



## Distribution of Class I Integron among Isolates of *Acinetobacter baumannii* Recoverd from Burn Patients

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### ABSTRACT

**Background:** *Acinetobacter baumannii*, is an important opportunistic pathogens responsible for nosocomial infections. The aim of this experiment was to determine prevalence of Class I Integron in *A. baumannii* strains isolated from burn patients in Mottahari Hospital and the drug susceptibility pattern.

**Methods:** There were 69 *Acinetobacter* isolates, 68 (98.5%) were identified as *A. baumannii*. Antimicrobial susceptibility of these isolates were determined by a disk diffusion method. PCR assay for detection of *bla*<sub>OXA-51</sub> like gene (for identity confirmation) and *intI* was performed.

**Results:** The most effective antibiotic for treating *A. baumannii* was colistin, followed by tetracyclin and tobramycin. The presence of Integron class I was detected in 14.49% of isolates. ESBL and carbapenemase production were observed in 10% and 24.6% of isolates, respectively.

**Conclusion:** Due to the high resistance of strains lacking Integron I, the findings are although class I integrons are disseminated among clinical isolates of *A. baumannii*, at present research, they they do not play important role in dissemination of antibiotic resistance genes in Mottahari Hospital in Tehran, Iran.

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## Introduction

Infection in the critically burned patient remains one of the most important contributors to morbidity and mortality (1). The emergence and rapid spread of multidrug resistant *Acinetobacter baumannii* (MDRAB) isolates causing nosocomial infections are a crucial concern (2). MDRAB is also an increasing problem in Iranian hospitals (3, 4, 5, 6).

Many antibiotic resistance genes are located on plasmids and transposons, enabling their transfer among a variety of bacterial species. The other mechanism involves DNA element that mediates the integration of resistance genes by a site-specific recombinational mechanism (7). Various studies have reported the existence of antibiotic resistance genes located on integrons among *Acinetobacter* spp. (8, 9). Transfer of integrons into new bacteria and insertion of gene cassettes encoding resistance genes result in the emergence of multiple antibiotic resistant strains (10). Several classes of integrons have been described, with class I integrons being the most common and widely distributed among Gram-negative bacteria. Integrons have been found in isolates of *Acinetobacter* spp. from different parts of the world. It has been suggested that multiresistant isolates of *Acinetobacter* spp. may act as a reservoir of integron-associated antibiotic resistance gene, that could then spread to other pathogens in the hospital environment (11). A distinguishing feature of an integron is the presence of three components within the conserved in fifty nine regions:

1. An integrase gene (*intI*) encoding the IntI integrase

2. A gene (*attI*) encoding the cassette integration site
3. One or more promoters responsible for the expression of gene cassettes if present. As of now, approximately sixty different gene cassettes have been identified, most of which encode resistance to antibiotics (12, 13).

Most of *A. baumannii* infections are caused by strains, which can spread widely and rapidly between patients. These strains also exhibit multiple antibiotic resistances. It has been suggested that epidemic potential among isolates of *A. baumannii* may be linked to the presence of integrons. The aim of this research was to investigate the distribution of type I integrons in *A. baumannii* isolated from burn patients of Motahhari Hospital in Tehran, Iran. The concern was detecting any correlations between antibiotic resistance and the carriage of class I Integron among isolates.

## Material and Methods

### *Bacterial strains*

There were sixty nine isolates of *Acinetobacter* were obtained from hundred sixty three patients attending the Motahhari hospital from June 2010 until April 2011. This hospital is one of the few highly out fitted tertiary burn centers in Tehran, Iran. It provides care to severely burned patients from the province of Tehran and to complicated cases referred from other centers across the country. The medical information of all hospitalized burn patients including age, sex, type of burn injury,

degree of burn injury, patient outcome, site of infection, and sample were recorded. *A. baumannii* was identified in the Antimicrobial Resistance Research Centre laboratory by Gram staining, standard biochemical tests including; oxidase, motility, glucose O/F, citrate test,

and growth at 42°C, TSI, MacConkey agar and EMB agar (14).

In addition, *A. baumannii* identification was confirmed by the presence of *bla*<sub>OXA-51</sub> like gene (15). The primers are shown in Table 1.

**Table 1.** Oligonucleotide primers used for detection of class I integron and identification of *A. baumannii*

Primers Disignation	Sequences	Product size	References
<b>OXA-51-likeF</b>	5' TAATGCTTTGATCGGCCTTG 3'	353	15
<b>OXA-51-like-R</b>	5' TGGATTGCACTTCATCTTGG 3'		
<b>IntI-F</b>	5' CAGTGGACATAAGCCTGTTC 3'	160	19
<b>IntI-R</b>	5' CCCGAGGCATAGACTGTA 3'		

#### *Antimicrobial Susceptibility test*

Antimicrobial susceptibility test was performed by disk diffusion, according to CLSI guidelines 2011 (16). The applied antimicrobials were as follows: piperacillin + tazobactam (100/10 µg), piperacillin (100 µg), azteronam (30 µg), ticarcillin (75 µg), tetracycline (30 µg), gentamicin (10 mg), tobramycin (10 µg), kanamycin (30 µg), amikacin (30 µg), colistin (10 µg), imipenem (10 µg), cefotaxime (30 µg), ceftazidime (30 µg), ceftazidime-clavulanic acid (30/10 µg), cefotaxime-clavulanic acid (30/10 µg), trimethoprim-sulfamethoxazole (25 µg) and ciprofloxacin (5 µg).

*A. baumannii* isolates which resistant to carbapenems, ceftazidime, cefotaxime, gentamicin, tobramycin, and fluoroquinolones were defined as multi drug resistant.

All strains that were resistant to ceftazidime and cefotaxime by inhibition zone less than 14 mm were evaluated for the producing of ESBL by synergy double disk method. This assay was performed following: disks of ceftazidime (30 µg), cefotaxime (30 µg), ceftazidime-clavulanic acid (30/10 µg), and cefotaxime-clavulanic acid (30/10 µg)

were placed on Mueller Hinton Agar (Merck, Germany). A difference of 5 mm between ceftazidime, ceftazidime-clavulanic acid, and cefotaxime, cefotaxime-clavulanic acid in the zone diameter was considered as a positive result (17).

Imipenem resistant isolates were examined for metallo-β-lactamase production by EDTA disk method as described previously (18). Briefly, test organisms were inoculated onto plates of Mueller Hinton agar. A 0.5 M EDTA solution was prepared by dissolving 186.1 g of EDTA in 1,000 ml of distilled water and adjusting it to pH 8.0 by using NaOH. The mixture was sterilized by autoclave. Appropriate amounts of 10 µl EDTA solution were added to 10-µg-imipenem disk (Mast) and were placed on the plate. The inhibition zones of the imipenem and imipenem-EDTA disks were compared. After sixteen to eighteen h of incubation in air at 35°C, difference of 7 mm in the zone diameter the control for positive result (18).

#### *PCR amplification for detection of Integron*

A loopful of bacteria was taken from each fresh overnight culture on Mueller Hinton

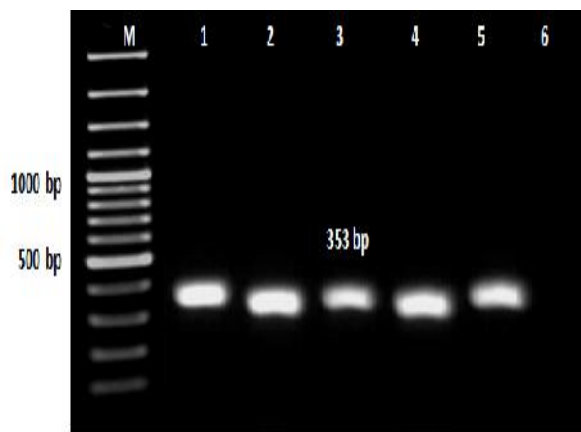
Agar plates, suspended in 200  $\mu$ L of sterile water was boiled for 10 min. After centrifugation, the supernatant was used as the template for PCR. Amplification was performed using a Sencoquest Thermal Cyclerin. There was 25  $\mu$ l volume containing 5  $\mu$ l of purified DNA, PCR Pre mix (bioneer, USA) and 10 pM of each primer. Primer sequences and the amplicon sizes are listed in Table 1 (19). The PCR conditions are listed: initial denaturation at 94°C for 5 min; 30 cycles with denaturation at 94°C for 45 s, annealing at 56°C and 58°C for 45s for blaOXA-51 like and *intI* and extension at 72°C for 60 s 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 against 100 bp DNA ladder as a size marker under UV doc apparatus.

## Result

### Bacterial strains

During this experiment time frame (June 2010 until April 2011), a total number of hundred sixty three severely burned patients were hospitalized and treated at Motahhari hospital. There were sixty nine isolates of *Acinetobacter* (97%) were obtained from wound and 3% from blood samples. The range of patients was between 20-35 years. The most common cause of burns was flammable liquids (e.g. gasoline, boiling water, etc.) (66.40%). Major burn degree in this study was degree II and III by 40.58% frequency. The mortality rate in this study was (14/69, 20%), this rate among females was higher than males: 21.4% (8/27) vs. 7.5% (6/42).

Ninety eight percent *Acinetobacter* isolates exhibited the presence of bla<sub>OXA-51</sub> like gene (Figure. 1) and identified as *A. baumannii*. One isolate was identified as *Acinetobacter lwoffii*.



**Figure 1.** PCR amplification of blaOXA51-like-F, R for identification of *A. baumannii* isolates. Lanes: 1-5 representative of *A. baumannii* strains; 6, *A. lwoffii*. M: DNA 100bp size marker

### Antimicrobial Susceptibility test

The most effective antimicrobial agents against *A. baumannii* isolates in this research are listed: colistin with 100% activity, tetracyclin (46.4%), and tobramycin (31.9%). Most isolates showed high resistance to piperacilin (97.1%), piperacillin + tazobactam (97.1%), imipenem (92.8%), cefotaxime (97.1%), ceftazidim (97.1), ticarcillin (92.6%), azteronam (98.6%), gentamicin (64.7%), amikacin (95.7%), kanamycin (97.1%), tobramycin (2.9%), tetracycline (40.6%), trimethoprim-sulfamethoxazole (98.6%) and ciprofloxacin (97.1%).

ESBL assay for cefotaxime and ceftazidime were positive in 10% and 5% the isolates, respectively. There are 24.6% of *A. baumannii* isolates exhibited Metalo - lactamase (carbapenemase) activity. In total 17 antibiotyps (antibiogram patterns) were

obtained in our study which are presented in Table 2. According to Table 2 A. *baumannii* are resistant to 3 families of antibiotics as a

result, they are considered as multi drug resistant.

**Table 2.** Antibiotyps (antibiogram patterns) of isolates

Pattern	cefotaxime	Cefotaxime+ clavulanate	cefazidim	Ceftazidim+ clavulanate	azteronam	imipenem	piperacilin	piperacilin + tazobactam	ticarcillin	Ticarcillin+clavulana te	gentamicin	amikacin	tobramycin	ciprofloxacin	colistin	kanamycin	tetracycline	trimethoprim-sulfamethoxazole	N. (%)*
P1	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	S	R	21 (30.4)
P2	R	R	R	R	R	R	R	R	R	R	R	R	S	R	S	R	S	R	12(17.4)
P3	R	R	R	R	R	R	R	R	R	R	S	R	S	R	S	R	S	R	8 (11.6)
P4	R	R	R	R	R	R	R	R	R	R	I	R	S	R	S	R	S	R	6 (8.7)
P5	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	I	R	6 (8.7)
P6	R	R	R	R	R	R	R	R	R	R	S	R	S	R	S	R	R	R	4 (5.8)
P7	R	R	R	R	R	R	R	R	R	R	R	R	I	R	S	R	S	R	2 (2.9)
P8	R	R	R	R	R	R	R	R	R	R	S	R	R	R	S	R	R	R	1 (1.45)
P9	R	R	R	R	R	R	R	R	R	R	R	R	R	I	S	R	S	R	1 (1.45)
P10	R	R	R	R	R	R	R	R	R	R	I	R	R	R	S	R	R	R	1 (1.45)
P11	R	R	R	R	R	R	R	R	R	R	S	R	R	R	S	R	I	R	1 (1.45)
P12	R	R	R	R	R	R	R	R	R	R	S	R	S	R	S	R	I	R	1(1.45)
P13	R	R	R	S	R	R	R	R	R	R	R	R	R	R	S	R	I	R	1 (1.45)
P14	R	R	R	R	R	S	R	I	S	S	R	R	S	R	S	R	S	R	1 (1.45)
P15	I	I	S	S	R	S	I	S	R	R	S	S	S	R	S	S	S	S	1 (1.45)
P16	R	R	R	R	R	R	R	R	R	R	R	S	R	R	S	R	R	R	1 (1.45)
P17	S	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	R	1 (1.45)
<b>Total</b>																			<b>69 (100)</b>

### Detection of *intI* genes

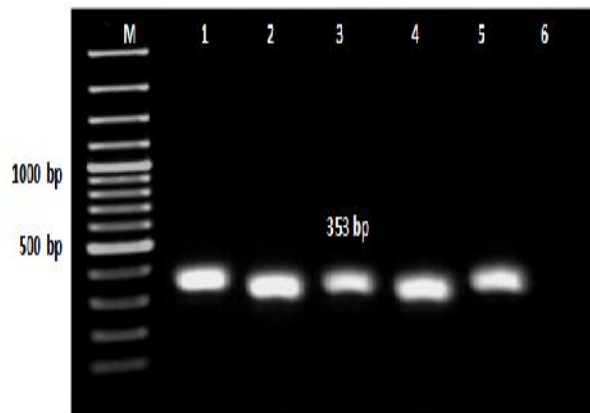
PCR amplification of *IntI*-F, R in *A. baumannii* isolated from patients show in Figure 2. The presence of Class I integrons are detected by amplification of the integrase genes in (10 of 68, 14.7%) of *A. baumannii* isolates, indicating that these elements are not widely spread among multiresistant *A. baumannii* isolated in Motahhari hospital in the period of experiment. These elements also are negative in *A. lwoffii*.

### Discussion

The mortality rate in the experiment was 20%, which is higher than other studies from

Iran (20-24). In female, the percentage was higher than male. Most of the existing literature on burns patients in economically developed countries has shown nosocomial infections are the most common causes of mortality and morbidity among severely burned hospitalized patients (24).

The most frequently encountered infections in burn patients, including: ventilator-associated pneumonia (VAP), catheter-related bloodstream infection, urinary tract infection, and wound infections. They are generally caused by known nosocomial pathogens (25).



**Figure 2.** PCR amplification of IntI-F, R in *A. baumannii* isolated from patients. Lanes: 1-6 representative of *A. baumannii* isolated from patients; 7, positive control; 8, negative control. M: DNA 100bp size marker

*A. baumannii* has emerged as a new intimidation for burn patient population. Serious infection with this organism are reported with increasing frequency, and national infection surveillance programs now recognize it as a major component of nosocomial pathogens (26-31). In addition the concerns are changing drug susceptibilities, which now display increasing drug resistance to conventional therapeutic agents.

Most samples in this experiment were isolated from wounds (97%). *A. baumannii* were common spp. isolated from wound infection in patients, these results are consistent with other studies (26-31).

Antimicrobial susceptibility results showed that *IntI* negative isolates like class I integron-positive *A. baumannii* were highly resistant to all tested antibiotics (except for colistin).

Seventeen different antibiogram patterns were obtained among sixty nine isolates (16 patterns belonged to *A. baumannii* and one

pattern for *A. lwoffii*) but there were no clear phenotypic differences between isolates of the strains either they have integron or not. Imipenem resistance was found in 92.8% of the entire isolates and 100% of class I Integron-positive isolates. The experiment shows there is a higher number of incidence than other studies from Iran (3, 5). Carbapenemase activity was reported in 24.6% of isolates in this study, the high carbapenem resistance that we observed in this study may be related to other resistant mechanisms.

The explanation may lie in numerous use of imipenem or piperacillin as the empiric regimen used for suspected gram-negative coverage in Motahhari hospital.

Antibiotic resistance to other antibiotics in this study was higher than other reports from Iran. Therefore, the use of carbapenems, previously recognized as drug of choice for *A. baumannii* infection (32). And other antibiotic should not be approved without a susceptibility test.

In the report by Hanberger in 1999 analyzing microbiological data from 5 European countries, *Acinetobacter* species were found to have highest increase in resistance to antibiotics in entire population of gram-negative bacilli studied (34). Interestingly, we did not encounter resistance to colistin in any of our isolates (34-36).

With increasing drug resistance, clinicians are faced with complicated decisions regarding therapeutic choices. Colistin has been reintroduced as a treatment option, and a few published reports have described its use in cases of *Acinetobacter* and *Pseudomonas* infections.

However, the use of colistin in a nebulized form has been rarely investigated. Despite being the most effective antibiotic against *Acinetobacter* in vitro, colistin use is limited only to life threatening conditions due to its serious side effects (37). Nevertheless, observation of high resistance rate of *Acinetobacter* to the majority of the tested antibiotics has limited the use of alternative effective antibiotics.

There have been newer agents recently introduced, including tigecycline, a glycylcycline, which shows in vitro and in vivo activity against *A. baumannii*. Nevertheless, it appears to be a promising option at this time.

Other effective antibiotics this experiment include tetracycline, tobramycin and gentamicin. It may be related to less use of these antibiotics in burn patients. High resistance to other aminoglycosids reported in our study, aminoglycoside resistance determinants are predominantly located on the integron in gram negative bacteria, many MDR *Acinetobacter* produce complex combination of aminoglycoside-modifying enzymes, many of which are not coded by integrons. Analysis of disc diffusion test indicated that this was the case for present isolates.

In the present experiment, two different PCR assays were used to identify and detect of *A. baumannii* and class I integrons by amplification of specific *bla*<sub>OXA-51</sub> like and *intI1* gene. *Bla*<sub>OXA51</sub>-like PCR were used for confirmation of identification. Integrase gene PCR 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 (38).

This study demonstrated detection of class I integron in *A. baumannii* from 14.42% of clinical isolates, in contrary to other reports from Iran and other regions (3, 5, 39).

However, some integron-negative isolates were resistant to six or nine drugs with the same antibiogram patterns of integron-positive strains. The antibiotic resistance genes of these isolates could be acquired by plasmid or other mobile elements (40). In addition, there is a possibility of the presence of other integrase gene homologues that could not be excluded as these genes may not be amplified by the primers used in this study.

The gaps from resistant gene determinants phenotypic characterization remain unsolved. Bratu *et al.* 2008 (41) demonstrated that multiple factors have contributed to antimicrobial resistance in clinical isolates of *A. baumannii*. Data from Yan *et al* 2010 (42) show 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 that *A. baumannii* is capable of possessing and the potential horizontal gene transfer between polyclonal MDRAB strains.

ESBL production is shown in 10% of isolates. Resistance to extended-spectrum -lactams could be due to a combination of integrons and resistance genes located on other genetic structures such as plasmids (43).

## Conclusion

Infections of burn site are serious problems that can compromise patient's survival and the outcome of reconstructive treatment. On the other hand, the antimicrobial pattern of resistance is an important option for treatment in burn patients. Using new extended-spectrum antibiotic can be useful for treatment.

It is well known in different events, such as infection control measures, antibiotic use policy, and susceptibilities in individual patients, can facilitate or prevent the dissemination of MDR bacteria (44), including *A. baumannii*.

Since did not detect any association between resistances to other antibiotics integrons. It can be conclude the role of other resistance determinants in Motahhari hospital.

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## Conflict of Interest

None declared conflicts of interest.

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