Republic of Iraq Ministry of Higher Education & Scientific Research AL-Muthanna University College of Science Department of Biology



Genetic variations of some mobile genetic elements and antibiotics susceptibility genes among *Pseudomonas aeruginosa* isolated from burn and wound units in Al muthanna province

A Thesis Submitted to the Council of collage of Science /Al Muthanna University as Partial Fulfillment of the Requirements for the Degree of Master of Science in Biology

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B.Sc. Biology/ 2011

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بِسْ اللهوالر مرزار الرجيب

يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ ⁵وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ (١١)

صَدَقَ اللَّهُ الْعَلِيُ الْعَظِيمُ

سورة المجادلة الآية ١١

Supervisor's Certification

I certify that this thesis entitled "Genetic variations of some mobile genetic elements and antibiotics susceptibility genes among *Pseudomonas aeruginosa* isolated from burn and wound units in Al muthanna Province" Was prepared by "Marwa Mezher kani" under my supervision at the Department of Biology the College of Science Al Muthanna University, as a part of the Requirements of the Master Degree of Science in Biology.

Signature

Assist. Prof. Dr. Yasir Adil Jabbar

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Date: / / 2022

Recommendations of the Head of the Biology Department

In view of the available recommendations, I forward this thesis for debate by the examining committee.

Signature

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Date: / / 20

Committees' Certification

We are certified as an examining committee, that we have read the thesis entitled "Genetic variations of some mobile genetic elements and antibiotics susceptibility genes among *Pseudomonas aeruginosa* isolated from burn and wound units in Al muthanna Province" and examined the student "Marwa Mezher Kani", in 19/4/2023 and found that the thesis meets the standards for the degree of Master of Science in Biology.

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Dedication

To the great creator ... Allah

To whom Allah has sent as a good tidings, a warner and a mercy to the worlds...

To his successor and progeny who followed the path of the manifest truth...

To their leader is the remnant of Allah on His earth, and the hopeful for the revival of this religion Al-Imam Al-Qa'im Al-Mahdi...

To those who have given their lives to our Iraq...

To the fountain of love and the symbol of sacrifice, my dear mother...

To the hope for which I live my dear father

To my soul my partner and support .. My Husband Ammar

To my little angels .. My lovey children Ruqayyah.. Ali.. Mahdi

To everyone who rejoices at my success and progress.. my dear sisters and my brothers

I dedicate my humble effort...

Marwa-2022

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Abstract

Pseudomonas aeruginosa is one of the most significant pathogens that causes nosocomial infections and has high levels of drug resistance. The rising rate of antibiotic resistance among clinical P. aeruginosa isolates is one major factor contributing to treatment failure. Therefore, identification of the underlying resistance mechanisms is essential for more effective management of this issue. This study aims to identify the integron system in the genomic DNA and plasmid of P. aeruginosa bacteria. Integrons are introduced as the most common mechanism for resistance gene dissemination in *P. aeruginosa*, in addition the purpose of the present study was to determine the diversity of the gene cassettes carried with class 1 integrons in clinical isolates of *P. aeruginosa*. In addition, in this work, the molecular characteristics of the integron class 1 gene and 16S rRNA gene in the bacterial isolates under study were studied and compared with the same gene in the bacterial isolates found in the international Gene Bank. To achieve this purpose, one hundred patients' samples from burn and wound swabs of both sexes (male and female) were collected for the period from November 2021 to March 2022 from Al-Muthanna hospitals in Iraq. The local (Iraq) strains of P. aeruginosa were isolated using conventional microbiological methods and genetic detection by 16S rRNA gene. Fifty-five bacterial isolates were obtain gave positive growth, in percentage 27/55 (49.09%) and 28/55 (50.9%) from wounds and burn samples, respectively. The genome of all of these isolates were successfully amplified and produced a single band for the 16S rRNA locus with a molecular weight of about 1500bp, which was used to confirm, at the molecular level, that all these isolates were indeed P. *aeruginosa*. The sequence results were then utilized to build a phylogenetic tree. All Iraq P. aeruginosa strains have shown a similarity rate of (90%-96%) with international isolates recorded in GenBank. Fifty-fife clinical isolates of *P*. *aeruginosa* were investigated for antimicrobial susceptibility, presence of class 1, 2 and 3 integrons and associated resistance gene cassettes in Genomic DNA and

Abstract

plasmid DNA. Bacterial isolates showed resistance in different levels toward all tested antibiotic, imipenem-EDTA was the most effective antibiotic in this study. The genomic DNA and plasmid DNA of all of these isolates were successfully amplified and produced a single band for the *intII* gene locus with a molecular weight of about 160 base pairs, which was used to confirm, at the molecular level, 12 isolates (21.81%) were contained class 1 integrons which all of them carried gene cassettes. while *intI2* and *intI3* genes were not found in any isolate in our study. There was a strong correlation between integron presence and resistance to several drugs. Amplification of the internal variable regions of class 1 integrons revealed 9 different arrays of gene cassettes ranging in different sizes (900bp, 1000bp, 2000bp, 3000bp). The amplicons were sequenced using the using ABI3730XL program, nucleotide sequences were compared to the GenBank databases and categorized by the phylogenetic tree analysis. According to the results of the phylogenetic tree, the Iraq isolates of *P. aeruginosa* (3, 31, 46 and 55) were very similarity rate of (95.10-95.35-95.02-99.44%) to the *P. aeruginosa* from national strains and shared the same sequence of *intI1* gene. DNA and plasmid sequencing analysis of class 1 integrons revealed the presence of several gene cassettes associated with resistance to aadB), aminoglycosides (aacA4 and β-lactams (blaOXA and blaIMP), chloramphenicol (*cmlA* and *catB*), trimethoprim (*dfrA*), Quaternary ammonium compounds and sulfonamides and an open reading frame (ORF) with unknown function (a protein or a functional noncoding RNA). The most prevalent gene cassette found within class 1 integrons was aacA4 (75%), which is the most prevalent gene discovered in this study, followed by blaOXA-10 (41.66%) and aadB (41.66%). The aacA4 and aadB genes which confirms the resistance to aminoglycoside antibiotics and *blaOXA-10* which confirms the resistance to beta lactam antibiotics in this bacteria. Most of gene cassette arrays in class 1 integron in this study are reported for the first time in Al Muthanna province hospitals, Iraq.