



Investigation of Class I, II and III Integrons among *Acinetobacter* Strains Isolated from Ventilator-Associated Pneumonia Patients in Intensive Care Unit of Rasoul Akram Hospital in Tehran, Iran

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ABSTRACT

Background: Multi-drug resistant strains of *Acinetobacter* spp. have created therapeutic problems worldwide. The objective of this study was to detect integrons in *Acinetobacter* spp. isolates from Ventilator-Associated Pneumonia patients using PCR method.

Methods: A total 51 Bronchoalveolar lavage samples were obtained from patients in ICU and examined for *Acinetobacter* spp. infection by biochemical and PCR methods using *bla*OXA51-like primers. Antimicrobial susceptibility testing was performed using disk diffusion and MIC methods.

Results: Among 51 patients with VAP (62.7% males, 35.2% females, mean age 53 year), 50 (98%) were positive, with a high prevalence of gram-negative bacteria, mainly *Acinetobacter* spp. (70%), from which *A. baumannii* was detected in 34 (68%) and *A. lwoffii* in 1 (2%) of isolates. More than 90% of isolates were resistant to imipenem, piperacillin+tazobactam, third generation cephalosporins and gentamicin, while the most effective antibiotic was colistin (100%). The correlation coefficient between disk diffusion and MIC was 0.808 ($p = 0.001$). Three *Acinetobacter* isolates (8%) harbored *integrase I* gene but none of isolates contained Class II or III integrons.

Conclusion: The results showed that colistin was an effective antibiotic and can be used for treatment of patients in ICU. Due to the high number of MDR isolates lacking Integrons it can be concluded that although class I integrons are important among clinical isolates of *A. baumannii*, they have no significant role in dissemination of antibiotic resistance genes in Rasoul Akram Hospital in Tehran, Iran. The presence of *IntI* in *A. lwoffii* may be related to transfer of integron to *A. baumannii* which can be considered as an important threat for hospitalized patients.

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Introduction

Acinetobacter baumannii is an important opportunistic pathogen responsible for a variety of nosocomial infections; including ventilator associated pneumonia, bacteremia, surgical site infections, secondary meningitis, and urinary tract infections (1). The treatment of such infections is difficult and related to *Acinetobacter* ability to rapidly become resistant to multiple antibiotics including aminoglycosides, extended-spectrum cephalosporines, carbapenems and fluoroquinolones (1, 2). Most of the *A. baumannii* infections are caused by outbreak strains, which can be spread widely and rapidly among patients. Since these strains also exhibit multiple-antibiotic resistance, it has been suggested that epidemic potential among isolates of *A. baumannii* may be linked to the presence of Integrons (3). Three main classes of integrons have been described with class I integron being the most common and widely distributed among gram negative bacteria (4). All have a 59 bp conserved segment, including an *intI* gene encoding an integrase and an *attI* recombination site, but have distinct 39 bp conserved segments (5). This structure is capable of capturing genes by a site-specific recombination mechanism that often carry gene cassettes containing antibiotic resistance genes (6). Transfer of integrons into new bacteria and insertion of gene cassettes encoding resistance genes result in the emergence of multiple antibiotic resistant strains (7). Since the integron mediated antibiotic resistance has been shown to be one of the factors which can influence the nosocomial dissemination of *A. baumannii* (8), the aim of this study was to investigate the role of 3 classes of inte-

grons in conferring antibiotic resistance in *Acinetobacter* spp. strains isolated from VAP patients in ICU of Rasoul Akram Hospital in Tehran, Iran.

Materials and Methods

Sample collection and clinical data

A total of 51 hospitalized patients in ICU of Rasoul Akram Hospital from October 2010 to September 2011 included in this study. The patients were hospitalized in MICU, SICU and Neuro-ICU and suffered from Ventilator Associated Pneumonia (VAP). VAP diagnosis criteria included radiographic image of a new and persistent pulmonary infiltrate and at least two of the following criteria, (I temperature above 38.5°C, ii) leukocytosis with a count higher than 10000 cells / mm³, iii) purulent bronchial secretions.

The following variables were recorded as predisposing factors for the development of VAP: age, sex, duration of hospitalization, chronic underlying disease and antibiotic used for treatment.

The Bronchoalveolar lavage (BAL) samples were obtained from patients and evaluated for microbiology analysis. *Acinetobacter* was identified in the Antimicrobial Resistance Research Centre (ARRC) laboratory by gram staining and standard biochemical screening tests including; oxidase, motility, glucose O/F, citrate test, and growth at 42°C, TSI, MacConkey agar or EMB agar (1). In addition, *A. baumannii* identification was confirmed by the presence of *blaOXA-51*-like gene (9) using oligonucleotide primers followed by OXA-51-like F: 5' TAATGCTTTG ATCG

GCCTTG 3' and OXA-51-like-R: 5' TGGAT TGCA CTT CATCTTGG 3'. PCR product should be observed in 353bp in size (10).

Antimicrobial Susceptibility test

Antimicrobial susceptibility testing was carried out by agar disk diffusion, according to CLSI guideline 2010 (12) and microdilution method.

The applied antimicrobials in disk diffusion method were as follows: ticarcillin (TC, 75 µg), ceftriaxone (CRO, 30 µg), aztreonam (ATM, 30 µg), cefotaxime (Ce, 30 µg), ceftazidime (CAZ, 30 µg), colistin (CO, 25 µg), amikacin (AK, 30 µg), piperacillin (PRL, 100 µg), piperacillin / tazobactam (PTZ, 100/10 µg), chloramphenicol (C, 30 µg), ciprofloxacin (CIP, 5 µg), levofloxacin (LEV, 5 µg), gentamicin (G, 10 µg), imipenem (IM, 10 µg), tetracycline (T, 30 µg), cotrimoxazol (TS, 25 µg) and cefepime (CPM, 30 µg). All the antibiotics were provided from Mast Company, UK. In microdilution method, we used MIC Sensititre® susceptibility plates, France, and results were interpreted according to manufacture guideline.

PCR amplification for detection of Integrons

For extraction of bacterial genome, one loopful of fresh overnight bacteria on Mueller Hinton Agar plates was picked up and suspended in 200 µL of sterile water and boiled for 10 min. After centrifugation, the supernatant was used as the template for PCR assay. Amplification was performed using a Sencoquest Thermal Cycler, Germany, in 25 µl volume containing 5 µl of purified DNA, PCR Pre-mix (20 µl) (bioneer, USA) and 10 pM of each primer. Primer sequences used for amplification were as follows:

IntI-F: 5'CAGTGGACATAAGCCTGTTC3', IntI-R: 5' CCCGAGGCATAGACTGTA3' (13), intIII-F: 5'TTGC GAGTATCCATAACCTG3', IntII-R: 5'TTACCTGCA CTGGATTAAGC3' (13), IntIII-F: 5'CCCTCCGGCA GCGACTTTCAG 3', IntIII-R: 5' ACGGATCTGCCA AACATGACT 3' (14) and the amplicon sizes were 160bp, 288bp and 979 bp, respectively (13, 14). The PCR conditions was as follows: initial denaturation at 94°C for 5 min; 30 cycles with denaturation at 94°C for 45s, annealing at 58°C for 45s and extension at 72°C for 60s followed by final extension at 72°C for 5 min. PCR products were separated by electrophoresis on a 1% agarose gel and were detected by comparison with a 100 bp DNA ladder as a size marker under UV doc apparatus.

Result

Among 51 patients diagnosed with VAP episode, 33 (62.7%) and 18 (35.2%) were male and female, respectively and the mean (\pm SD) age was 53.8 (\pm 12.35). Duration of hospitalization of patients was 17.63 \pm 3.4 days. The main chronic underlying disease and cause of hospitalization were brain surgery and high blood pressure in 28.5% and 20% of patients, respectively. Most of the antibiotics used for treatment were vancomycin (77.1%), ciprofloxacin (51.4%), imipenem (45.7%), meropenem (42.8%) and amikacin (42.8%). The clinical and microbiological analysis showed that 50 (98%) of patients were positive, with a high prevalence of gram-negative bacteria, mainly *Acinetobacter* (70%), from which *A. baumani* was detected in 34 (68%) and *A. lwoffii* in 1 (2%), other samples contained different isolates, including *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Serratia rubidaea*.

Antimicrobial Susceptibility test

A total of 5 antibiotypes were obtained in our study by disk diffusion assay. Antimi-

crobial susceptibility testing results by disk diffusion and MIC methods are presented in Table 1 and 2.

Table 1. Antibiotyping of *Acinetobacter* isolates from the VAP samples (P1-4 in *A. baumannii* and P5 in *A. lwoffii*)

Pattern	colistin	chloramphenicol	ciprofloxacin	levofloxacin	gentamicin	amikacin	sulfamethoxazole	trimethoprim-sulfamethoxazole	piperacillin	piperacillin + tazobactam	ceftioxone	tetracycline	imipenem	azteronam	ticarcillin	ceftazidim	Ceftazidim+ clavulanate	cefotaxime	Cefotaxime+ clavulanate	cefepime	Number (%)	
P1	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	26 (74.3)
P2	S	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	4 (11.43)
P3	S	R	R	R	R	S	R	R	R	R	R	S	R	R	R	R	R	R	R	R	R	3 (8.57)
P4	S	R	R	I	S	R	S	R	S	S	R	R	S	R	R	R	S	R	R	S	1(2.86)	
P5	S	I	S	S	S	S	S	S	S	S	I	R	S	I	S	S	S	S	S	R	1(2.86)	
Total																						35 (100)

PCR amplification for detection of Integrons

Thirty four isolates were identified as *A. baumannii* using OXA 51-like primers (figure 1). The presence of class I integrons was detected by amplification of the integrase (*intI*) genes (3 of 35 *Acinetobacter* isolates, 8%); this element was also present in *A. lwoffii* isolated in this study. There were no *intII* or *intIII* in *Acinetobacter* spp. isolates.

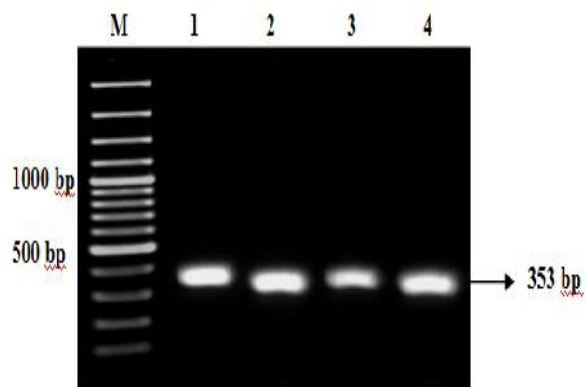


Figure 1. PCR amplification of *blaOXA51*-like-F, R for identification of *A. baumannii* isolates. Lanes: 1-3 representative of *A. baumannii* strain -s; 4, *A. lowfeii*. M: DNA 100 bp size marker.

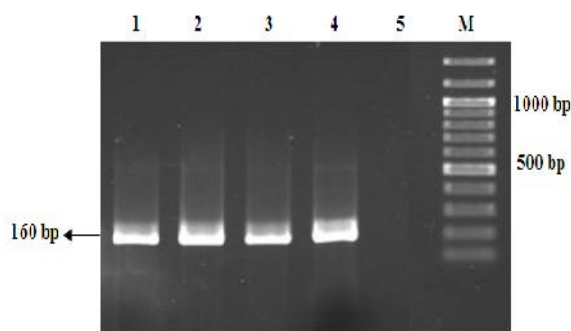


Figure 2. PCR amplification of *IntI-F, R* in *A. baumannii* isolated from patients. Lanes: 1-3 representative of *A. baumannii* isolated from patients; 4, positive control; 5, negative control. M: DNA 100bp size marker.

Discussion

Several studies have suggested that the occurrence of VAP increases the risk of death in critically ill patients, especially when the episode of pneumonia is due to a multidrug-resistant pathogen (15). VAP due to MDR *Acinetobacter* spp. has been reported by many workers (16-18). It is well known that this organism rapidly develops resistance to various groups of antimicrobials including aminoglycosides, fluoroquinolones, and carbape-

nems. In our study, all of *A. baumannii* isolates were resistant to 3 families of antibiotics and considered as multi drug resistant (19).

Imipenem resistance was seen in 90% of total isolates and 100% of class I integron-positive isolates.

Table 2. Antibiogram results using disk diffusion and MIC assays

Antibiotics	Sensitive (%)		Intermediate (%)		Resistant (%)	
	disk diffusion	MIC	disk diffusion	MIC	disk diffusion	MIC
Ceftazidime	1 (2.8)	0	0	0	34 (97.14)	35 (100)
Cephotaxime	1 (2.8)	0	1 (2.8)	2 (5.7)	33 (94.3)	33 (94.3)
Ceftriaxone	1 (2.8)	1 (2.8)	0	0	34 (97.14)	34 (97.14)
Cefepime	1 (2.8)	2 (5.7)	0	0	34 (97.14)	33 (94.3)
Cefoperazone	-	2 (5.7)	-	0	-	33 (94.3)
Carbenicillin	-	0	-	1 (2.8)	-	34 (97.12)
Aztreonam	1 (2.8)	0	0	0	34 (97.14)	35 (100)
Piperacillin	1 (2.8)	1 (2.8)	0	0	34 (97.14)	34 (97.14)
Piperacillin/Tazobactam	2 (5.7)	1 (2.8)	0	0	33 (94.3)	34 (97.14)
Ticarcillin	1 (2.8)	1 (2.8)	0	0	34 (97.14)	34 (97.14)
Ticarcillin/clavulanic acid	-	1 (2.8)	-	0	-	34 (97.14)
Imipenem	2 (5.7)	1 (2.8)	0	0	33 (94.3)	34 (97.14)
levofloxacin	1 (2.8)	2 (5.7)	1 (2.8)	0	33 (94.3)	33 (94.3)
Ciprofloxacin	1 (2.8)	1 (2.8)	0	0	34 (97.14)	34 (97.14)
Sulfisoxazole	-	4	-	0	-	31 (88.6)
Trimethoprim/sulfamethoxazole	3 (8.8)	3 (8.8)	2 (5.7)	2 (5.7)	30 (85.7)	30 (85.7)
Amikacin	8 (22.9)	12 (34.3)	0	0	27 (77.1)	23 (65.8)
Chloramphenicol	0	2 (5.7)	0	0	35(100)	33 (94.3)
Tobramycin	-	12 (34.3)	-	0	-	23 (65.8)
Gentamicin	2 (5.7)	2 (5.7)	0	0	33 (94.3)	33 (94.3)
Tetracycline	3 (8.8)	11 (31.4)	0	0	32 (92)	24 (68.6)
Colistin	35 (100)	-	0	0	0	-

This rate of resistance to imipenem is higher than previously studied isolates from Iran (13). In contrary to our study, Basustaoglu and colleagues (2001) reported that more than 90% of their isolates were sensitive to imipenem.

The high rate of resistance to carbapenem that we observed in ICU may due to the abundant use of imipenem as the empiric regimen for suspected gram-negative coverage of *Acinetobacter* infections (20-21).

In addition, resistance to carbapenem among *int* negative strains may be related to other resistant mechanisms.

Antibiotic resistance to other antibiotics (including amikacin, piperacillin, piperacillin / tazobactam, chloramphenicol, ciprofloxacin, gentamicin, tetracycline, cotrimoxazol and cefepime) in this study was higher than previous reports from Iran (13). Therefore, carbapenems, previously recognized as drug of choice for *A. baumannii* infection (15) and other antibiotic should not be prescribed without a susceptibility testing. We found that 100% of our isolates were sensitive to colistin. With increasing rate of drug resistance, clinicians are faced with confusion in decisions regarding drug choices.

Colistin has been re-introduced as a treatment option, and a few published reports have described its use in cases of *Acinetobacter* spp. and *Pseudomonas* spp. infections.

Moreover, despite being the most effective antibiotic against *Acinetobacter* in vitro, colistin use is limited only to life threatening conditions due to its side effects (22). Nevertheless, observation of high resistance rate of *Acinetobacter* to the majority of the tested antibiotics has limited the use of alternative effective antibiotics. In this study, Prescription of colistin was rare due to its limited distribution in lung and it has been shown that treatment of lung infection by colistin is about 25% (3).

Other effective antibiotics (30%) in our study was amikacin, tobramycin and tetracycline by MIC method, it may be related to lower consumption of these antibiotics in VAP. Newer agents have been recently introduced, including tigecycline, a glycylcycline, which show in vitro and in vivo activity against *A. baumannii* and appears to be a promising option at this time.

High resistant to gentamicin was seen in our study (Table 1). Aminoglycosid resistance determinants are predominantly located on the integrons in gram negative bacteria. Many MDR *Acinetobacter* produce complex combination of aminoglycoside-modifying enzymes, many of which are not coded by integrons and analysis of disc diffusion test indicated that this was the case for present isolates. PCR of integrase gene is a rapid, valuable procedure, which can be easily used in routine clinical microbiology laboratories for the detection of integrons in clinical *A. baumannii* isolates (3).

This study demonstrated detection of class I integron in *A. baumannii* from 8% of the clinical isolates, in contrary to other reports from Iran and other countries (13-14, 23-24), while none of isolates harbored class II or class III integrons.

Antimicrobial susceptibility results in our study showed that five different antibiogram patterns were obtained for 35 *A. baumannii* isolates (4 patterns for *A. baumannii* and 1 pattern for *A. lwoffii*), but no difference was seen to antibiotic resistance profile of *intI* negative *A. baumannii* and class I integron-positive isolates and a high rate of resistant was seen to all tested antibiotics (except for colistin). Some integron-negative isolates were resistant to 3 antibiotic families with the same antibiogram patterns of integron-positive strains. The antibiotic resistance genes of these isolates may have been acquired by plasmid or other mobile elements and possibility of the presence of other integrase gene homologues could not be excluded as those genes may not be amplified by the primers used in this study.

The gaps from resistant gene determinants to phenotypic characterization remain unsolved. Bratu, *et al* (24) demonstrated that multiple factors have contributed to antimicrobial resistance in clinical isolates of *A. baumannii*. Data from Yan and colleagues 2010 (25) showed a high distribution of integrons, transposons, resistant gene determinants and efflux pumps in genotypically related and unrelated MDRAB strains, emphasizing the multitude of resistance genes.

Conclusion

Base on results, we concluded that colistin was an effective antibiotic and can be used

for treatment of patients in ICU and although class I integrons are important among clinical isolates of *A. baumannii*, they have no significant role in dissemination of antibiotic resistance genes in Rasoul Akram Hospital in Tehran, Iran. The presence of *IntI* in *A. lwoffii* may be related to transfer of integron to *A. baumannii* which can be considered as an important threat for hospitalized patients.

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None declared.

Conflict of Interest

None declared conflicts of interest.

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