



ISSN: 0067-2904

## The Prevalence of Integron Classes Genes Among *A. Baumannii* Isolates

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Received: 19/11/2021

Accepted: 19/12/2021

Published: 30/5/2022

### Abstract

*Acinetobacter baumannii* has been recently classified as a major threat to public health because it has resistant to almost all antibiotics and there are many reasons that are responsible for conferring this feature to *A. baumannii*. One of these reasons is integrons so in this study we show the role of the integrons in providing resistance to some antibiotics. A number of 60 isolates were collected from different clinical sources of patients who were admitted to Baghdad hospitals and all isolates were diagnosed using biochemical tests and confirmed using Chrom-ager culture media, and Vitek 2 compact system. The antibiotic susceptibility test was determined during this study using Kirby-Bauer method and the results of susceptibility demonstrate that these bacteria are responsible for providing resistance to Amikacin, Trimethoprim, Piperacillin, Cefepime, Tetracycline, Ampicillin-sulbactam, Imipenem, and levofloxacin. All isolates show high resistance to trimethoprim and low resistance to tetracycline. The presence of integrons in *A. baumannii* was detected using conventional polymerase chain reactions. The results showed integron class I was found in all 60 isolates with a percentage (100%) while integron class II was found only in 7 isolates with a percentage (11.6%) and the results of detection showed integron class III are not found in the examined isolates. This study conclude that all *A. baumannii* isolates had the strongest resistance to various antibiotics, and the class I integron appeared to be the most dominant class among class II and III.

**Keywords :** Antibiotics resistance ,Gram negative bacteria ,Mobile elements

### انتشار جينات اصناف الانتكروونات بين عزلات *A. baumannii*

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#### الخلاصه

تم تصنيف بكتريا *A. baumannii* مؤخرًا على أنها تهديد كبير للصحة العامة لامتلاكها مقاومة لجميع المضادات الحيوية تقريبًا وهناك العديد من الأسباب المسؤولة عن منح هذه الميزة لـ *A. baumannii*. أحد هذه الأسباب هو الإنتكروونات ، لذا في هذه الدراسة نوضح دور الإنتكروونات في توفير مقاومة لبعض المضادات الحيوية. تم جمع عدد 60 عزلة من مصادر سريرية مختلفة لمرضى دخلوا مستشفيات مختلفة في بغداد وتم تشخيص جميع العزلات باستخدام الاختبارات البيوكيميائية والتأكد من ذلك باستخدام وسط زراعة الكروم ونظام Vitek 2 . تم تحديد اختبار الحساسية للمضادات الحيوية خلال هذه الدراسة باستخدام طريقة ( karby bauer ) وأظهرت نتائج الحساسية أن هذه البكتيريا مسؤولة عن توفير مقاومة للميكاسين وتريميثوبريم وبيبراسيلين وسيفيديم وتتراسيكلين والأمبيسلين-سولباكتام وإيميبينيم وليغوفلوكساسين. أظهرت جميع

العزلات مقاومة عالية لميثوبريم ومقاومة منخفضة للتتراسيكلين. تم الكشف عن وجود Integrons في *A. baumannii* باستخدام تفاعلات اللمرة المتسلسلة التقليدية. أظهرت النتائج أن Integron class I وجدت في جميع العزلات الستين بنسبة (100%) بينما تم العثور على عدد Integron class II في 7 عزلات فقط بنسبة (11.6%) وأظهرت نتائج الكشف أن Integron class III غير موجودة في العزلات المختبرية. خلصت هذه الدراسة إلى أن جميع عزلات *A. baumannii* كانت تتمتع بأقوى مقاومة للمضادات الحيوية المختلفة ، ويبدو أن الصنف I integron هو الأكثر انتشارًا بين الصنفين II و III .

## Introduction

*A. baumannii* is an important pathogen in hospitals[1]. It is a widely distributed opportunistic pathogen and the Infectious Disease Society of America has designated it as a dangerous pathogen. [2]. As it has developed widespread resistance to antibiotics, it become a major threat in intensive therapy units (ITUs), and the number of *A. baumannii* infections has continued to rise internationally since the beginning of antibiotics to used in the treat of infections [3-5]. These bacteria can come from many sources (such as patients or the environment ) and require very little nourishment to develop and may live in a variety of settings, including both dry and wet habitats[6]. In people with normal immunity, *A. baumannii* is rarely the source of serious illnesses, and it is also infrequently seen in healthy people [6]. *A. baumannii* is classified as the most common cause of nosocomial infection, which includes meningitis, pneumonia, urinary tract infections, and lung infections. This bacterium is also seen in wound infections(burns) and patients hospitalized in intensive care units (ICUs) [7,8]. *A. baumannii* is also very prone to antimicrobial resistances and possesses intrinsic resistance to a number of drugs [9, 10]. Mutations in target genes, changes in enzymes, outer membrane permeability, mobile genetic elements, and increased production of efflux pumps all contribute to antibiotic resistance in these bacteria[11,12]. In Gram-negative bacteria like *A. baumannii* and other bacteria, integrons are a major source of resistance genes [13, 14]. Integrons are elements of DNA that capture genes using a specific process known as (site-specific recombination) and often contain gene cassettes including resistance genes [15]. Only 6 types of integrons have been found (based on the *intI* gene), with classes 1 and 2, which have a significant role in the transfer of antibiotic resistance genes [16]. In *A. baumannii*, class 1 integrons have a crucial role in resistance by expressing genes for lactamases, aminoglycoside, Metallolactamases, and oxacillinase [17]. Resistance to streptomycin, aminoglycosides, trimethoprim, and chloramphenicol is linked to integrons (Class II), which are carried out using gene cassettes inside the transposon Tn7 [18]. So ,this study aimed to detect the prevalence of integrons classes among clinical isolates of *A. baumannii* bacteria.

## Methods

### Bacterial isolation and identification

Totally, 60 bacterial isolates were collected from different sources of patients who were admitted to different hospitals in Baghdad city. All isolated were successfully diagnosed with biochemical tests and all of them were confirmed using both Vitek 2 compact system and CHROMagar culture media.

### Antibiotic susceptibility assay

All of the successfully diagnosed isolates were subjected to the test of antibiotic susceptibility using the Kirby-Bauer method to determine it is ability in providing sensitivity/ resistance to some antibiotics (Amikacin, Trimethoprim, Ampicillin-sulbactam, Imipenem, Piperacillin, Cefepime, Levofloxacin, and Tetracycline). In which the bacterial inoculum was taken from the overnight cultured bacterial and transferred into 5 ml of normal saline and the bacterial suspension was adjusted to 0.5 McFarland by determining the optical density using a spectrophotometer, after adjustment of bacterial suspension was completed the bacterial

suspension was spread on the surface of Muller Hinton agar by using a cotton swab and the antibiotics discs were placed in the surface of the agar and incubated for 24hrs at 37°C. The results were interpreted according to the guidelines of CLSI (2020).

#### DNA extraction

The DNA was extracted in this study by using Wizard® Genomic DNA purification kit (Promega) and According to the manufacturer's instructions

#### Detection and amplification of integrons by conventional PCR

Three sets of primers were used in the current study to determine the presence of integrons (class I, II, and III) in *A.baumannii*. All primers were designed with the Primer 3 software (<https://primer3.ut.ee>) and synthesized by an alpha DNA manufacturer in (Canada) as shown in table 1. The detection of integrons was performed using a conventional PCR program. The PCR mixture was as follows: 12µl Master Mix (Go Tag) was mixed with 1µl forward and reverse primers, 3µl template DNA was added to the mixture, and the mixture was completed to a volume of 25µl by adding 6.5µl nuclease-free water. One cycle of denaturation at 95°C for 5 minutes was used in the PCR reaction. The reaction was then followed by 35 cycles of denaturation at 95°C for 30 seconds for each step, annealing at 58°C for integrons (class I), 60°C for integrons (class II), and 62°C for integrons (class III) for 30 seconds, elongation at 72°C for a minute, and finally, one cycle of final extension at 72°C for 5 minutes. PCR products were electrophoresed in 2 gram of agarose dissolved in 100ml of 1X TBE buffer.

**Table 1**-primers sequences of integrin classes I.II.III

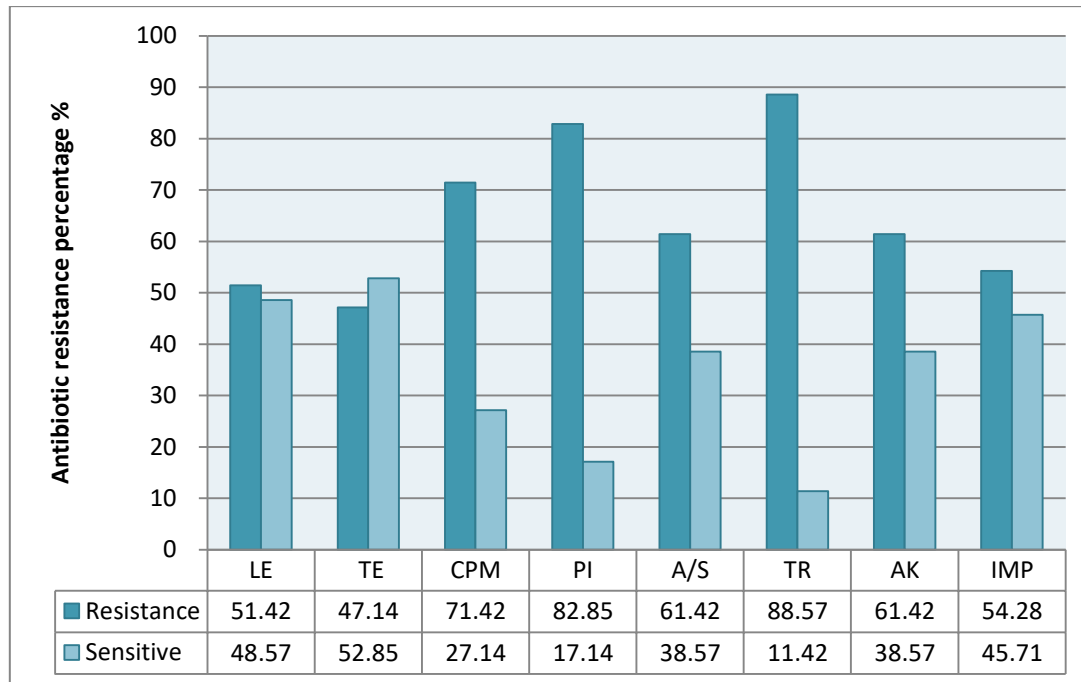
| GENE         | Primer ( 5' - 3')                                     | Size of product (bp) | References |
|--------------|---|----------------------|------------|
| <i>intI1</i> | F- ACATGTGATGGCGACGCACGA<br>R-ATTTCTGTCCTGGCTGGCGA    | 569 bp               | 19         |
| <i>intI2</i> | F-CACGGATATGCGACAAAAAGGT<br>R-GTAGCAAACGAGTGACGAAATG- | 789 bp               | 20         |
| <i>intI3</i> | F-GCCTCCGGCAGCGACTTTTCAG<br>R -ACGGATCTGCCAAACCTGACT  | 980 bp               | 20         |

#### Results and discussion

About 60 bacterial isolates were collected from different clinical sources (burns, wounds, and medical devices) from patients who visited different hospitals in Baghdad .

#### Antibiotics susceptibility test

The results of antibiotics susceptibility test using the Kirby-Bauer method demonstrated that *A.baumannii* isolates were resistant to Levofloxacin, Trimethoprim, and Imipenem with a percentage (51.42%, 88.57%, and 54.28%) respectively while it is resistant to Ampicillin, Cefepime, Amikacin, and Tetracycline in a percentage (61.42%, 71.42%, 61.42%, and 47.14 %) respectively, and finally it is resistance to piperacillin was (82.85%) as shown in the Figure 1. The fast development of *A. baumannii* to become broadly multidrug-resistant is regarded to be a major critical problem that may be explained by several ideas; one of them is the incorrect usage of antibiotics, which makes these bacteria the focus of study.



**Figure 1**-Results of antibiotic susceptibility tests of *Abaumannii* isolates .LE(levofloxacin ),TE(tetracycline),CPM(Cefepime),PI(piperacillin),A/S(Ampicillin),TR(Trimethoprim),AK(A mikacin),IMP(Imipenem)

### The results of integrons detection

The results of integron classes genes detection using conventional PCR program showed that integron class I was found in all 60 isolates with a percentage (100%) while integron class II was found only in 7 isolates with a percentage (11.6%) and the results of detection showed integron class III are not found in the examined isolates. As shown in the following Figure 2.

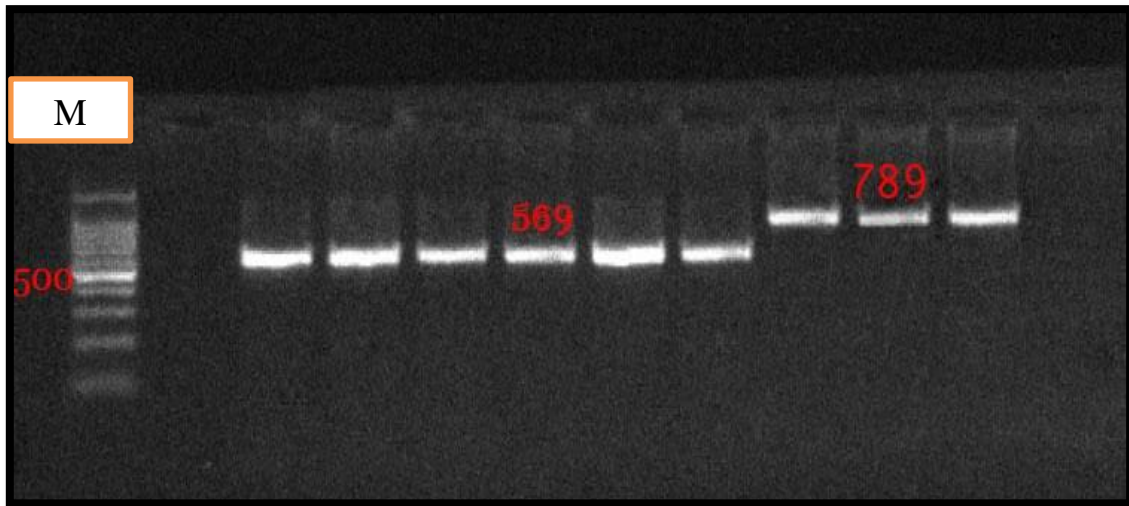
The current study leads to a better understanding of the distribution of integrons (class 1, 2, and class 3) in *A. baumannii* isolates.

*A. baumannii* is participate in causing nosocomial infection due to its resistance to a variety of antibiotic classes [16]. Antibiotic resistance is developed in a number of methods, and the transfer of antibiotic resistance genes via integrons is represent a major problem in the illnesses treatment caused by this bacteria [4,17].

The high prevalence of integron (class I) in *A. baumannii* isolates can be attributed to several factors, including inappropriate antibiotic use for *A. baumannii* infections, which leads to high expression of integrons (class I), and, secondly, the ability of integrons' to obtain new gene cassettes, which leads to antibiotic resistance spreading among clinical isolates.

Furthermore, integrons Class 1 are more common than the other classes of integrons, presumably because they are found on genetic elements such as transposons and conjugative plasmids [21].

Finally, failing to develop a nationwide surveillance program in medical facilities as part of the Global Strategy for containment of Antimicrobial Resistance results in MDR survival (2).



**Figure 2.1-** integrone class I and integron class II. In which the first 5 bands represent the integron class I with product size 569bp while the last three bands represent the integron class II with product size 789bp.

### Conclusions

The *A. baumannii* isolates had the strongest resistance to various antibiotics, and the class 1 integron appeared to be broadly spread among clinical isolates, according to this study. Integron and *A. baumannii*'s widespread dissemination may represent a severe danger to the development of future effective antimicrobial treatments. This is the first comprehensive investigation of integron class distribution in *A. baumannii* in Iraq.

### Ethical clearance

This research was ethically approved by the Research Ethical Committees of the Ministry of Environmental and Health and the Ministry of Higher Education and Scientific Research, Iraq.

### Conflict of interest:

The authors declare that they have no conflict of interest.

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