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GENETIC VARIATION OF *ACINETOBACTER BAUMANII* ISOLATED FROM DIFFERENT SOURCES

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Abstract

Samples were collected from Kirkuk governorate during the period between December 2019 to March 2020. 8 bacterial isolates were collected and diagnosed from clinical infections (wounds, burns, urinary tract infection, Sputum) and environmental sources (operating room bed, split in operating room, wall in a preterm room). RAPD reactions were carried out using the Accupower PCR pre mix kit supplied by the Korean company Bionneer, according to the instructions attached to it, and each tube contains the basic components of the polymerase chain reaction, which include one unit of dNTPs, Tris-HCL 10Mm PH= 9, MgCL2 5.1, 30Mmkcl, and Taq DNA Polymerase enzyme, and samples were prepared for migration by PCR. Random primers whose sequences range from 5 to 3 were used, as they included the primer OPA-01 with the CAGGCCCTTC nucleotide sequence and the OPA-06 primer with the GGTCCCCTGAC nucleotide sequence. The initiator OPA-01 showed the location of the bands, and they were all different, their molecular sizes ranged between (50-2000bp), the number of bands reached (53) bands, and ranged between (4-8) bands for the sample in the results of this primer. Sample (3) was characterized by a unique band with a molecular size (50bp). Sample (6) was characterized by the appearance of a unique band of molecular size (200bp). Sample (5) was characterized by the presence of an absent band of molecular size (2000bp). Sample 1) was characterized by the presence of an absent band of molecular size (1000bp). The sample (4) was characterized by the presence of an absent packet with a molecular size of (900bp) and the contrast ratio of this primer was 100%, and its efficiency was 7.11 and its discriminatory ability 3. The results of the OPA-06 primer showed the location of the packets, and they were all different, their molecular sizes ranged between (200-2000bp) amounting to a number of Packages (47) ranged between (3-7) per sample, in the results of this primer. Sample (3) was characterized by the presence of a unique bundle of molecular size (1000bp). The sample (5) was characterized by the presence of a unique bundle of molecular size, respectively (2000 bp, 400, 250, 200). The sample (6) was characterized by the presence of a unique band with a molecular size of (300bp). And sample (7) has a unique band of molecular size (225bp). Sample (2) was characterized by the presence of an absent band with a molecular size of (600bp), and the contrast ratio was 100%, and its efficiency was 3.10 and its discriminatory ability was 9.10.

Keywords: Acinetobacter baumanii, Gram-negative, beta-lactam antibiotics

Introduction

Acinetobacter baumannii is Gram-negative, and it causes the spores to be motile, as well as oxidase-negative and catalase-positive [1]. *Acinetobacter baumannii* is one of the species that

causes many pathological conditions, it constitutes about 80% of human disease cases in hospitals, as well as being a pathogen and the phenomenon of opportunistic pathogens [2]. The danger and increase in the spread of this bacteria is related to its tolerance of drought conditions and the speed of its spread in the external environment [3]. As it causes many diseases that affect humans, such as meningitis and skin infection, as well as infecting the respiratory tract and respiratory tracts, Respiratory tract Infection, Wound Infection and Pneumonia [4]. The cause of the pathogenicity of these bacteria is due to the virulence factors possessed by these bacteria such as (necrotizing factor, cytotoxic, curli fiber, siderophores, Capsular, Polysaccharides, Gelatinase, Lipase) [5]. These bacteria are also characterized by high resistance to beta-lactam antibiotics such as broad-spectrum penicillins, ampicillin, cephalosporins, fluoroquinolones and carbapenems [6].

Random Amplified Polymorphic DNA (RAPD) is an adaptation of the PCR technique that relies on the rationale that in the presence of a low amount of nucleic material, it is a cost-effective technique for taxonomic studies, potentially finding a rudimentary synthetic oligonucleotide a number of sequences in a DNA template. which can combine with it when these sites are close to each other and lie in opposite directions, and the DNA sequences between sites will be amplified to produce DNA pieces characteristic of that genome to be amplified. Multiple domains of different sizes that are produced from the same genomic DNA constitute an "indirect fingerprint" of this genome [7]. The process of conservation and continuous use of genetic resources is the essential step to reduce the process of genetic drift, as the presence of descriptive data based on accurate scientific criteria to characterize these sources include physiological, molecular and morphological studies, as they contribute effectively to the assessment of their genetic diversity in breeding programs. The rapid progress in molecular biology has created many means and methods that have been used in studies and evaluation of the variances and relationships between genotypes. There are many modern techniques based on the study and analysis of genetic material, among these techniques Random Amplified Polymorphic DNA (RAPD) [8].

Materials and methods

Samples were collected from Kirkuk governorate during the period from December 2019 to March 2020, 8 bacteria isolates were collected and diagnosed from clinical infections and environmental sources, as shown: **Clinical Isolates**: Five samples were collected from bacterial infections of patients hospitalized in Kirkuk hospitals and from various infections (wounds, burns, urinary tract infection, Csf, sputum) by using a sterile cotton swab, after which they were cultivated directly or within a period not exceeding two hours on special culture media. bacteria for diagnostic purposes. **Environmental Isolates:** Three environmental areas were collected from Kirkuk General Hospital, where samples were collected (operating theater bed, split in operating room, wall in neonatal room) by placing them in the Specimen Container. After that, drops of isolates were spread on the dishes containing their culture media. Incubated at C 37 for 24 hours, then each colony was transferred separately on another plate to grow on its own to obtain pure bacteria.

Samples Culture

The collected isolates were cultured on MacConkeys agar blood agar Nutrient agar culture media. Then the plates containing the bacterial isolates were incubated at 37 C for 24 hours, then the growing isolates were transferred to different media to complete the diagnosis process.

Cultural Characteristics

The characteristics of the developing colonies were observed on Nutrient agar media and Blood agar media. The medium of MacConkys agar in terms of size, shape, edge, color, and whether it is fermented or not fermented with lactose.

Preservation of Bacterial Isolates

Short term maintenance: This preservation was carried out by culturing Acin etobacter baumani isolates on Nutrient broth medium, by culture method on the tube containing the medium, then the tubes were incubated at 37 hours for 24 hours, then kept at a temperature of 4 C, taking into account the renewal of the isolates monthly.

Long term maintenance: This preservation was carried out through the use of nutrient Broth Heart Infusion Broth with glycerol at a rate of 15%, after which it was placed in an osmosis for the purpose of sterilization. An hour later kept at -20C in freezing.

Preparation of RAPD reactions

RAPD reactions were carried out using the Accupower PCR pre mix kit supplied by the Korean company Bionneer, according to the instructions accompanying it. PH=9) , MgCL2 5.1 , 30Mmkcl. Enzyme Taq DNA Polymerase.

Table (1): Names and Sequences of Random Prefixes Used

NO	Primer code	nucleotide sequence 5 to 3
1	OPA-01	**CAGGCCCTTC**
2	OPA-06	**GGTCCCTGAC**

Statistical analysis

Estimation of competency and discriminatory capacity of RAPD prefixes: The efficiency capacity of each initiator can be found using the equation (Grudman et al., 1995). Efficiency (number of packets per initiator / total number of multiply packets per prefix) x 100.

As for the discriminating ability, it was found through the following equation Discriminant power (number of divergent packets per initiator / total number of variant packets of all inequalities) x 100.

Determination of the genotypic dimension of samples of the bacteria *Acinetobacter baumannii*

As the differences in (DNA) genetic material, which can be obtained from the application of indicators of RAPD, where it can be adopted to determine the genetic dimension between the genotypes (study bacteria samples) that will be obtained by converting the results we obtain that appear in the gel into tables Characterization by placing (1) when the package is present and (0)

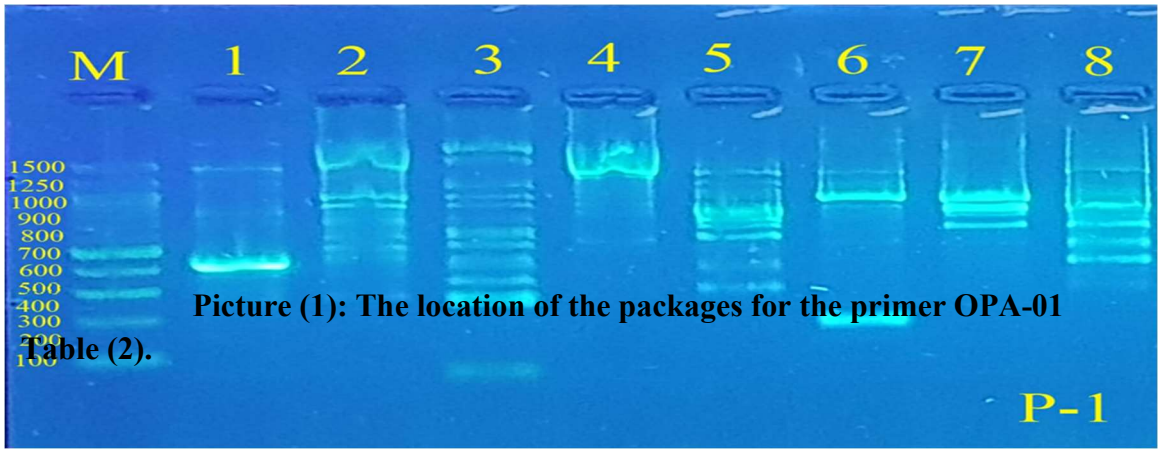
when the package is absent in order to find the genetic relationship between (the studied bacteria samples). Using the program (NTSYS-pc, ROHLF, 1993), and where the conclusion of the results of this program is based on the equation [9]. in order to detect genetic similarity by creating a sequential table that includes all the results of the prefixes.

Results:

Primer results for bacteria *Acinetobacter baumanii*

1- Primer OPA-01:

The initiator OPA-01 showed (13) sites for the bands, and they were all different, their molecular sizes ranged between (50-2000bp), the number of bands reached (53) bands, and ranged between (4-8) bands for the sample in the results of this initiator. Sample (3) was characterized by a unique band with a molecular size (50bp). Sample (6) was characterized by the appearance of a unique band of molecular size (200bp). Sample (5) was characterized by the presence of an absent band of molecular size (2000bp). Sample 1) was characterized by the presence of an absent band of molecular size (1000bp). The sample (4) was characterized by the presence of an absent band with a molecular size of (900bp) and the contrast ratio of this initiator was 100%, and its efficiency was 7.11 and its discriminatory ability was 3.12 as shown in figure (1) and a table (2).



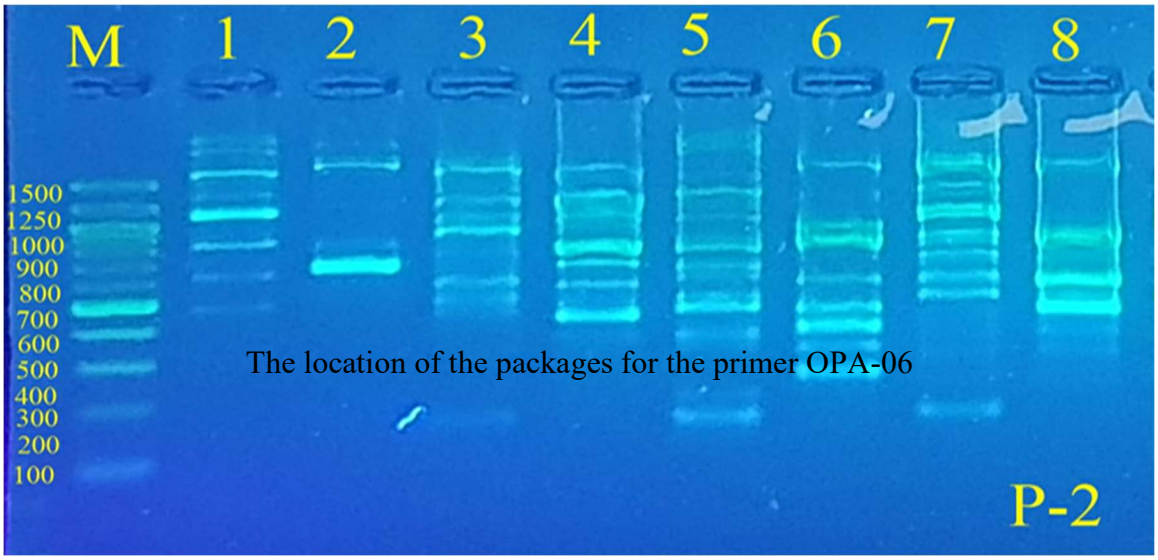
Picture (1): The location of the packages for the primer OPA-01

Table (2).

2- Primer OPA-06

Name the primer	molecular size	1	2	3	4	5	6	7	8
OPA-01	2000bp	1	1	1	1	0	1	1	1
	1500bp	1	1	0	1	1	0	0	1
	1250bp	1	0	1	0	1	0	0	0
	1000bp	0	1	1	1	1	1	1	1
	900bp	1	1	1	0	1	1	1	1
	800bp	1	0	0	0	1	1	0	0
	700bp	0	1	0	0	1	0	1	1
	600bp	0	1	0	1	0	0	1	1
	500bp	1	1	1	0	0	0	0	0
	400bp	1	0	1	0	1	1	0	0
	300bp	1	1	0	0	1	0	0	1
	200bp	0	0	0	0	0	1	0	0
	50bp	0	0	1	0	0	0	0	0

The results of the initiator OPA-06 showed (15) sites for the bundles, and they were all different, their molecular sizes ranged between (200-2000bp) and the number of bundles reached (47) ranged between (3-7) for one sample, in the results of this initiator. Sample (3) was characterized by the presence of a unique bundle of molecular size (1000bp). The sample (5) was characterized by the presence of a unique bundle of molecular size, respectively (2000 bp, 400, 250, 200). The sample (6) was characterized by the presence of a unique band with a molecular size of (300bp). And sample (7) has a unique band of molecular size (225bp). The sample (2) was characterized by the presence of an absent band with a molecular size of (600bp), and the contrast ratio was 100%, and its efficiency was 3.10 and its discriminatory ability was 9.10, as shown in the picture (2) and table (3).



The location of the packages for the primer OPA-06

Table (2).

Name the primer	Molecular size	1	2	3	4	5	6	7	8
OPA-06	2000bp	0	0	0	0	1	0	0	0
	1800bp	0	1	0	0	1	0	1	0
	1500bp	1	0	1	1	1	0	1	0
	1300bp	1	0	1	1	1	1	1	1
	1250bp	0	1	1	1	1	0	1	0
	1000bp	0	0	1	0	0	0	0	0
	800bp	0	0	0	1	1	1	1	1
	700bp	0	1	1	1	0	0	0	0
	600bp	1	0	1	1	1	1	1	1
	500bp	1	0	1	0	1	1	0	1
	400bp	0	0	0	0	1	0	0	0
	300bp	0	0	0	0	0	1	0	0
	250bp	0	0	0	0	1	0	0	0
	225bp	0	0	0	0	0	0	1	0
	200bp	0	0	0	0	1	0	0	0

Table 3: Genetic Dimensions Values for the Eight Studied Bacterial Isolates Using (2) RAPD Primers:

0.000							
0.509	**0.000**						
0.749	**0.522**	**0.000**					
0.682	**0.405**	**0.477**	**0.000**				
0.559	**0.294**	**0.503**	**0.402**	**0.000**			
0.784	**0.392**	**0.476**	**0.382**	**0.463**	**0.000**		
0.570	**0.305**	**0.637**	**0.478**	**0.428**	**0.439**	**0.000**	
0.821	**0.510**	**0.583**	**0.402**	**0.452**	**0.428**	**0.660**	**0.000**

Discussion

The genetic distance was estimated from the results of the interactions of RAPD indicators among the eight isolates of *Acinetobacter baumannii* bacteria, using the genetic program (NTSYS_PC). Version 2.10), which depends on the presence of common packages between each of the genotypes, and in its analyzes it depends on the equation [9]. Table (3) shows the values of genetic dimensions for the eight bacterial isolates studied using (2) primers of the RAPD. The

genetic measure of the degree of genetic similarity between any two individuals, or between any two groups of individuals, or even between species or structures belonging to the same genus [10, 11]. Therefore, it is equal to zero and the congruence here occurs when there is no genetic variation between the eight bacterial isolates using a small number of primers, but when using more than one primer due to the difference in the linkage regions according to the primer sequence used, which determines the genetic proximity or distance between the eight bacterial isolates is the number of bundles The greater the number of those bundles, the lower the genetic dimension, and vice versa. These shared bundles indicate a similarity in the genetic material in that region of the genome of the studied isolates, which may represent a similarity in phenotypic traits, pigmentation, resistance to antigens, reproduction and resistance to phages, or similarity in genetic adaptation to environmental requirements appropriate for growth. The production or other of the many characteristics or the similarity may be in one of the non-coding regions that have no gene expression and is known as non-coding DNA. As for the isolates that are genetically distant from each other, they are the ones that share the least number of bands with each other; This is due to the presence of differences in the nucleotide sequences in the genome of those isolates. It is clear here the role of using different primers targeting several regions of the genome, thus showing the difference, if any, between the studied isolates according to the sequence of the initiator and the degree of difference between the genomes of the studied isolates [12]. Through the values of the genetic dimension indicated in Table (3), it was found that the values of the genetic dimension ranged between (0.294 - 0.821), where the lowest genetic dimension was between isolates 2 and 5, reaching 0.294, and this is the highest similarity between the two isolates included in the study, noting that the isolate 2 It was isolated from the air-conditioning device located in the surgical operating room, while isolate 5 was isolated from inflamed (csf) meninges, and the highest genetic dimension was 0.821 between isolates 8 and 1, noting that isolate 1 was isolated from the operating room bed and isolation 8 was isolated from urinary tract infection. (UTI) This is considered the least genetic similarity among the isolates under study, while the values of the genetic dimension for the rest of the isolates ranged between those values. The RAPD indicators were used to determine the genetic variance of the eight genotypes in this study, where the variances were found based on the analysis of the results of the RAPD by finding the genetic dimension and genetic fingerprint between samples depending on whether they appear or not, as well as the difference in the molecular sizes of those bundles that differ according to the number of The complementary sites of the initiator sequences on the genomic DNA strand as well as the difference in the distance from one site to another [13]. It was found through the results of this study that the concentration of template DNA visually affects the results, especially in the density of the band appearing on the agarose gel. It is a discriminatory characteristic that can be used in the diagnosis [14]. The arrangement of the genotypes and depending on the values of the genetic dimension results in a form called the genetic kinship tree or the dendogram, and it depends on the genetic range in which the main groups are related. group [15, 16]. This is called cluster analysis.

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العلاقة الوراثية لعزلات الراكدة البومانية المعزولة من مصادر مختلفة

ماهر رشيد حميد العزاوي زيدان مخلف السكماني سهى ماهر عبد

الخلاصة

تم جمع العينات من محافظة كركوك خلال المدة ما بين شهر كانون الاول 2019 الى شهر اذار 2020 تم جمع وتشخيص 8 عزلات بكتيرية من اصابات سريرية (جروح, حروق, التهاب مسالك بولية, Sputum) ومصادر بيئية (سرير صالة عمليات, سبلت في صالة عمليات, حائط في غرفة خدج). اجريت تفاعلات تقنية التضاعف العشوائي متعدد الاشكال RAPD باستعمال العدة Accupower PCR pre mix المجهزة من شركة Bionneer الكورية, وفقا للتعليمات المرفقة معها, وتحتوي كل انبوبة على المكونات الاساسية لتفاعل البلمرة المتسلسل, والتي تشمل وحدة واحدة من مزيج القواعد النتروجينية ,dNTPs (Tris-HCL 10Mm PH=9), MgCL2 5.1, 30Mmkcl, وانزيم Taq DNA Polymerase, وتمت عملية تحضير العينات لغرض الترحيل بواسطة جهاز PCR. وتم استعمال البادئات العشوائية والتي يكون تسلسل النيوكلوتايد فيها من 5 الى 3, اذ شملت البادئ OPA-01 ذو تسلسل نيوكلوتيدي CAGGCCCTTC والبادئ OPA-06 ذو تسلسل نيوكلوتيدي GGTCCCTGAC. اظهر البادئ OPA-01 موقع للحزم, وكانت جميعها متباينة تراوحت احجامها الجزيئية مابين (50-2000 bp), بلغت عدد الحزم (53) حزمة , تراوحت مابين (4-8) حزمة للعينة في نتائج هذا البادئ. تميزت العينة (3) بحزمة فريدة حجمها الجزيئي (50bp). تميزت العينة (6) بظهور حزمة فريدة حجمها الجزيئي (200bp). وتميزت العينة (5) بوجود حزمة غائبة حجمها الجزيئي (2000bp). وتميزت العينة 1)) بوجود حزمة غائبة حجمها الجزيئي (1000bp). وتميزت العينة (4) بوجود حزمة غائبة حجمها الجزيئي (900bp) وكانت نسبة التباين لهذا البادئ 100%, وكفائتة 7.11 وقدرتة التمييزية 3. وبينت نتائج البادئ OPA-06 موقع للحزم, وكانت جميعها متباينة تراوحت احجامها الجزيئية مابين (200-2000bp) بلغت عدد الحزم (47) تراوحت مابين (3-7) للعينة الواحدة , في نتائج هذا البادئ . تميزت العينة (3) بوجود حزمة فريدة حجمها الجزيئي (1000bp). وتميزت العينة (5) بوجود حزمة فريدة حجمها الجزيئي على التوالي (2002000,250,400bp,). وتميزت العينة (6)بوجود حزمة فريدة حجمها الجزيئي (300bp) . والعينة (7) بوجود حزمة فريدة حجمها الجزيئي (225bp). وتميزت العينة (2) بوجود حزمة غائبة حجمها الجزيئي (600bp), وكانت نسبة التباين 100% , وكفائتة 3.10 وقدرتة التمييزية9.10.