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ORIGINAL: Molecular Diagnosis of classI integron in *Acinetobacter baumanii* strains isolated from Patients admitted in hospitals of sari

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ABSTRACT

Introduction: *Acinetobacter baumannii*, is an opportunistic and gram negative, anaerobic and non-fermentative bacteria. It's highly resistance to last line of antibiotics and variable resistance pattern in patients whose infected by this microorganism, makes a potential reason for recognition related genes and it help us to choosing the best strategy for using antibacterial substances in correct way. So, the aim of this study is molecular identification of integron class I in *Acinetobacter baumannii* strains in hospitalized patients in north of IRAN.
Material and Methods: 28 samples of *Acinetobacter baumannii* were collected from hospitalized patients in burned and infected units of three hospitals in Sari during the 6 months from February to July of 2019. demographic information was collected from patients and susceptibility pattern of isolates was examined by Kirbey- Bauer method and presence of Integron class I were identified by PCR method.
Results: In this study, the highest resistance and susceptibility to antimicrobial agents in strains with integrons, were related to Amikacin (95.2%) and Cefipim (90.5%), respectively. Frequency of *IntI* was 75% in all isolated strains. Most samples were related to wound but isolates from sputum were rare.
Conclusion: Upon on results of our study ,the high resistance to different classes of antimicrobial agents of isolates have been observed and so high prevalence rate of intI were existing. Statistical analysis showed correlation between integron class I and antimicrobial resistance in all isolates, too.

Introduction

Acinetobacter baumannii is a Gram-negative coccobacillus that was first identified in the early 20th century and has since emerged as a prominent cause of nosocomial infections, largely due to its high level of antibiotic resistance. Although it

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is generally considered to possess low virulence, *A. baumannii* can lead to severe infections in immunocompromised individuals, particularly those with neutropenia, cystic fibrosis, or undergoing chemotherapy [1]. It poses a significant threat in hospital environments, especially in intensive care units, burn units, and surgical wards. The spectrum of infections caused by *A. baumannii* includes hospital-acquired infections, urinary tract infections, wound infections, respiratory tract infections such as pneumonia, meningitis, endocarditis, peritonitis, skin and soft tissue infections, and bloodstream infections including bacteremia and sepsis [2]. Among the species of this genus, *Acinetobacter baumannii* is particularly noteworthy due to its increasing colonization rates among hospitalized patients, especially those with prolonged hospital stays or those receiving broad-spectrum antibiotics or anticancer therapies. *A. baumannii* has been isolated from various sources within hospital environments, including air, water taps, ventilation systems, and medical equipment such as ventilators [3]. Notably, it can survive on dry surfaces for extended periods,up to 11 days at 31% relative humidity and 4 days at 10%,which significantly facilitates its persistence and spread in healthcare settings. The bacterium can also be found in human secretions such as sputum, urine, feces, and vaginal discharge. Approximately 25% of individual's harbor *A. baumannii* on their skin, and it can be detected in the oropharynx of 7% of adults and neonates. *A. baumannii* exhibits high-level resistance to antimicrobial agents, which may be either intrinsic or acquired through genetic elements. Most clinical strains are resistant to a wide range of antibiotics, including ampicillin, amoxicillin/clavulanic acid, anti-*staphylococcal penicillins*, broad-spectrum cephalosporins (with the exception of ceftazidime and cefepime), tetracyclines, macrolides, rifampin, and chloramphenicol [4].

Integrons are genetic elements capable of capturing and mobilizing genes, particularly those embedded within gene cassettes. Structurally, integrons consist of an integrase gene, two conserved regions known as *sul1* and *intI*, and a variable region containing gene cassettes [5]. Based on the type of integrase gene they possess, integrons are classified into four major classes, among which class 1 integrons have been the most extensively studied and are the most prevalent [5]. Because integrons can be located on plasmids, they are readily disseminated among different bacterial species. The resistance genes carried within these gene cassettes can be excised and incorporated into other integrons, a process that significantly contributes to the emergence and spread of novel resistance cassettes and the evolution of plasmids and chromosomes [6].

The coexistence of multiple antibiotic resistance genes and the presence of integrons plays a critical role in the development of multidrug resistance. *Acinetobacter baumannii* can acquire resistance either through spontaneous mutation or horizontal gene transfer mechanisms such as transformation, conjugation, and others [7]. Among these, conjugation is recognized as the most common route for the transfer of antibiotic resistance. During this process, plasmids, transposons, and integrons serve as vehicles for resistance genes, facilitating their transfer between bacterial cells [8].

A major challenge associated with *A. baumannii* is the emergence of multidrug-resistant (MDR) strains that exhibit resistance to multiple classes of antibiotics, including β-lactams, aminoglycosides, and fluoroquinolones. This resistance is frequently mediated by genes located on mobile genetic elements such as transposons and integrons, which enable rapid dissemination among bacterial populations [8]. Given the limited availability of novel antimicrobial agents effective against this pathogen, the continued efficacy of existing therapeutic options becomes increasingly critical. This study aims to investigate the molecular detection of class 1 integrons in *Acinetobacter baumannii* strains isolated from hospitalized patients in Sari, Iran.

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Methods

Sample Collection

In this study, a total of 28 clinical isolates of *Acinetobacter baumannii* were collected over a six-month period (from February 2019 to July 2019) from three hospitals in Sari, Iran: Zare, Imam Khomeini, and Shafa. The study protocol was approved by the Ethics Committee of the Faculty of Medicine, Islamic Azad University, Sari Branch, under the code IR.IAU.SARI.REC.1401.067. During this period, 28 bacterial culture plates obtained from clinical specimens of hospitalized patients were submitted to the microbiology laboratory. Each isolate was anonymized and assigned a unique code.

Bacterial Cultivation, Isolation, and Identification

Upon arrival at the research center laboratory, all culture plates were recultured, and the biochemical identification of isolates was conducted following purification. Initially, the samples were streaked using a four-quadrant method on MacConkey agar and blood agar plates, and incubated at 37°C for 18–24 hours. Colonies were evaluated based on their morphological characteristics: on MacConkey agar, presumptive *A. baumannii* colonies appeared as mucoid with pale pink to purple pigmentation; on blood agar, they presented as smooth, translucent to opaque colonies without hemolysis or pigment production. Suspicious colonies were subcultured again on MacConkey agar and incubated for an additional 18–24 hours at 37°C. Colonies with morphology consistent with *A. baumannii* were then subjected to Gram staining. Microscopically, *A. baumannii* appears as short, thick, Gram-negative coccobacilli, often observed in pairs. Catalase activity was assessed by transferring a few colonies from a 24-hour culture using a wooden applicator stick onto a microscope slide containing two drops of 3% hydrogen peroxide solution. The presence of bubble formation indicated a positive catalase reaction, which is characteristic of *A. baumannii*. Catalase-positive colonies were subsequently tested using an oxidase disc. As *A. baumannii* is oxidase-negative, any oxidase-positive isolates were excluded from further analysis. Triple Sugar Iron (TSI) agar tubes showing an alkaline/alkaline (Alk/Alk) reaction, along with a positive oxidative/fermentative (OF) glucose test only in the aerobic tube, were selected for further testing. To confirm the identity of the isolates, inoculation into SIM (sulfide, indole, motility) medium was performed to assess motility, indole production, and hydrogen sulfide (H_2S) generation. Non-motile isolates with negative results for both indole and H_2S production were identified as *Acinetobacter baumannii*. Final confirmation included evaluation of growth at both 37°C and 44°C, with *A. baumannii* demonstrating the ability to grow at 44°C.

Antibiotic Susceptibility Testing

In the next phase of the study, *A. baumannii* isolates were subjected to antimicrobial susceptibility testing against ten commonly used antibiotics for the treatment of infections caused by this pathogen. The antibiotics tested included: imipenem (10 µg), meropenem (10 µg), ciprofloxacin (5 µg), gentamicin (10 µg), amikacin (30 µg), trimethoprim-sulfamethoxazole (cotrimoxazole; 1.25 + 23.75 µg), ceftriaxone (30 µg), cefepime (30 µg), ceftazidime (30 µg), and cefotaxime (30 µg). The susceptibility profiles were determined using the standard disk diffusion method on Mueller-Hinton agar. Antibiotic disks were purchased from Mast Group (England). The testing procedure followed the Clinical and Laboratory Standards Institute (CLSI) 2018 guidelines and was quality-controlled using the reference strain *A. baumannii* ATCC 19606.

DNA Extraction

Genomic DNA was extracted from both clinical isolates and the standard reference strain *A. baumannii* ATCC 19606 using a commercial Gram-negative bacterial DNA extraction kit (Bioneer, South Korea), following the manufacturer's protocol with

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minor adjustments. Briefly, 1.5 mL microcentrifuge tubes were filled with bacterial culture medium and centrifuged at 14,000 rpm for 10 minutes. The supernatant was discarded, and 100 µL of Prelysis Solution and 20 µL of lysozyme were added to the pellet, followed by incubation at 55°C for 30 minutes. Subsequently, 400 µL of Lysis Solution was added, followed by 30 µL of Precipitation Solution.The lysate was transferred to a spin column and centrifuged at 13,000 rpm for 1 minute. After discarding the flow-through, 400 µL of Wash I buffer was added and centrifuged again. This step was repeated using 400 µL of Wash II buffer. The column was then transferred to a clean microtube, and 50 µL of Elution Buffer was added. The tube was incubated at 65°C for 2–3 minutes, followed by centrifugation at 13,000 rpm for 1 minute. The resulting eluate contained the purified genomic DNA.

Polymerase Chain Reaction (PCR)

PCR amplification was carried out in a total reaction volume of 25 µL. Each reaction mixture contained 12.5 µL of Master Mix (Andisheh Karaban Co., Iran), 1 µL of primer (Bioneer, South Korea) at a concentration of 0.8 pmol, 10 µL of template DNA, and 1.5 µL of nuclease-free water. Primer sequences used for amplification are presented in Table 1. Amplification of the class 1 integron gene (*intI1*) was performed using a Bio-Rad thermal cycler (Singapore) under the following cycling conditions: an initial denaturation at 94°C for 5 minutes; followed by 35 cycles of denaturation at 94°C for 1 minute, annealing at 55°C for 30 seconds, and extension at 72°C for 30 seconds; and a final extension step at 72°C for 5 minutes. PCR products were electrophoresed on a 1.5% agarose gel stained with RedSafe, and visualized using a gel documentation system. Images were captured for record and analysis.

Statistical Analysis

The association between the presence of integrons and antibiotic resistance was analyzed using SPSS software. The chi-square (χ^2) test was employed to assess statistical significance, and a *p*-value of less than 0.05 was considered statistically significant ($p < 0.05$).

Results

In this study, a total of 28 clinical isolates of *Acinetobacter baumannii* were collected over a six-month period (February to July 2019) from three hospitals in Sari, Iran: Zare (50%), Imam Khomeini (25%), and Shafa (25%). Among the collected samples, the majority originated from wound specimens (64.28%), followed by urine (17.85%), pulmonary secretions (10.71%), and sputum (7.14%).

Patient demographic analysis revealed that 71% of the infected individuals were male, while 29% were female. The highest prevalence was observed in the age group of 41–50 years (39.28%), whereas the lowest frequency was seen in patients under 30 years of age (7.14%). The most common length of hospital stay among patients was between 15 and 21 days (39.28%), while the shortest duration, up to 7 days, accounted for the lowest proportion (14.28%).

Molecular analysis indicated that 75% of the *A. baumannii* isolates carried class 1 integrons . Antibiotic susceptibility testing demonstrated the highest rates of resistance to amikacin (95.2%), ceftazidime (90.5%), gentamicin (90.5%), ciprofloxacin (85.7%), ceftriaxone (76.2%), and sulfamethoxazole/trimethoprim (66.7%). In contrast, lower resistance rates were observed for meropenem (23.8%), imipenem (19%), cefotaxime (14.3%), and cefepime (9.5%). Correspondingly, the highest susceptibility rates were recorded for cefepime (90.5%), cefotaxime (85.7%), imipenem (81%), meropenem (76.2%), sulfamethoxazole/trimethoprim (33.3%), ceftriaxone (23.8%), ciprofloxacin (14.3%), gentamicin (9.5%), ceftazidime (9.5%), and amikacin (4.8%) (Table 2).

A statistically significant association was identified between the presence of class 1 integrons and resistance to specific antibiotics. Significant correlations were observed for cefotaxime, cefepime, amikacin, gentamicin, ciprofloxacin, imipenem, and ceftazidime. However, no significant association was found with resistance to

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sulfamethoxazole/trimethoprim, meropenem, or ceftriaxone.

Table 1. Primer sequences used for detection of class 1 integron genes in *Acinetobacter baumannii* isolates.

Primer Name	Primer Sequence	Amplified Product Size (bp)
intI	F: 5′-CAG TGG ACA GCC TGT TC-3′ R: 5′-CCC GAC GCA TAG ACT GTA-3′	160 bp

Table 2. Antibiotic resistance and susceptibility patterns in *Acinetobacter baumannii* isolates with and without class 1 integrons.

Antibiotic	Integron-positive isolates (N = 21)		Integron-negative isolates (N = 7)	
	Resistant	**Susceptible**	**Resistant**	**Susceptible**
Imipenem	4 (19%)	17 (81%)	4 (57.1%)	3 (42.9%)
Meropenem	5 (23.8%)	16 (76.2%)	1 (14.3%)	6 (85.7%)
Ciprofloxacin	18 (85.7%)	3 (14.3%)	7 (100%)	0
Gentamicin	19 (90.5%)	2 (9.5%)	6 (85.7%)	1 (14.3%)
Amikacin	20 (95.2%)	1 (4.8%)	6 (85.7%)	1 (14.3%)
Sulfamethoxazole	14 (66.7%)	7 (33.3%)	4 (57.1%)	3 (42.9%)
Ceftriaxone	16 (76.2%)	5 (23.8%)	6 (85.7%)	1 (14.3%)
Cefepime	2 (9.5%)	19 (90.5%)	1 (14.3%)	6 (85.7%)
Ceftazidime	19 (90.5%)	2 (9.5%)	5 (71.4%)	2 (28.6%)
Cefotaxime	3 (14.3%)	18 (85.7%)	1 (14.3%)	6 (85.7%)

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Discussion

Gram-negative bacteria continue to be among the most significant causative agents of hospital-acquired infections within healthcare systems. *Acinetobacter baumannii* is recognized as the second most common Gram-negative pathogen responsible for nosocomial infections, following *Pseudomonas aeruginosa* [9]. As part of the normal skin flora, *A. baumannii* can be isolated particularly from moist areas of the human body. Infections caused by this organism are largely considered hospital-acquired, due to its remarkable ability to persist in the hospital environment and colonize both patients and healthcare personnel, thereby facilitating the onset of healthcare-associated infections.

In Iran, as in many developing countries, hospital-acquired infections represent a major contributor to escalating healthcare costs and are often difficult to prevent entirely. According to national surveillance data collected from 2007 to 2010 across 100 hospitals with more than 200 beds, the highest incidence of nosocomial infections was reported in burn units, followed by intensive care units (ICUs) and hematology/oncology wards. The overall mortality rate associated with these infections was reported at 14.8% [10].

Acinetobacter baumannii is currently the second most prevalent non-fermenting bacterium implicated in hospital-acquired infections, accounting for 7.8% to 23% of all cases in general hospital wards, and 10% to 43% of cases in ICU settings. The rise of antibiotic resistance, particularly among Gram-negative pathogens such as *A. baumannii*, has become a major therapeutic challenge. Excessive and, in some cases, inappropriate use of antibiotics in treating infections caused by this species has contributed to the emergence of multidrug-resistant (MDR) strains [9]. The clinical and economic consequences of MDR *A.baumannii* are profound, including prolonged hospital stays, increased financial burden on patients, and significant strain on healthcare systems [11]. Of particular concern is the dissemination of antibiotic resistance genes via integron structures, which facilitate the acquisition of multiple resistance traits. This genetic mechanism has played a central role in the rapid evolution of *Acinetobacter* spp. into formidable MDR pathogens, complicating the treatment of associated infections and underscoring the urgent need for improved antimicrobial stewardship and infection control strategies.

In the present study, the highest levels of antibiotic resistance were observed against amikacin (95%), gentamicin (90%), ceftazidime (90%), ciprofloxacin (85%), ceftriaxone (76%), and sulfamethoxazole (66%). In contrast, the isolates exhibited greater susceptibility to cefotaxime (85%), cefepime (90%), meropenem (81%), and imipenem (76%). These findings are consistent with those reported by Japoni et al. (2013)[12], Lin et al. (2013) [2], Peymani et al. (2012) [13], and Mostofi et al. (2010)[5], but differ from the results of Sirichot et al. (2009)[14].

Previous studies by Baiouga and Yoshi have highlighted a concerning trend in the increasing antibiotic resistance among *A. baumannii* strains, attributing this phenomenon to the widespread and often inappropriate use of antimicrobial agents. In their work, 82% of the *A. baumannii* isolates demonstrated a multidrug-resistant (MDR) phenotype. They reported MDR prevalence rates ranging from approximately 45% to 75% across different settings [15].

Global studies have shown considerable variability in the prevalence of integrons among *A. baumannii* strains, with reported rates ranging from 5% to 80% [15]. In the present investigation, class 1 integrons of 160 base pairs in size were detected in 75% of the isolates using specific primers. This prevalence differs from the findings of Ruiz, Ribera, and Coleman, who reported lower frequencies (27.5% to 44%) of integrons, typically ranging in size under 3000 base pairs.

Based on the findings of this study, 75% of

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the isolates harbored class 1 integrons. When compared with previous research, these results indicate a notably high prevalence of integrons and elevated levels of antimicrobial resistance in *Acinetobacter baumannii* strains. For instance, the study by Peymani et al. (2012) reported that 80% of *A. baumannii* isolates exhibited a multidrug-resistant (MDR) phenotype, with class 1 integrons widely distributed among the MDR isolates (92.5%) [13]. Similarly, in the study conducted by Taheri Kalani et al. (2011) [16], 58% of the isolates carried class 1 integrons. In another investigation by Japoni et al. (2011) [12], 53.4% of *A. baumannii* strains were found to possess class 1, class 2, or both integron types, with frequencies of 47.7% for class 1 and 3.4% for class 2 integrons. Notably, class 3 integrons were not detected in that study.

Lin et al. (2013) reported a class 1 integron prevalence of 72%, further underscoring the global dissemination of these genetic elements among *A. baumannii* populations [2]. In the study by Koczura et al. (2014) [17], 63.5% of the isolates were found to contain class 1 integrons. Conversely, Mangaloglu et al. (2014) reported a markedly lower prevalence of class 1 integrons, with only 33% of the isolates carrying them [18]. The relatively lower prevalence of class 1 integrons and associated antimicrobial resistance observed in the latter two studies may be attributable to regional differences in antibiotic stewardship practices and more stringent antimicrobial usage policies in those countries.

In this study, a statistically significant association was found between the presence of integrons and resistance to certain antibiotics, specifically cefotaxime, cefepime, amikacin, gentamicin, ciprofloxacin, meropenem, imipenem, and ceftazidime. However, no significant relationship was observed with sulfamethoxazole and ceftriaxone. A study by Burak et al. in Turkey did not find a statistically significant correlation between integron gene presence and resistance to specific antibiotics [10]. In contrast, studies conducted by Gu et al. and Fonseca et al. in other regions of the world reported a significant association between multidrug-resistant isolates and the presence of integron genes [12]. Similarly, Khorramrooz et al. (2015) did not observe a statistically significant correlation between integron presence and resistance to specific antibiotics in their study [19].

In 2011, Peymani and colleagues investigated 100 clinical isolates of *Acinetobacter baumannii* collected from various clinical specimens in hospitalized patients in Tabriz. Their findings indicated resistance rates of 92% to ceftazidime, 88% to cefepime, and 54% to imipenem. These discrepancies may be attributed to differences in treatment strategies across hospitals or to the nature of the clinical samples from which the bacteria were isolated. The high level of antibiotic resistance observed in Iran may be linked to the widespread use of broad-spectrum antibiotics, whether for infection treatment or, in some cases, prophylaxis. Inappropriate prescribing practices further contribute to selective pressure, promoting the emergence of multidrug-resistant organisms, including those resistant to last-line therapies [13].

Iran ranks among the top 20 countries in global drug consumption and holds the second position in Asia after China. On average, each Iranian consumes 341 pharmaceutical items annually, placing the country at the top in terms of per capita drug consumption. Although antibiotic prescription is often essential in bacterial infections and withholding treatment can be life-threatening, studies suggest that 30% to 60% of prescriptions are inappropriate or incorrect. These errors are frequently attributed to physicians, distributors, or self-medication.

Conclusion

Based on the findings of the present study, the high prevalence of *A. baumannii* strains in healthcare facilities, organisms associated with significant morbidity and mortality, underscores the urgent need for comprehensive measures in the prevention,

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treatment, and control of infections caused by this pathogen to improve patient outcomes. A strikingly high proportion of *A. baumannii* isolates exhibited resistance to key therapeutic antibiotics. Therefore, ongoing surveillance of antibiotic susceptibility patterns is critical for informing treatment decisions and developing strategies to curb the emergence of further resistance. It is recommended that hospital-acquired infections be closely monitored, reviewed, and controlled, particularly in inpatient wards and during wound care procedures, as antibiotic resistance hampers timely and effective treatment of infectious diseases, especially in hospitalized patients. Future studies should investigate the dissemination of integrons in other regions of Iran to better estimate the prevalence and distribution of these important genetic elements in *Acinetobacter* species across the country.

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Authorship

all authors meet the ICMJE authorship criteria.

Conflicts of interest

No potential conflict of interest relevant to this article was reported.

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