Original Article

Prevalence of Integron Classes in *Acinetobacter baumannii* Isolated from Patients in Diyala

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Abstract

Background: *Acinetobacter baumannii* is a worldwide threat that causes infections in hospital settings because of its multidrug resistance to widely available antibiotics. **Objectives:** This study aimed to assess the antimicrobial susceptibility of *A. baumannii* isolates from clinical specimens in Diyala, Iraq, and to investigate the prevalence of integrons and associated gene cassettes. **Materials and Methods:** The specimens came from patients at Iraq's Diyala hospitals. Thirty distinct isolates were identified as *A. baumannii*. Both traditional procedures and the VITEK 2 system were used to identify isolates. By using the disc diffusion method, the bacterial antibiotic-resistant *A. baumannii* isolates were established. **Results:** All isolates of *A. baumannii* were resistant to (Piperacillin, Ceftazidime, Cefepime, Cefotaxime, Ceftriaxone, Ampicillin–sulbactam, Piperacillin–tazobactam, Ticarcillin–clavulanic acid, and Gentamicin) in percentage (100%), While resistance to Amikacin 96.7%, Imepenem and Meropenem 76.7% and finally to Ciprofloxacin 63.3%. Eeleven isolates (36.7%) were MDR, and nineteen isolates (63.3%) were XDR. PCR analysis proved that 19 (100%) of 19 extensively drug-resistant *A. baumannii* isolates were integrase positive, 10 (52.63%) had Class II integrons, and 16 (84.21%) of isolates harbored Class III integron. An analysis of Class I integron variable regions indicated the presence of three distinct segment dimensions, ranging in size from 750 to 3000 bp. A variety of genetic cassette records, including *AddB*, *TrpA*, *Accd*, *aadA1,* and *aadA2*, were found in the DNA of PCR-amplified Class II integrons. **Conclusion:** The acquired data strongly support the hypothesis that Class I integrons are associated with antibiotic resistance in these *A. baumannii* isolates.

Keywords: *Acinetobacter baumannii*, antibiotic resistance, integrons, PCR, XDR

Introduction

Acinetobacter baumannii is a type of opportunistic infectious agent that has the ability to cause a variety of nosocomial infections, including, among others, restricted to catheter-related infections of the bloodstream, secondary meningitis, infections of the skin and soft tissue, urinary tract infections (UTI), and pneumonia caused by ventilators.[1] *Acinetobacter baumannii* has been classified as a priority one threat to human health worldwide by the World Health Organization due to its high level of resistance to multiple antibiotics.[2] *Acinetobacter baumannii* has shown a wide variety of intrinsic with acquired resistant mechanisms, which include the drug's enzyme deactivation, changes to target sites' constructions that inhibit the attaching of antibacterial substances, and traits or tools that block a sufficient amount of the antimicrobial agent from reaching the location of target.[3] Integrons are genetic elements that can move within bacteria and are often found in plasmids, chromosomes, and transposons. They play a crucial role in the complex genetic processes that lead to the emergence of various phenotypes and bacterial adaptation.[4] There are five classes of integrons based on the amino acid sequence of their integrase, with only Classes I–III strongly associated with the dissemination and maintenance of antibiotic resistance genes (ARGs).[5,6] Class I integrons play a significant role in antibiotic resistance and commonly contain genes that confer resistance to aminoglycosides, β-lactamases, Metallo-β-lactamases, and oxacillinases.[7]

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Integrons are prevalent in bacteria, with approximately 15% of all sequenced bacterial genomes containing these elements.[8]

The basic components of an integron consist of *intI* integration genes, one of the members of the tyrosine recombinase family, the *attI* recombination site, the *PintI* integrase promoter, and the common promoter (Pc) that is typically located within *intI* and controls the expression of cassette-associated genes that lack their own promoters, effectively making integrons natural expression vectors.[9] Structurally, integrons are made up of a 5' conserved segment (5'CS), a 3' conserved segment (3'CS), and an internal variable region consisting of one or more resistance gene cassettes that are captured by the integron.[10] Gene cassettes are typically small segments of DNA, ranging from 500 to 1000 base pairs, consisting of one or rarely two genes and a short sequence called the "59 base element," which acts as a recombination site. As the genes in the cassette lack their own promoter, they rely on the promoter of the integron for expression.[11] Integrons have an important role in the spread of antibiotic resistance; thus, the purpose of this work was to investigate the prevalence of integron classes in extensively drug-resistant *A. baumannii* isolates from hospital settings in Diyala Province, Iraq.

Materials and Methods

Bacterial isolation and identification

From October 2022 to February 2023, a total of 200 clinical samples in this study were collected from various sources, such as wounds, burns, sputum, and urine from both males and females, from different wards, including intensive care wards, wounds, and burns wards, consulting clinics, and microbiology laboratories at Baquba Teaching Hospital and Al-Batoul Hospital. All *A. baumannii* isolated were diagnosed with MacConkey agar, CHROMagar culture media, and biochemical tests, and it was confirmed that it was *A. baumannii* bacteria using the Vitek 2 compact system.

Antibiotic sensitivity test

The antibiotic sensitivity test (AST) of all 30 isolates was performed following the method described by Benson.[12] Mueller-Hinton agar plates were prepared as per the manufacturer'se's the instruction. The inoculum colonies were prepared overnight, and a culture of the isolates was transferred to a 3 mL tube of normal saline. The turbidity was then adjusted to a level of 0.5 McFarland. The isolate was interpreted as either sensitive, intermediate, or resistant to particular antibiotics (Piperacillin, Ceftazidime, Cefepime, Cefotaxime, Ceftriaxone, Imipenem, Meropenem, Ampicillin-sulbactam, Piperacillin-tazobactam, Ticarcillin-clavulanic acid, Gentamicin, Amikacin, Ciprofloxacin, Levofloxacin) by comparing the inhibition zone with the standards set by CLSI.[13]

DNA extraction

Among 30 *A. baumannii* isolates, only 19 were extensively drug resistant (XDR). In this study, only 19 XDR isolates have been used for genotyping study. The genomic DNA was isolated from bacterial growth following the ZR fungal/yeast/bacterial DNA MiniPrep protocol according to manufacturers' instruction (ZYMO) from the USA.

Thermal cycling condition protocols

PCR tests were performed on only 19 XDR isolates using primers of *intI1, intI2, intI3*, and conserved segments of Class I integron (CS) (Integrated DNA Technologies). The PCRs were prepared at a final volume of 25 µL. The PCR component mix was as follows: Taq PCR pre-mix 5 µL (Taq DNA polymerase, DNTPs, reaction buffer containing MgCl2, gel-loading buffer), 1.5 µL of DNA template, 1 µL of each primer (forward and reverse; 10 pmol/µL) [Table 1], and nuclease free water 16.5 µL. One cycle of denaturation at 94°C for 5 min was used in the PCR reaction. The reaction was then followed by 35 cycles of denaturation at 94°C for 45 s for each step, annealing at 58°C for integrons (Class I), 60°C for integrons (Class II), 60°C for integrons (Class III), and 64°C for the conserved segment of Class I integrons (CS) for 30 s, elongation at 72°C for 45 s, and finally, one cycle of final extension at 72°C for 7 min. All PCR results were run on 1.5% agarose gel containing 30 µL of Red Safe nucleic acid staining solution (Intron-Korea) and then visualized under ultraviolet (UV) light.

Table 1 : Primers used for conventional PCR to detect (*intI1, intI2, intI3,* and *InCS*) genes of *Acinetobacter baumannii*

Primer	Oligo sequence (5–3)	Product size (bp)	Annealing temp	Reference
intI1	F: CCTCCCGCACGATGATC R: TCCACGCATCGTCAGGC	280	58	[10]
intI2	F: TTATTGCTGGGATTAGGC R: ACGGCTACCCTCTGTTATC	233	60	
intI3	F: AGTG F:GGTGGCGAATGAGTG R: TGTTCTTGTATCGGCAGGTG	600	60	
In5`CS* *In3`CS	F: GGC ATC CAA GCA GCA AG R: AAG CAG ACT TGA CCT GA	Variable	64	[14,15]

CS = conserved segment of Class I integrons

Sequencing of Class I integron gene cassette (CS)

Amplified gene cassettes were sent for sequencing (Macrogen Research, Seoul, Korea).

The sequences obtained were compared with those deposited in the NCBI database using the BLAST program https://blast.ncbi.nlm.nih.gov/Blast.cgi to determine the cassette arrays of integrons.

Statistical analysis

Data are represented as percentage frequency, and statistical analysis has been conducted using SPSS version 23. To compare percentages, the chi-square test was performed. The cutoff for statistical significance was $P = 0.05$.

Ethical approval

The study was conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki. It was carried out with patient verbal and analytical approval before subjects were recruited for the study. The study protocol, subject information, and consent form were reviewed and approved by Diyala University, College of Sciences, Department of Biology approved this work (research approval number 41463, dated September 6, 2022).

Result

Isolation of *A. baumannii*

The results of this study observed only 30/200 of *A. baumannii* bacterial isolates could be isolated from different clinical samples and 12/30 (40.0%) *A. baumannii* bacterial isolates were identified from wound samples. Eight (26.7%) *A. baumannii* bacterial isolates from burn swab samples, followed by 6/30 (20.0%) and 4 (13.3%) *A. baumannii* bacterial isolates were identified from sputum and urine samples, respectively [Table 2]. The results of this study also found that 34/200 had no growth of any bacteria from the aforementioned clinical samples, while 136/200 of other species of bacteria could be isolated. These differences were statistically nonsignificant (chi-square = 10.07, $P = 0.12$).

Antibiogram pattern of *Acinetobacter baumannii*

The result of antibiotic susceptibilty test revealed that all isolates of A. baumannii were resistant to Piperacillin, Ceftazidime, Cefepime, Cefotaxime, Ceftriaxone, Ampicillin–sulbactam, Piperacillin–sulbactam, Ticarcillin–clavulanic acid and Gentamicin in percentage (100%). While resistance to Amikacin 96.7%, Imepenem and Meropenem 76.7%, Levofloxacin 66.7% and finally to Ciprofloxacin 63.3%, as show in Figure 1. The percentage of Cephalosporins antibiotics resistance was 100%.

The results of carbapenems resistance (Imipenem and meropenem) are considered a drug choice of treatment was 76.7%. This study showed that *A. bumannii* isolates multiple antibiotic resistance was observed in isolates, which means they were resistant to less than one drug from three or more among the 10 antibiotic groups examined in this investigation. Multiresistant isolates were categorized as MDR, potential XDR, or possible PDR based on this standard. Eleven of 30 isolates (36.7%) were MDR, and 19 of 30 (63.3%) isolates were XDR.

Detection of integron classes

Depending on the detection of the Integrase gene for the Investigation of class I Integron (280 bp), PCR assay revealed that 19 (100%) out of 19 extensively drug-resistant *Acinetobacter baumannii* isolates were integrase positive as shown in Figure 2, which confirms the extremely high dissemination of class I integron at the hospitals in Baquba /Diyala province.

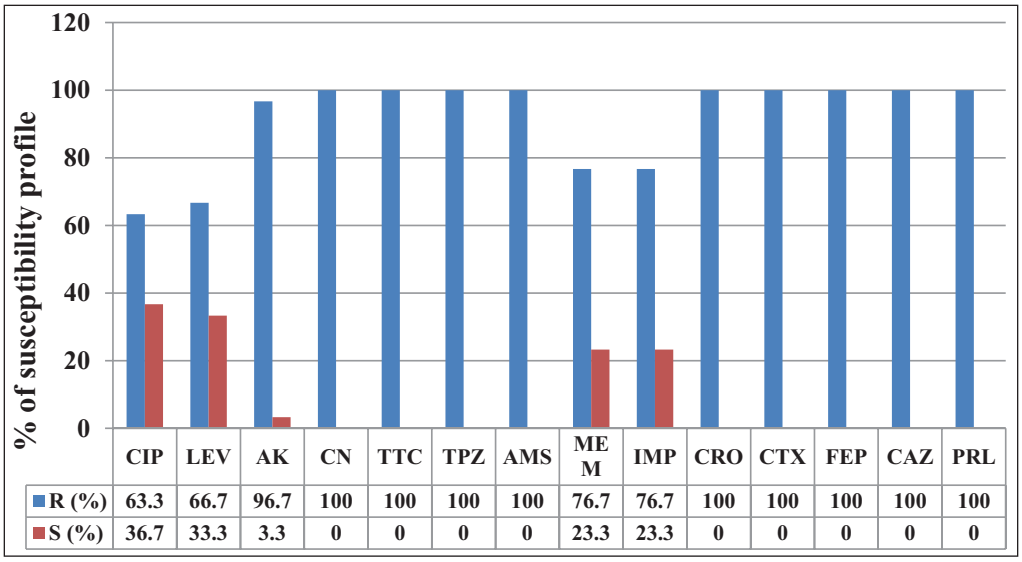
The result in this study shows that 10 (52.63%) of 19 extensively drug-resistant *Acinetobacter baumannii* have Class II integron [Figure 3].

Class 3 Integron gene was detected after amplification with PCR technique using the primer Int III with amplicon size (600 bp) was detected on agarose gel Figure 4. The result appears that 16 (84.21%) of 19 extensively drug-resistant *Acinetobacter baumannii* isolates harboring Class III integron.

The results of this study observed that all *A.bumannii* isolates (n=19) harboured integron classs1 gene , while only 10/19 (52.63%) *A.bumannii* isolates harboured both integron class1 and class2 gene, while 15/19 (78.94%) *A.bumannii* isolates harboured integron class1 and class3,

Table 2: Distribution of *A. baumannii* in the clinical samples ($n = 200$)

Clinical samples	Diagnosis of bacteria			Total	*P* Value
	No growth	Other species	*baumannii*		
Wound	5 (14.7%)	33 (24.3%)	12 (40.0%)	50 (25.0%)	Chi-square =10.07 $P = 0.12$ (N.S)
Burn	13(38.2%)	29 (21.3%)	8 (26.7%)	50 (25.0%)	
Urine	9 (26.5%)	37 (27.2%)	4 (13.3%)	50 (25.0%)	
Sputum	7 (20.6%)	37 (27.2%)	6 (20.0%)	50 (25.0%)	
Total	34 (100.0%)	136 (100.0%)	30 (100.0%)	200 (100%)	



	CIP	LEV	AK	CN	TTC	TPZ	AMS	MEM	IMP	CRO	CTX	FEP	CAZ	PRL
R (%)	63.3	66.7	96.7	100	100	100	100	76.7	76.7	100	100	100	100	100
S (%)	36.7	33.3	3.3	0	0	0	0	23.3	23.3	0	0	0	0	0

Figure 1: Antibiotic susceptibility profile of *Acinetobacter baumannii* (*n* = 30). CIP = ciprofloxacin, LVE = levofloxacin, AK = amikacin, CN = gentamicin, TTC = ticarcillin–clavulanic acid, TZP = piperacillin-tazobactam, AMS = ampicillin-sulbactam, MEM = meropenem, IPM = imipenem, CRO = ceftriaxone, CTX = cefotaxime, FEP = cefepime, CAZ = ceftazidime, PRL = piperacillin

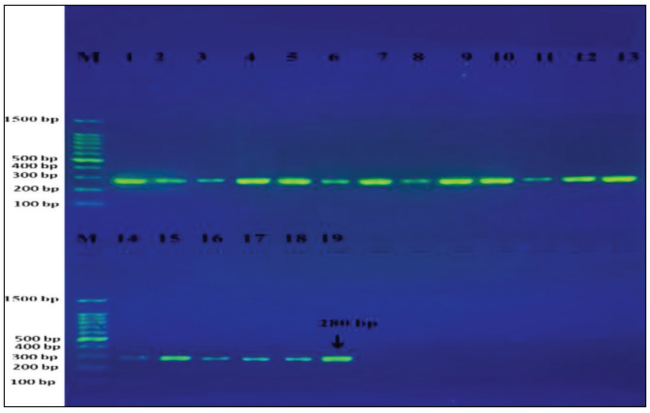


Figure 2: Agarose gel electrophoresis of amplified PCR product for the detection of integron Class I (*Int*I1) gene (280 bp) run on 1.5% agarose (90 min at 70 V), stained with Red Safe, lane 1–19 *Acinetobacter baumannii* isolates. All lanes positive for *Int*I1 gene. M = marker DNA ladder (100 bp)

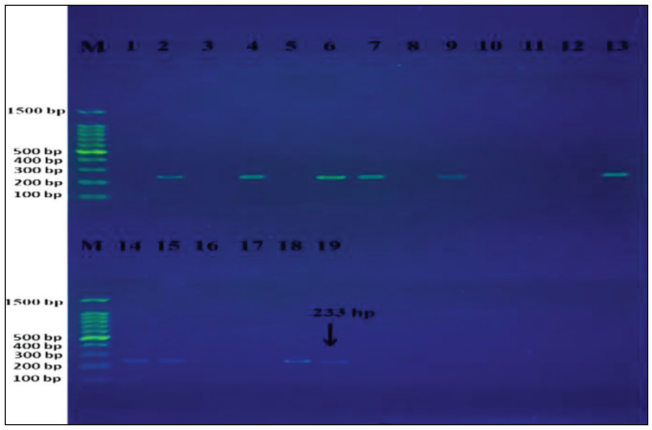


Figure 3: Agarose gel electrophoresis of amplified PCR product for the detection of integron Class II (*Int*I2) gene (233 bp) run on 1.5% agarose (90 min at 70 V), stained with Red Safe, lane 1–19 *Acinetobacter baumannii* isolates. Lanes 2, 4, 5, 6, 7, 9, 13, 14, 15, 18, and 19 positive for integron Class II gene. M = marker DNA ladder (100bp)

while 9/19 (47.36%) *A.bumannii* isolates harboured integron class1,2 and 3 gene as in Figure 5.

The conducted sequencing reactions indicated the presence of variable lengths of the amplified integrin sequences in the investigated isolates of *Acinetobacter baumannii*. The alignment of nucleic acid sequences of these isolates with the most relevant reference sequences of *Acinetobacter baumannii* revealed the presence of variable nucleic acid variants distributed in the investigated samples. Furthermore, the amplified fragments were found to exhibit different lengths despite using the same amplicons. These lengths were varied from 750 bp to 3000 bp.

The Class I integron variable areas were analyzed, and it was discovered that there were three variable segment lengths ranging from to 750, 2500, and 3000 bp [Figure 6]. Fourteen isolates included Class I integrons with sizes ranging from approximately 750 bp, three (3) isolates with sizes of approximately 2500 bp, and two (2) isolates with sizes of approximately 3000 bp.

Sequencing was used to pick two amplicons of varying lengths, evaluate them, and compare the results in Gen Bank. The findings showed that two distinct isolates with a 3000 bp genome size carried two different types of Class I integron gene cassettes, including *aadA2*, *aadB* (aminoglycoside (2') adenylyltransferase), and *Accd*, which encodes a regulator of transcription from the AcdS family involved in stress response and virulence regulation. The

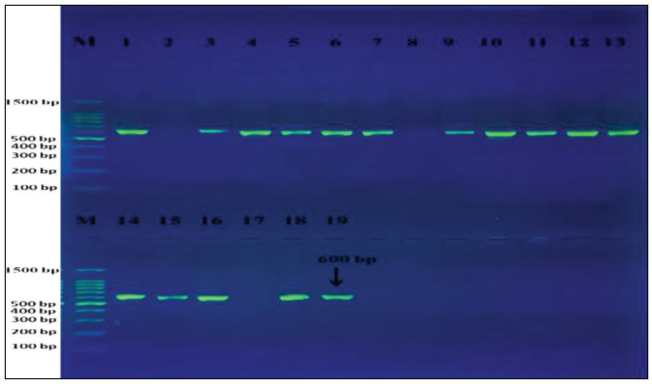


Figure 4: Agarose gel electrophoresis of amplified PCR product for the detection of integron Class III (*Int*I3) gene (600 bp) run on 1.5% agarose (90 min at 70 V), stained with Red Safe, lane 1–19 *Acinetobacter baumannii* isolates. Lanes 1, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, 15, 16, 18, and 19 positive for Integron Class III gene. M = marker DNA ladder (100 bp)

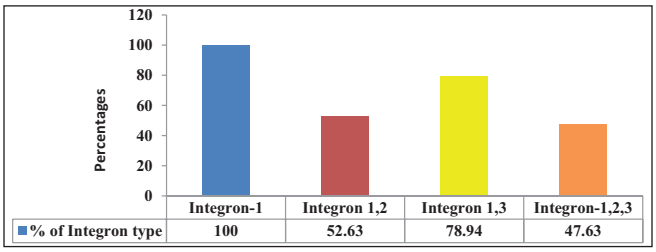


Figure 5: Prevalence of integron Class I–III genes among *A. bumannii* isolates (n = 19)

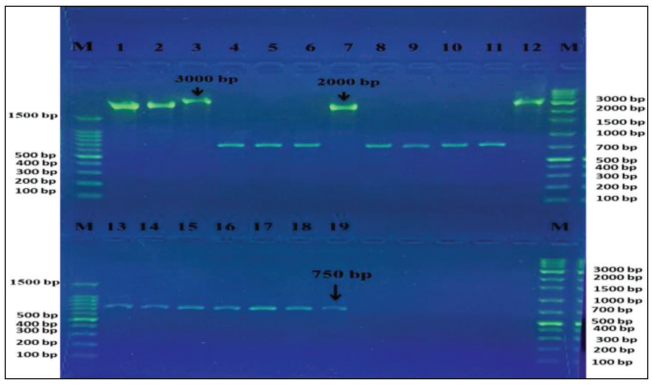


Figure 6: Class I integron variable regions using primers In5'CS and In3'CS the band size variable. The product was electrophoresis on 1.5% agarose at 7 V/cm. 1x TBE buffer for 1 h. M = DNA ladder (100) and ladder (1000 plus)

relevant data are displayed in [Table 3] below. ADditionally, our findings showed that isolates with identically sized gene cassettes nearly displayed comparable resistance to antimicrobial mechanisms.

The aminoglycoside adenylyltransferase gene, which causes resistance to aminoglycoside antibiotics like amikacin and gentamycin, is associated to the expression of the aadA2 and aadB proteins. Our findings showed that 96.7% and 100%, respectively, of the isolates were resistance to amikacin and gentamycin.

Discussion

The emergence and global expansion of *A. bumannii*-XDR isolates have caused grave concerns and may make it more challenging to select empirical medicines. The very rapid spread of *A. baumannii* resistance to antibiotics is due to the wrong and irregular use of antibiotics. Results from this study are consistent with a Iraqi study in Al-Najaf City by Al-Ghazaly and Tuwaij[16] which reported that *A. baumannii* isolates resistance 100% to Cephalosporins. The current results disagree with other local Iraqi study in Erbil hospitals,[17] which shows resistance in percentage 86% for Ceftazidime and 4% for Ceftriaxone and Cefepime. The resulting closely near Iraqi study that shows resistance against meropenem and imipenem was 78% and 75%, respectively, by Hamad and Alzubaidy,[18] but disagree with a study in Thailand that shows the highest resistance rates were observed against meropenem (100%), imipenem (100%).[19] The current results disagree with other local Iraqi study reported significantly higher rate of multidrug drug resistance-MDR *A. baumannii* (70%) in Babylon hospitals.[20] It should be stated that the epidemiologic value of XDR bacteria results from both their particular capacity to develop antibiotic resistance to each or many antibacterial drugs as well as their resistance to a number of antimicrobial medications.[10] The result agrees with an Iraqi study by Mahmood[21] whose PCR results showed that 100% of all 60 *Acinetobacter baumannii* Isolates have Class I integron. But disagree with an Iranian study that shows 70% of *Acinetobacter baumannii* isolates have Class I integron.[22] The result in this study appears that 10 (52.63%) out of 19 extensively drug-resistant *Acinetobacter baumannii* have Class II integron [Figure 4]. The result by Al-Kadmy[23] shows that 64% of *Acinetobacter baumannii* isolates harbored the integron Class II gene. But disagree with the Iranian study that appears (16.39%) of *A. baumannii isolates* carried Class II integrin.[24] The result in Korea by Jeon[25] shows that Class I integrons (*intI1*, 99.7%) showed the highest prevalence in *A. baumannii*, whereas Class II integrons (*intI2*, 0.3%) were rare. The result disagrees with an Egyptian study by Khodier *et al*[26] that shows none of Class II or Class III integrons have been found. These findings were at odds with those of an Iranian investigation, which found Classes I–III of integrons to be present in 90.2% (n = 1571), 72.4% (n = 1261), and12.1% (n = 211) of cases, respectively.[10] Additionally, our findings showed that isolates with identically sized gene cassettes nearly displayed comparable resistance to antimicrobial mechanisms. The aminoglycoside adenyltransferase gene, which causes resistance to aminoglycoside antibiotics like amikacin and gentamycin, is associated with the expression of the aadA2 and aadB

Table 3: Size of amplicons and gene cassettes associated with intI

Acinetobacter baumannii						
No. and BP	**Type of substitution**	**Location**	**Nucleotide**	**Sequence ID with compare**	**Identities**	**Gene cassette**
Ab1	Gap	1259	G	ID: AJ289190.2	95%	aadA1, aadB
2500bp	Gap	1260	C			
	Gap	1261	A			
Ab3	–	–	–	ID: CP079943.1	100%	aadB, TrpA, Accd
3000bp						
	–	–	–			
Ab6	–	–	–	ID: AJ289190.2	100%	aadB
750bp						
Ab7	Gap	1267	A	ID: AJ289190.2	91%	aadA1, aadB
2500bp	Gap	1268	G			
	Transversion	1269	(C,T)Y/G			
	Transversion	1270	(G,T)K/G			
	Transversion	1271	(C,T)Y/C			
	Transition					
Ab11	–	–	–	ID: AJ289190.2	100%	aadB
750bp						
Ab12	Transversion Transition	1224	(C,T)Y/C	ID: CP121615.1	90%	aadA1, aadA2
3000bp	Transversion	1256	A/T			
	Transition	1259	A/G			
	Gap	1259-1260	R			
	Transversion	1263	(C,G)S/C			

proteins. Our findings showed that 96.7% and 100%, respectively, of the isolates were resistant to amikacin and gentamycin. This gene cassette has been reported in other regions in Iran by Japoni *et al*,[27] Khoramrooz *et al*.,[10] and Saudi Arabia.[28] Gene cassettes in *A. baumannii* that contain genes for antibiotic resistance are linked to MDR patterns, which can cause outbreaks and treatment failure in medical facilities.[29-31] Therefore, determining the gene cassettes in clinical isolates of *A. baumannii* from each geographic region and analyzing antimicrobial susceptibility profiles highlight the significance of epidemiological research for the efficient treatment and appropriate management of MDR and pandrug-resistant pathogens. *Acinetobacter baumannii* specimens hospital outbreaks.

Conclusions

The current analysis discovered a considerable incidence of *A. baumannii* isolates carrying integrons, which may indicate the spread of multidrug resistance events in our examined medical hospitals. The numerous gene cassette arrays highlight the crucial role that geography plays in the dissemination of isolates that are multidrug-resistant (MDR). This could be connected to a variety of treatment treatments in several fields. In order to stop the spread of antibiotic resistance among *A. baumannii* strains in Diyala/Iraq, the findings emphasize the significance of ongoing surveillance monitoring.

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Conflicts of interest

There are no conflicts of interest.

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