Molecular Study of Multidrug-Resistant Integrons Associated with Acinetobacter baumannii Isolated from Clinical Samples

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Abstract: The study investigated the prevalence of class one, two, and three integrons in A. baumannii isolates obtained from patients admitted to government hospitals in Diwaniyah (General Diwaniyah, Women and Children, al Hussein, and Burns Hospital) in Al-Qadisiyah Governorate, Iraq. From September 2024 to January 2025, 150 non-duplicate samples were collected, including 21 (14%) wound swabs, 36 (24%) burn swabs, 32 (21.33%) blood samples, 34 (22.66%) urine samples, and 27 (18%) tracheal swabs. After initial identification on MacConkey agar, blood agar, and chromium agar, the samples were chemically tested and Gram-stained. The VITEK2 compact system was used to confirm the diagnosis. This study showed that 117 (78%) of the samples showed bacterial growth, of which 40 (34.18%) were confirmed as A. baumannii, distributed as follows: 3 isolates (14.28%) from wounds, 6 isolates (16.66%) from burn swabs, 13 isolates (40.62%) from blood, 6 isolates (17.64%) from urine samples, and 12 isolates (44.44%) from tracheal swabs. The data indicated that tracheal swabs had the highest percentage of A. baumannii isolation, followed by blood, urine, burns, and wounds (44.44%, 40.62%, 17.64%, 16.66%, and 14.28%, respectively). Antibiotic susceptibility testing was performed using the Vitek device for 20 antibiotics. The purity and concentration of genomic DNA were measured using a Nanodrop instrument, and a conventional PCR reaction was set up to detect integrons using specific primers provided by Promega. The products were analyzed by agarose gel electrophoresis. The highest prevalence was found for class I integrons (23 samples, 57.5%), followed by class III (9 samples, 22.5%), and class II (6 samples, 15%) out of the 40 A. baumannii-positive samples.

1 INTRODUCTION

Acinetobacter baumannii is one of the most significant challenges facing healthcare facilities and hospitals worldwide, as very few effective antibiotics are available to treat the conditions it causes. It has attained therapeutic significance due to its exceptional capacity to acquire or control resistance factors, establishing it as one of the most notable multidrug-resistant (MDR) organisms. Discontinue the existing antibiotic treatment owing to the increasing incidence of antimicrobial resistance [1].

Acinetobacter baumannii surfaced as one of the most aggressive and dangerous organisms in ESKAPE, an acronym for the group of microorganisms that includes(Enterococcus faecium, Staphylococcusaureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonasaerugnosa, and Enterobacter species) [2]. Due to its introductory or easy accession of several mechanisms of resistance to multiple antibiotics and its veritably long survival in the terrain, survival indeed in dry environmental elements is linked to its capability to form biofilms.This point, together overuse of antibiotics and rwiththe magpie, has allowed A. baumannii to live and acclimatize impeccably to healthcare surroundings, therefore representing an important source of spread of this opportunistic bacterium [3] Acinetobacter baumannii, classified as an ESKAPE pathogen, accounts for 2-10% of clinical Gram-negative infections. It induces complications in long-term hospitalized persons and those who are immunocompromised. It is often established as colonies in the esophagus and respiratory tract. It can beget sanitarium- acquired pneumonia and generally targets wettish apkins similar as mucus, bloodstream infections, and meningitis [4]. To date,thir were multi classes of integrons have been described in colorful Gram-negative bacteria, and these play a crucial part in the rearrangement and spread of resistance genes. All integrons consist of two primary components: a conserved 5' segment, which includes the Int.ase gene, and the major integration site (attI), which has a unique 3' conserved segment. The most common

introns are first class, and integrons have an important part in the emergence of multidrug resistance patterns through the development of antimicrobial resistance in Gram-negative bacteria [5]. Class I integrons comprise three open reading frames in the 3' conserved sequence(3' CS) region. Class II integrons, whose conserved 3' member contains five tns genes, are responsible for the movement of transposable rudiments, generally associated with the Tn7 transposable element family [6], Class 3 introns have also been reported, but their conserved 3' member has not been characterized.[7] This work aims to investigate the frequency of class one, two, and three integrons obtained from A. baumannii isolates [8].

2 MATERIAL AND METHODS

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2.1 Patient

A total of one hundred fifty different clinical samples were collected from visitors and hospitalized patients in governmental hospitals in Al-Qadisiyah province. (Al- Diwanyiah Teaching Hospital, Maternary and Children Teaching Hospital and Al-Diwanyiah Burns Center), during the period from septmber/ 2024 to jenuary /2025. The clinical specimens were randomly collected from patients, and checked to recognize Acinetobacter baumannii isolates in this cross-sectional study. which included 21 (14%) wound swabs, and 36 (24%) burn swabs, 32 (21.33%) blood samples, 34 (22.66%) urine samples and 27 (18%) tracheal swabs were collected for analysis. These patients were males and females of different age groups, ranging from 1 month to 80 years including 26 males and 14 females.

2.2 Isolation and Identification of Isolates

Traditional methods have been used to identify bacteria, as well as by the phenotype of the causative organism, using bacteriological methods, including culture on selective media,Gramstaining, microscopic,characteristics,colony morphology,and biochemical identification tests. A. baumannii can be difficult to identify using traditional culture media, especially In this study, Acinetobacter chromium agar was used, which is a selective medium used for the accurate and rapid detection of A. baumannii, as it grows in the form of red colonies after an incubation period of 24 hours.[9].

2.3 Primers DNA Extraction and Polymerase Chain Reaction

Using the NCBI Genbank database, PCR primers were designed in this study, as shown in Table 2.

By using (Presto™ Mini gDNA Bacteria Kit) Bacterial genomic DNA was extracted from bacterial isolates as and done according to company instructions gene was calculated by using Optimase Protocol Writer™ online application and done by PCR thermocycler. See table 1.

2.4 Antibacterial Agents Susceptibility of A. baumannii Isolates

The isolates of Acinetobacter baumannii were subordinated to antibiotics vulnerability test by using Vitek2 compact system. All isolates were examined against 20 antibiotics agents related to 8 antibiotic classes. According to Clinical and Laboratory Norms Institute (CLSI 2024).

2.5 Ethics in Study Management

The present study has been managed according to recommendations companion gained from the College of biology, University of Al- Qadisiyah. The task of collecting samples from rehabilitated cases was eased according to an sanctioned executive order (numbered as 30/4161 in 1/9/2024) issued by the College of biology, University of Al- Qadisiyah, and it was approved by the directors of visited hospitals. The study did n't include interdicted natural accoutrements or genetically modified organisms.

Table 1: Thermocycler PCR reaction condition.

NO	Integron name	PCR Amplicon (bp)	Initial denaturation Temp./time	Denaturation Temp./time	Annealing Temp./time	Extension Temp./time	Cycles	Final extension Temp./time
1	*IntI-I*	243	95°C /5 min.	95°C/30sec	52.7°C/30sec	72°C/1min.	35	72°C/5min.
2	*IntI-II*	788	95°C /5 min.	95°C/30sec	55.2°C/30sec	72°C/1min.	35	72°C/5min.
3	*IntI-III*	600	95°C /5 min.	95°C/30sec	56.7°C/30sec	72°C/1min.	35	72°C/5min.

Table 2: The Primers Sequences used in this study.

Primer	Sequence (5'-3')	Product Size	Reference orGenbank
IntI-I	F- TCTCGGGTAACATCAAGG	243	10
	R- AGGAGATCCGAAGACCTC		
IntI-II	F- CACGGATATGCGACAAAAAGG	788	11
	R TGTAGCAAACGAGTGACGAAATG		
IntI-III	F- AGTGGGTGGCGAATGAGTG	600	11
	R- TGTTCTTGTATCGGCAGGTG		

3 RESULTS

3.1 Isolation of Bacterial Growth in Clinical Specimens

An aggregate of one hundred fifty different clinical samples were collected from callers and rehabilitated cases in governmental hospitals in Al- Qadisiyah fiefdom.(Al- Diwanyiah Teaching Hospital, Maternary and Children Teaching Hospital and Al-Diwanyiah Burns Center), during the period from septmber/ 2024 to jenuary/ 2025. The clinical samples were aimlessly collected from cases, and checked to fete Acinetobacter baumannii isolates in thiscross-sectional study.

The results in the current study revealed that 147 (81.6) samples had been given positive growth while 33(18.3) samples showed no growth asappeare (Figure 1).

3.2 Identification of A. baumannii Isolates

The confirmatory diagnosis of the suspected isolates was made by using the GN ID Card of the VITEK 2 compact system, (about 99% accuracy).The results confirm that all 40 (34.18%) of the collected isolates were identified as Acinetobacter baumannii isolates . According to the definite diagnosis of A. baumannii isolates , they distributed as showed in Figure 2. The number and percentage of A. baumannii isolates recorded in Table was 40 isolates distributed as 3 isolates (14.28%) from wound ,6 isolates (16.66%) from burn swabs , 13 isolates(40.62%) from blood, 6 isolates (17.64%) from urine samples and 12 isolates (44.44%) from trachea swabs, however, the data showed the rise percentage of A. baumannii were isolated are blood samples , trachea swabs followed by urin, bourn and followed by wound (44.44%),(40.62%),(17,64%),(16.66%)and (14.28%) respectively.

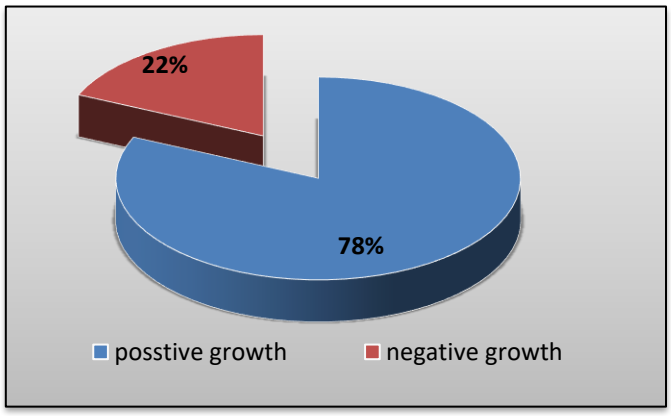

Figure 1: The percentages of clinical specimens.

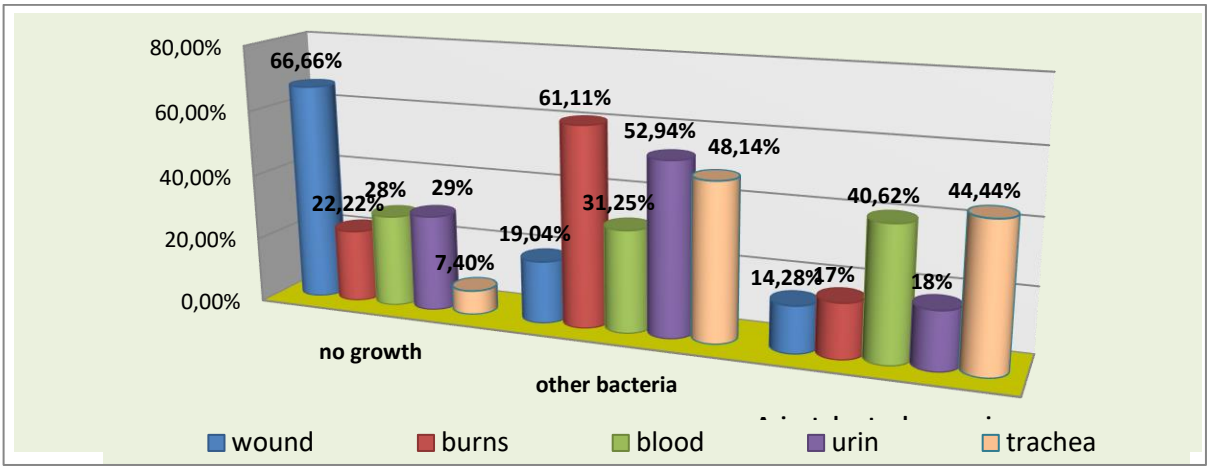

Figure 2: Distribution of bacterial growth according to source of samples.

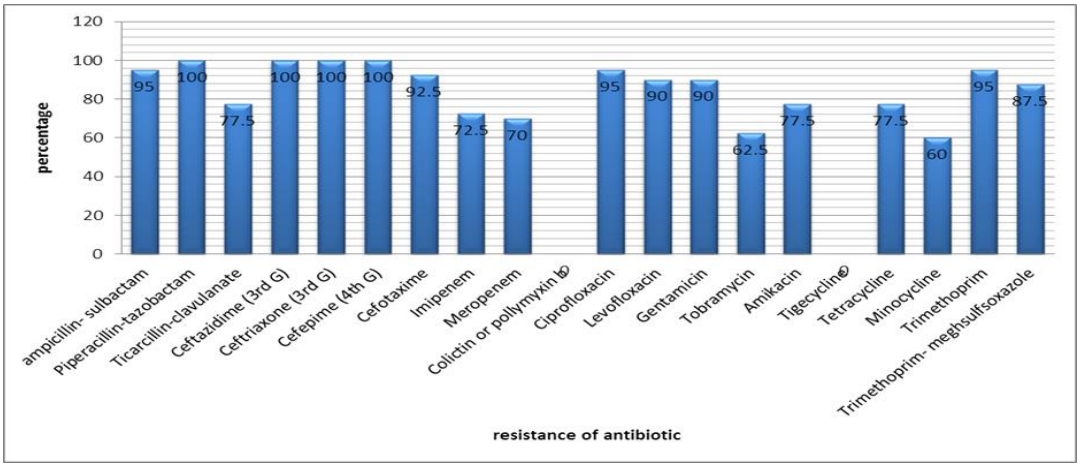

Figure 3: Antibiotics susceptibility pattern of 40 A. baumannii isolate.

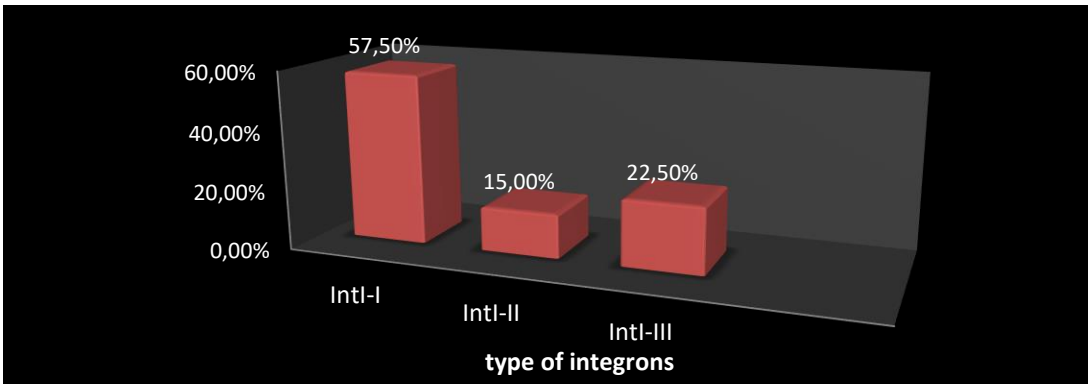

Figure 4: Distribution of integrons of 40 A. baumannii isolates.

3.3 Antibacterial Agents Susceptibility for Isolates of A. baumannii

Forty Acinetobacter baumannii isolates underwent antibiotic susceptibility testing with the Vitek2 compact system. recommendations all results were interpreted and all isolates were classification as very susceptible, intermediate, or resistant to each tested antibiotics agent show as Figure 3 . The antibiotic susceptibility test revealed the maximum resistance (100%) was recorded for Cephalosporins (3th and 4th G) and for penicillin (Piperacillin) . There was growing resistance to carbapenems, Imipenem (82.5%), and Meropenem (70%). According to present results the isolates showed a considerable resistance to Levofloxacin and Ciprofloxacin (95%). (90%). For Ampicillin/Sulbactam (95%), Piperacillin/Tazobactam (100%) and for Ticarcilline/Clavulanic Acid the resistance rate were (77.5%). The lowest resistance rates were found to found minocycline (60%) and tobramycin (62.5%). While antibiotics that are very sensitive are tigecycline (0%) and colistin or pollymyxin(0%).

4 DISCUSSION

The current study suggest that the percentage of A. baumannii isolated from the trachea and blood samples were (44.44%), (40.62) respectively. it differs from where's a study that obtained highest percentage (72.2%) of A. baumannii isolates from burn patients ,otherwise in Egypt, a study documented the prevalence of A. baumannii in patients with burns and wound infections were (3.8% and 4.8%) respectively,in this study the percentage of A. baumannii isolated from burn and wound swabs were (16.66%) and (14.28%) respectively [12]. this result in concordance with local study done in Al-Diwanyiah city who documented that A. baumannii isolated from burn and wound swabs were (7.36%) and (3.63%) respectively [13]. Moreover Previous local study had documented that A. baumannii rates isolated from burn and wound swabs were (36.5%) and (34.1%).While, A. baumannii isolation rates from burns and wound were recorded as (12.5% , 8.5%) in Baghdad Hospitalsand (2.8% and 0.6%) in Babylon [14].

A. baumannii can cause a wide variety of opportunistic infections due to its ability to infect virtually anywhere in the human body when the host's immune system is compromised, making it dangerous especially for the elderly and patient in ICUs [15]. additionally, patients with immunocompromised Due to weak immunity in some patients, they are more susceptible to the risk of developing urinary tract infections, which may be asymptomatic, as urinary tract infections can develop into symptomatic infections and eventually into bacteremia and then sepsis, which may lead to death [16].

PCR was used to detect several classes of integrons. produced by the genes encoding produced by the genes encoding Class 1 Integrons were found in 23 (57.5%) out of 40 samples, class 2 Integrons in 6 (15%) out of 40 samples, class 3 Integrons in 9(22.5%) out of 40 samples (Fig. 4). Although the emergence of multidrug resistance in A. baumannii isolates is highly complex and requires further study to understand the mechanism, it can be linked to the fact that one of the ways bacteria acquire antibiotic resistance is through resistance genes located on integrons. Transposable elements among bacteria facilitate the dissemination of antibiotic resistance,

especially in Gram-negative bacteria. Integrons were identified by the amplification of an internal segment of the intronase gene in 77.5% (31 of 40) of A. baumannii isolates, suggesting that these elements are prevalent among multiresistant isolates of this species. Among the 40 clinical isolates, Class 1 introns, recognized as the predominant category of mobile introns in multidrug-resistant Acinetobacter baumannii clinical strains, have been validated globally.

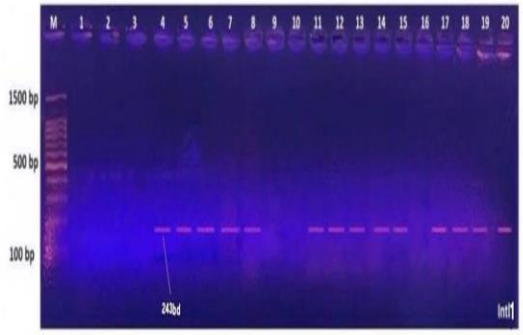

Figure 5: Distribution of class 1 integron (243 bp) among 40 A. baumannii isolates.

Figure 5 shows the frequency of the first class integrons among the isolates. The result of the polymerase chain reaction (PCR) was 243 base pairs. 23 samples appeared to carry the first integron out of 40 samples.

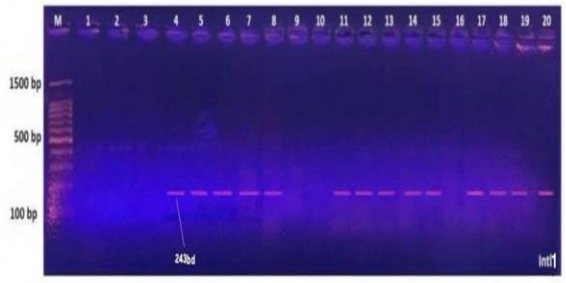

Figure 6: Distribution of class 1 integron (243 bp) among 40 A. baumannii isolates.

Figure 6 shows the frequency of the first class integrons among the isolates. The result of the polymerase chain reaction (PCR) was 243 base pairs. 23 samples appeared to carry the first integron out of 40 samples.

The current analysis revealed the presence of class 1 integrons in clinical isolates of A. baumannii, aligning with prevalence rates documented in other locations, such as Poland, where the occurrence of class 1 integrons was 63.5%, and Taiwan. The present investigation indicates a prevalence of class 1 integrons at 57.5%. Nevertheless, it significantly exceeds the percentages seen in Turkey (6.4%) and Iran (7.5%). 22 Substantial and notable percentages have also been documented in Korea (89.3%) and Egypt (85%) (18). This data indicates that class 1 integrons are the most common and extensively dispersed among multidrug-resistant Acinetobacter baumannii in the critical care units of Diwaniyah hospitals. Iraq. 2(5) samples contained both class I and class II integrins. While 5(12.5) samples contained both class I and class III integrins. The spread of integrins and their appearance in further than one class may be related to the increase in antibiotic resistance of Acinetobacter baumannii and the expansion of the range of resistance, as the maturity of samples came XDR.

A research on MDRAB in Brazil identified class 2 integrons in 23 samples, aligning with the present work that discovered 6 class 2 integrons in A. baumannii strains. Taheri-Kalani's research examined the prevalence of class 1, 2, and 3 integrons in A. baumannii isolates in Tehran, revealing that class 2 integrons were present in 14 samples.

Despite contradicting the findings of the present research, which revealed the existence of class 2 integrons in a restricted number of multidrug-resistant A. baumannii strains, this strain was recognized as the predominant type in investigations by Mirnejad (82) and Kamalbeik (67.5) (20). It also contradicts studies conducted in Korea, China, Poland, Iran, and Thailand could not identify any class 3 integrons. In the present investigation, we detected class 3 integrons with a prevalence of 22.5%. A. baumannii isolates in our present investigation conducted in Iraq [21].

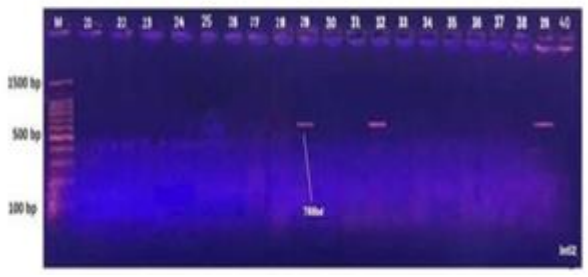

Figure 7: Distribution of class 2 integron (788 bp) among 40 A. baumannii isolates.

Figure 7 shows the frequency of the first class integrons among the isolates. The result of the polymerase chain reaction (PCR) was 788 base pairs. 6 samples appeared to carry the second integron out of 40 samples.

5 CONCLUSIONS

The studies demonstrate the presence of integrons, namely class II and I integrons harboring antibiotic resistance genes, which are instrumental in the virulence characteristics of multidrug resistance and extended drug resistance (XDR) in Acinetobacter baumannii.

A. baumannii was isolated from burns, wounds, and urine samples using conventional methods and the Vitek2 compact system. Antibiotic susceptibility and multidrug resistance tests were performed on the isolates. Most isolates were found to be extensively resistant (XDR) and resistant to most available antibiotics, with the exception of tigecycline and colistin, which are susceptible. This resistance may result from the acquisition of integrons containing antibiotic resistance genes. The majority of positive isolates were obtained from patients in intensive care units and premature infants. This is attributable to the bacteria's adaptation and capacity to endure prolonged periods on desiccated surfaces, including instruments, materials, and devices, as well as the utilization of ventilators, catheters, and incubators for premature infants. In addition, the infection may be transmitted from healthcare workers.

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