	0	18 (39.1)	11 (23.9)	17 (37)
Aztreonam	66 (100)	0	0	23 (50)	20 (43.5)	3 (6.5)
Meropenem	66 (100)	0	0	45 (97.8)	0	1 (2.2)
Piperacillin/ Tazobactam	11(16.7)	3 (4.5)	52 (78.8)	32 (69.6)	5 (10.8)	9 (19.6)
Ceftizoxime	66 (100)	0	0	42 (91.4)	2 (4.3)	2 (4.3)
Carbenicillin	66 (100)	0	0	43 (93.5)	0	3 (6.5)
Netillmicin	52 (78.8)	2 (3)	12 (18.2)	5 (10.8)	0	41 (89.2)
Cefixime	66 (100)	0	0	46 (100)	0	0
Tobramycin	33 (50)	1 (1.5)	32 (48.5)	9 (19.6)	5 (10.8)	32 (69.6)
Ticarcillin	66 (100)	0	0	37 (80.5)	2 (4.3)	7 (15.2)
Ceftriaxone	66 (100)	0	0	42 (91.3)	0	4 (8.7)
Cephotaxime	66 (100)	0	0	43 (93.5)	0	3 (6.5)
Polymixin B	0	10 (15.2)	56 (84.8)	0	0	46 (100)
Amikacin	37 (56)	0	29 (44)	11(23.9)	10 (21.7)	25 (54.4)
Colistin	0	0	66 (100)	1 (2.2)	0	45 (97.8)

R: resistant, I: intermediate, S: sensitive.

In contrast to *A. baumannii* strains, non-*A. baumanni* isolates showed lower resistance to the used antibiotics ($p < 0.05$). These 46 isolates had diversity in antibiotic resistance patterns (more than 10 resistance patterns). The most resistant strains had resistance to 16 antibiotics, and most sensitive strains were resistant to four antibiotics, but in general, 97.8 % of isolates had resistance to meropenem.

Interestingly, the antibiotic resistance profile of *A. baumannii* strains were associated with both plasmid profiles and RAPD PCR profiles ($p < 0.05$) (**Fig. 1**), while antibiotic profile of non-*A. baumannii* was not associated with plasmid and RAPD PCR profiles ($p > 0.05$). This indicates that plasmid and genotypic background of *A. baumannii* strains can be used for tracing and profiling the antibiotic resistance. However, antibiotic profiles were not associated with site of infection ($p > 0.05$).

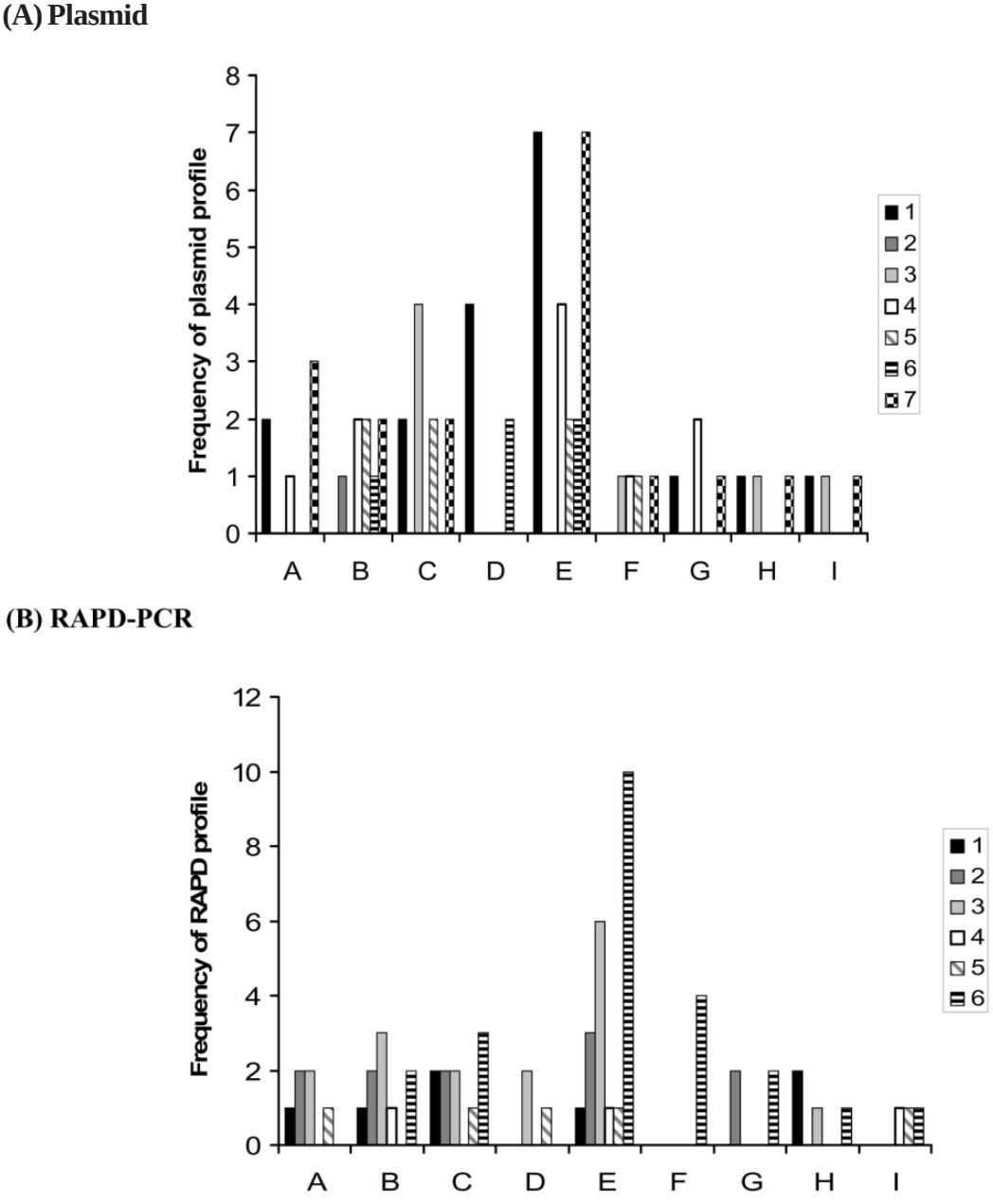

Fig. 1 The frequency of plasmid profiles (**A**) and RAPD-PCR profiles (**B**) in nine different profiles (**A-I**) of antibiotic resistance of *A. baumannii*.

Plasmid profiles

Seven different plasmid profiles were observed among 112 *Acinetobacter* isolates, designated arbitrarily from 1 to 7. Plasmids were found in 107 (95.5%) of 112 isolates, and no plasmids were found in five isolates (4.5%) including three *A. baumannii* and two non- *Acinetobacter baumannii* isolates. The sizes of the plasmids were in the range between 1 and >21 kb. Representative plasmid profiles are shown in **Fig. 2**. Similar plasmid profile was seen in two different specimens (blood and wound specimens) from two different hospitals.

Plasmid profiles 1-7 were all observed in isolates recovered from the blood culture. All profiles except profile 2 were seen in strains isolated from urine cultures (**Table 3**). Plasmid profiles 1, 3, 4, 5, and 7 were seen in all types of specimens. Plasmid profile 7 was the most common pattern among isolates. It was seen in 23 isolates. Nevertheless, there was no significant difference between the type of *Acinetobacter*, whether *A. baumannii* strains or non-*A. baumanni,* and plasmid profiles ($p > 0.05$). Moreover, there was no significant association between plasmid profiling and site of infection ($p > 0.05$).

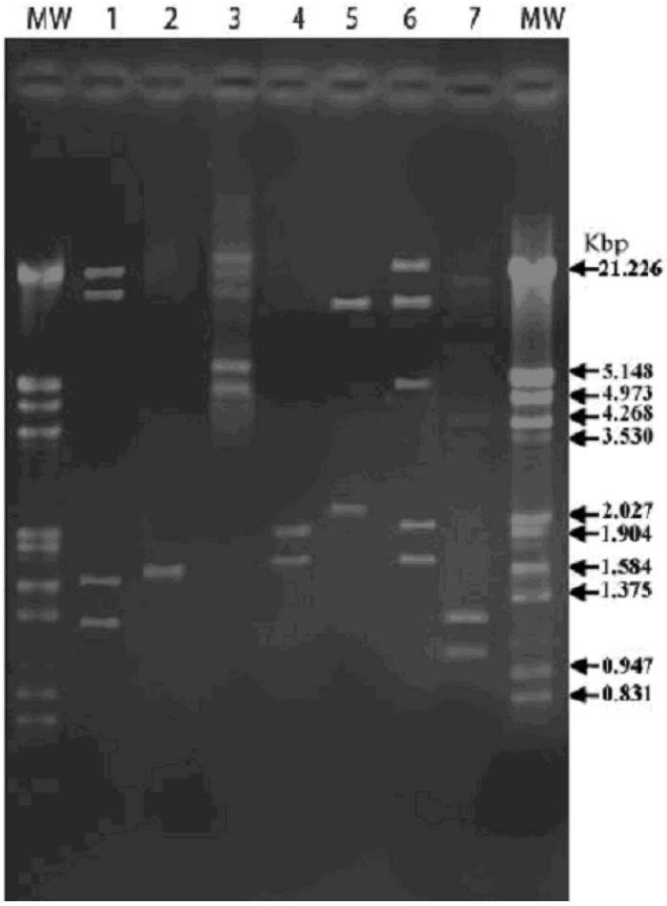

Fig. 2 Representative plasmid profiles. Lanes 1-7 are seven different plasmid profiles observed in this study. Molecular weight marker is lambda DNA with *Hind* III + *Eco*RI.

Table 3. Plasmid profiles observed in *Acinetobacter* strains from different sources.

Plasmid profiles	Blood	Urine	Wound	Respiratory tract
1	2	12	3	3
2	1	0	1	0
3	5	3	3	4
4	7	6	3	3
5	3	6	3	2
6	3	8	0	3
7	4	13	3	3

RAPD-PCR

There was a significant difference between the type of *Acinetobacter*, whether *A. baumannii* strains or non-*A. baumanni*, and RAPD-PCR profiles ($p < 0.05$). RAPD-PCR assay of *A. baumannii* strains showed that six different PCR patterns (designated arbitrarily from 1 to 6) were revealed in the isolates (**Fig. 3, Table 4**). Among these, 24 (36.4%) isolates out of 66 belonged to pattern 6, 16 (24.2%) isolates had pattern 3, 11 (16.7%) isolates had pattern 2, seven (10.6%) isolates had pattern 1, five (7.6%) isolates had pattern 5, and three (4.5%) had pattern 4.

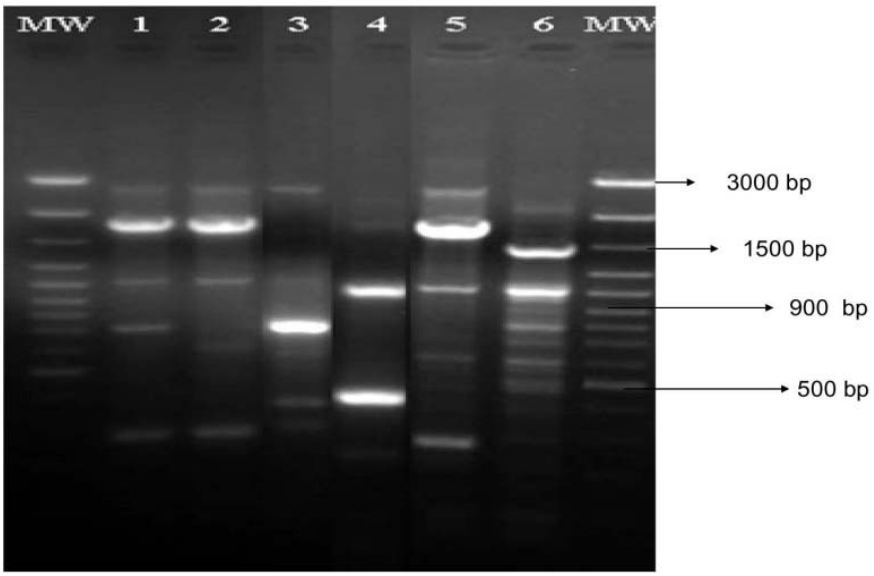

Fig. 3 Representative RAPD-PCR patterns. Lanes: 1- 6. Six different RAPD-PCR patterns were observed among *Acinetobacter baumannii* isolates. MW: DNA size marker 100 bp.

Table 4. RAPD- PCR patterns observed in *Acinetobacter* strains from different sources.

RAPD-PCR	*Acinetobacter baumannii*						Non-*Acinetobacter baumannii*					
Pattern	1	2	3	4	5	6	1	2	3	4	5	6
Blood	2	1	5	0	2	7	0	0	5	2	0	2
Urine	1	7	6	2	3	12	0	2	6	4	4	4
Wound	1	1	2	0	0	3	0	3	3	2	2	0
Respiratory tract	3	2	3	1	0	2	4	0	0	2	0	1

Among *non-A. baumannii* isolates, six different RAPD-PCR patterns were observed as shown in **Fig. 4.** Among them, 14 (30.4%) out of 46 isolates belonged to pattern 3, nine (19.6%) isolates had pattern 4, eight (17.4%) isolates had pattern 6, six (13%) isolates had pattern 5, five (10.9%) isolates had pattern 2, and four (8.7%) isolates had pattern 2. It is noteworthy to mention that RAPD PCR profiles in both *A. baumanni* and non-*A. baumanni* were not associated with site of infection ($p > 0.05$).

Discussion

Hospital-acquired infection caused by multiple resistant gram-negative bacilli has proved to be a problem over the last 20 years. An increasing incidence of resistant members of the family *Enterobacteriaceae* involved in nosocomial infections was observed following therapeutic introduction of newer broad-spectrum antibiotics in hospitals. A remarkable increase in the importance of strictly aerobic gram-negative bacilli was observed including *Pseudomonas aeruginosa, Stenotrophomonas (Xanthomonas) maltophilia,* and *Acinetobacter spp.* [1].

All *Acintobacter spp.* isolated in this study were multiple-drug resistant bacteria that *Acinetobacter baumannii* strains had 100 % resistance to at least 11 used antibiotics. Non-*A. baumannii* strains had resistance to at least four used antibiotics. This presented huge ability for *Acinetobacter* to acquire resistance genes and cause serious infection in humans. Until now, no case of resistance to meropenem has been reported in the region of the Middle East. This study is the first report of resistance of pathogenic bacteria to meropenem in this region. Despite the increasing significance and frequency of multiple resistant *Acinetobacter* infections, many clinicians still lack an appreciation of the potential importance of these organisms in hospitals. More care should be taken for preventing the spread of resistance to this antibiotic and dissemination of such strains in the community.

In our study, the issued antibiotic profiles were significantly associated with plasmid and RAPD-PCR profiles of *A. baumannii* rather than non-*A. baumannii* bacteria. This provided evidence on the feasibility of using more advanced and precise molecular typing and detection methods for tracing and predicting *A. baumannii* resistance against antibiotics. In fact, traditional methods for recognizing and typing *Acinetobacter* have often lacked sufficient reproducibility, typeability, and discriminatory power

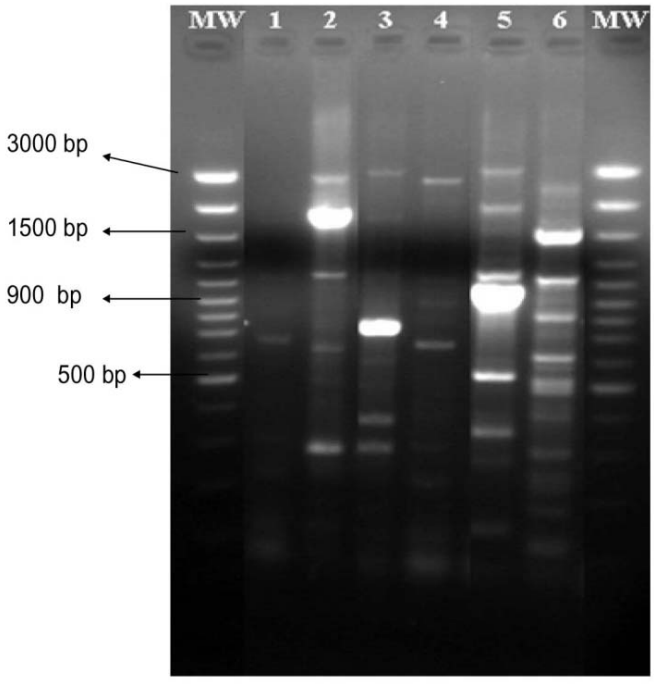

Fig. 4 Representative RAPD-PCR patterns. Lanes: 1- 6. Six different RAPD-PCR patterns were observed among Non-*Acinetobacter baumannii* isolates. MW: DNA size marker 100 bp.

[20]. Antimicrobial susceptibility patterns may be suitable as screening methods in epidemiological investigations, but they need to be confirmed by more precise and complementary techniques. New molecular identification and typing methods for members of this genus have now been developed, and these should form a rational scientific foundation for proper epidemiological studies of genotypically related strains involved in outbreaks of hospital infection [1]. The current study showed that the resistance rate in *A. baumannii* was remarkably higher than in non-*A. baumanii*. This directs attention to the great importance of monitoring *A. baumanii* more carefully given that *A. baumannii* is a major pathogen involved in nosocomial infections causing epidemic outbreaks or endemic occurrence with documented high mortality rates [13, 17].

In our study, 23.2% of *Acinetobacter* isolates were obtained from blood. Therefore, these bacteria can enter and persist into the blood stream and cause life-threatening multiple-drug resistant septicemia and disseminated infections. This refers clearly to the serious pathogenecity of this bacteria. *Acinetobacter spp*. is dangerous because it has no fastidious growth requirements and it is able to grow at various temperatures and pH conditions. Hence, it can persist in either moist or dry conditions in the hospital environment, thereby contributing to transmission. This hardiness, combined with its intrinsic resistance to many antimicrobial agents, contributes to the organism fitness and enables it to spread in the hospital setting [21, 22].

Similar to our observations, two separate studies reported that all *Acinetobacter* isolates were sensitive to colistin. However, this antibiotic has low clinical value because of its toxicity [18, 23]. In a previous study, all *A. baumannii* isolates from an outbreak in an intensive care unit setting were resistant to aztreonam, amikacin, cefatzidime, imipenem, meropenem, ticarcillin/clavulanic acid, piperacillin, and tazobactam but were sensitive to polymixin B [24]. Thirty-two tested *A. baumannii* isolates were resistant to ceftazidime, cefixime, ceftriaxone, and aztreonam [25], which is exactly as the same as our results. In a previous study [26], all 52 tested isolates of *A. baumannii* were shown susceptible to meropenem. Interestingly, all isolates in our study were resistant to meropenem. This imposes an urgent alarm for the hazardous and quick development of *A. baumannii* resistance against most powerful antibiotics, which in turn needs close monitoring and precise typing techniques as these highly resistant strains might spread to other regions of Asia or the whole world in a short time.

In our study, minor variations were frequently observed among tested isolates and were difficult to interpret without the help of a complementary typing system. Antimicrobial susceptibility patterns may be suitable as a screening method in epidemiological investigations, but requires confirmation by complementary techniques. Plasmid profile analysis has proved to be useful for the study of outbreaks of *Acinetobacter* infections. This technique is simple, requiring a minimum of equipment and expenses, and is accessible for most diagnostic laboratories [10, 13]. In our study, 108 (96.4%) of 112 tested isolates harbored plasmids. This result is close to a study by Seifert et al. [2] where plasmids were seen in 86.7% of tested isolates. In addition, according to another study by Seifart et al. [15], all *A. baumannii* strains in an outbreak, except for one, harbored plasmids. On the other hand, others reported much lower rate of plasmids detection. In another way, plasmids were detected in only 42% of the tested strains [16]. This pinpoints to the conspicuous role of the geographical variation in controlling plasmids transfer among different strains of *Acinetobacter*. Moreover, sizes of the plasmids might vary with geographical region. In our study, sizes of the plasmids were in the range between 1 and 21kb. A previous study [10] showed that the sizes of the plasmids were in the range from 2 to >30kb [10].

In our study, an identical plasmid profile was seen in two different specimens (blood and wound specimens) from two different hospitals. Plasmid profiles were also identical in some isolates recovered from blood and urine cultures. This might be explained because they were probably derived from a common ancestral strain. Siefert et al. [15] reported similar plasmid profiles in two different samples from two hospitals. Plasmid profiles were also similar in two isolates obtained from two different patients in the same hospital. Similar to our results, same plasmid profile in two isolates were obtained from two different patients in the same hospital. The resultant plasmid profiles were not related to the source of infection significantly. In addition, *A. baumannii* and non-*A. baumannii* showed similar association with plasmid profiles. This indicated that plasmid transfer among different strains is highly active at all sites of infection

and at all strains of *Acenitobacter spp.* Nevertheless, plasmid profiles were significantly associated with antibiotic resistance profiles. This association provided evidence that plasmid typeability can be used reliably to predict antibiotic resistance on molecular bases.

Unlike plasmid profiling, RAPD-PCR profiling was associated, via different profiles, with *A. baumannii* and non- *A. baumannii* strains. We demonstrated six different RAPD-PCR patterns in 66 *A. baumannii* isolates and six patterns among 46 isolates of non- A. baumannii strains. In a study by Prashanth et al. [17] using AP-PCR with M13 primer, they distinguished eight different PCR patterns in *A. baumannii* which were different form that in non- *A. baumannii* [17]. In our study, similar RAPD-PCR pattern was seen in four different specimens (wound specimens) from three different hospitals. All patterns among *A. baumannii* isolates were seen in all urine cultures. Interestingly, RAPD profiles were significantly associated, like plasmid profiles, with antibiotic resistance profiling of *A. baumannii* as well as, unlike plasmid profiles, with type of *Acinetobacter*, whether *A. baumannii* or non- *A. baumannii*. Accordingly, RAPD typing was of particular significance in the epidemiological tracing and study of *Acinetobacter* more than plasmid profiling. This can be attributed to the nature of RAPD profiling, which depends on DNA analysis. Therefore, RAPD typing is more conservative and stable than that of plasmids. However, using both arms of molecular typing for *Acinetobacter* strains (namely, plasmid and DNA analysis), it can provide robust typeability of pathogenic bacteria in association with antibiotic resistance.

Conclusion

Nosocomial infections caused by *Acinetobacter* spp. occurred in high rate in the Middle East region. Most of isolates are actually multi-drug resistant. The current finding provided baseline information for future surveillance and prevention programs for *Acinetobacter* spp. In addition, this study highlighted the wide spread of the multiple-drug resistance of this bacterium in the region of the Middle East, showing the suitability and high precision of both plasmid and RAPD-PCR typeability in predicting and/or tracing different antibiotic profiles.

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