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Molecular characterization of *Acinetobacter baumannii* isolated from different clinical sources

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Abstract:*Acinetobacter baumannii* is one of the most important multidrug bacterial types (MDR)opportunistic pathogenic, in addition to its high ability to gain resistance to various antibiotic groups, collected (25) from various clinical sources of infections: wound, burns, sputum , blood, Urine and csf . the distribution of isolations according to the sources of injury burn samples (7)% 28 and then the urine (4)%16, blood (4)%16, wounds (3)12%, sputum (3)%12 csf (2) 8% .Antibiotic susceptibility testing varied (Tetracyclin, Pipercillin, Trimethoprim, Ceftazidime, Amikacin, Ciprofloxacin, Cefepime, Meropenem, Imipenem, Colistin) and by (100%,92%,92%, 96%,84%, 80%, 88%, 76%,56.60%) respectively. The 25 isolations of *A.baumannii* showed a differentiation of two patterns according to their resistance to antibiotics, with 18 (72%) XDR isolation being resistant to(7-10)antibodies, while 5 (20%) of MDR isolationswere resisted for (3-6) antibodies. The genetic variation in this study was a description of the molecular properties related to the relationship of 10 isolations of *A.baumannii* bacteria andusing Randomly Amplified Polymorphic with RAPD DNA and 16sRNAtechnique where genetic DNA was extracted from 10 isolations from isolated *A.baumannii* from various local clinical sources. In addition, the genetic distance and cluster analysis between the various *A.baumannii* isolations were analyzed and estimated using upgma computer software based on10 genetic patterns distributed in two groups, 9 of which were similar to molecular weights and one single isolation ranging from(2000-100 base pairs)This study demonstrated genetic differences (polymorphisms of DNA) between the10 *A.baumannii* isolations isolated from different sources. Sequencing was conducted for five isolations taken from different local clinical sources, where the isolations showed a different variation and the evolutionary tree was painted, with MW599783, MW599779 and MW599782. Close to global isolations, MW599780 and MW599781 are close but far from the insulation MW599783, MW599779 and MW599782 .

Keywords: Molecular characterization , MDR, XDR,RAPD,16sRNA

Introduction:*Acinetobacter baumnnii* is an opportunistic pathogen, which plays a vital role as a major cause of healthcare - associated infection . [(1)]

A. baumannii strains are found in different environments and have the ability to colonize many environmental outlets including soil, water, and animals, including humans .Italso has the ability to survive in low PH (3.37),dehydration and a high temperature of up to 40°C [(2)].

These bacteria *A. baumannii* cause diseases and infections including pulmonary associated with secondary meningitis respiratory system, urinary tract infections, wound infections andblood stream infections [(3)]. These bacteria have become a major cause ofconcern in conflict zones, and have gained a special reputation in desert conflicts in Iraq, making them the

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nickname "Iraq bacteria." [4].

A. baumannii is one of the most important types of multi-drug resistant bacterial(MDR) opportunistic pathogens in hospitals due in part to its high ability to gain resistance to various antibiotic groups [5] due to its diseases due to its possession of multiple virulent factors, the most important of which are: multi-diabetes Fatty (LPS)has Pili capillaries, which are important factors for sticking to living and non-living surfaces and therefore their ability to form a biosphere, as well as containing the proteins of the outer membrane(OMPs)that contribute to their formation of the membrane [6,7]

Determining the source of infection and the extent of epidemics within hospitals are important factors for reducing disease outbreaks, especially in bacteria causing hospital infections, so genetic variation is found between bacterial isolations, and one of the efficient ways to find the difference between isolations A. *baumannii* is the Random Amplified Polymorphisim DNA (RAPD)method to determine the genetic fingerprint at the strain levelIn addition, a sequential analysis of the DNA sequencing of isolates that show a difference in the phenotype is performed in order to investigate the extent of their compatibility with global isolates.

Materials and methods

Bacterial isolation :

In this study, 180 clinical samples of lying and non-lying patients were collected at Baquba Educational Hospital and City of Medicine Hospitals (Baghdad Teaching Hospital, Ghazi Hariri Specialist Surgery Hospital, Specialist Burns Hospital), including 55 samples of burns and 40 of the urine. Thirty blood, 30 wounds, 20 sputum and 5 spinal cord fluid, taken from males and females, of different ages and various local areas, from September 1, 2020 to the end of January 2021.

Each swab was carefully taken from the site of the injury and placed in tubes containing ready media(swab with media)to keep the swab wet during transfer to the laboratory, each sample was found on the center of MacConky agar and the middle of the blood dens, and all dishes were incubated at 37°C 24-48 hours to determine the bacteria *A.baumannii* stagnant according to biochemical tests and physiological tests.

diagnosis:

In this study, based on the appearanceal qualities of *A.baumannii*bacteria colonies, isolations were implanted on MacConky and blood rent. *Colonies of A.baumannii* isolation appeared on white to creamy blood trees.

Isolations based on biochemical, appearance and physiological tests included: catalase test, jacket test, blood decomposition test, 44m growth, oxidas test, indol test, Citraet test , Voges- Proskauer test , urea test, Tsi iron medium [8] . and then diagnosis of VITEK2.

Antibiotic susceptibility testing:

The sensitivity of *A.baumannii* antibiotic isolations was determined bythe Kirby-Bauermethod, where 10 different types of antibiotics were used in this test, including:

Tetracycline(10 µg),(Trimethoprim(10 µg), Ceftazidime (30 µg), Piperacillin(100 µg), Meropenem(10 µg), Amikacin(10 µg) ,Cefepime(30 µg), Ciprofloxacin (10 µg), Colistin (10µg) , Imipenem (10 µg)according to CLSI Guide 2019.

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DNA extraction :

The genetic DNA of isolated *A.baumannii* bacteria was extracted from 180 bacterial isolations in accordance with ABIOpure's DNA extraction of protocol for analysis of PCR stored at a temperature (-20).

Total genomic DNA was extracted from only ten isolates having six different sites (wounds ,blood , burns, urine, sputum, and csf) using a method described by [9]. .A single colony was inoculated on 5ml of brain heart infusion broth and incubated over night at 37ºC. Then 1.5 ml of a saturated culture was harvested with centrifugation for 5 min. at 14,000 rpm. The cell pellet was resuspended and lysed in 200µl of lysis buffer (40 mM Tris-acetate pH 7.8, 20 mM sodium-acetate, 1 mM EDTA, 1% SDS) by vigorous pipetting. To remove most proteins and cell debris, 66 µl of 5M NaCl solution was added and mixed well, and then the viscous mixture was centrifuged at 12,000 rpm for 10min. at 4ºC. After transferring the clear supernatant into a new eppendorf tube, an equal volume of chloroform was added, and the tube was gently inverted at least 50 times when a milky solution was completely formed. Following centrifugation at 14,000 rpm for 5min., the supernatant is then removed to another eppendorf tube and double volume of 100% ethanol was added. The tubes were inverted 5 to 6 times gently, then centrifuged at 10,000rpm for 5minutes. The supernatant was discarded and 1ml of ethanol (70%) was added to the pellet, and tubes centrifuged at 10,000 rpm for 5 minutes. Finally the supernatant discarded and the pellet was dried for 10 min at room temperature, The pellet was resuspended by 100µl H2O. The stock was kept at -20ºC until use. The DNA concentration has been determined by measuring absorbance of the sample at 260 nm using spectrophotometer [10].

Random Amplified Polymorphic DNA(RAPD)Fingerprinting This technique was implemented according to [11] with some modificationsAGAGTTTGATGATCCTCTCAG F, GGTTACCTTTTTTACGT R used in the , study,Amplifications were performed in 25µl consist of 10x reaction buffer with MgCl2 (Promega), 200µm dNTPs, 10 pmol of primer , 1 unit of Taq DNA polymerase and 25 ng of genomic DNA. The amplification were performed in thermocycler programmed as follow: 1 cycle 94ºC for 2 minutes, 40 cycles (92 ºC for 1 minutes; 37 ºC for 1 minutes; 72 ºC for 1 minutes) and 1 cycles 72 ºC for 7 minutes. The amplification products were resolved by electrophoresis in a 1.5% agarose gel in 1X TBE buffer at 75 volts for 1.5 hr (5 volt/cm) [12] . Gels stained with Ethidium bromide, visualized under UV light and photographed using a high resolution digital camera (12.1 mega pixel),tandard molecular weight markers also used in each electrophoresis run .[13]

RAPD Data Scoring and Analysis:

The PCR-based DNA marker RAPD was used in this study as an accessible tool for studding the polymorphism, genetic variation and fingerprinting of *A.baumannii* isolates collected from different sites of infections. RAPD results were analyzed by using all information obtained from tables and figures. This information included; the presence or absence of amplified DNA bands, total number of amplified bands across all isolates of *A.baumannii*, the number of polymorphic bands, which can be detected horizontally.The Numerical Taxonomy System

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(NTSYS) 1.8 software, using the Jaccard coefficient of similarity [14] , and Unweight pair group method arithmetic (UPGMA) averages cluster analysis were used to calculate genetic distance and obtaining phylogenetic tree [15] . Primer efficiency and discriminatory power were calculated for each primer using two equations as described by [16]

Gel preparation, sample loading and detection

The products were dissolved by electrical relay on 1.2% acaroz gel prepared from solution 1×TBE(Tris/Borate/EDATA)and mixed with 0.5micrograms/ml of ethereum bromide×.TBE. 20 microliters of each sample were mixed with 4 primary load dyes and loaded into gel and 5ulof DNA markers (gene100pb plus, lambda DNA/HINDIII)(Fermentas,ThermoScientific) and sigmaAldrichloaded into the gel pit, 80 volts for two hours as well as the placement of 7.5 volts/cm of gel that was equipped, DNA packs were filmed using 366 nm transilluminatorUV rays and photographed usingUVP GelDoc It imaging system

Results:

This study included the collection of 180 clinical samples(55 burns samples, 40samples of urine , 30 blood,30 wounds, 20 sputums and 5 spinal cord fluid). The growth on the MacConkey agar revealed a pale pink color, due to the fact that its colonies are not fermentation lactose sugar. Colonies of *A.baumannii* isolation appeared on white to creamy blood trees blood agar [17].These isolations appeared negative for the pigment of dignity, coarse cyclics and positive results for both catalase testing, sitrite testing, growth at a temperature of 44 m, and a negative result for both oxidase test, and idol test, and Fox Proscauro's test of the of kigler and iron, while the uris test showed different results [18]as shown in the table.

Table (1): Biochemical characteristics of *A. baumannii*

Biochemichal tests	Result
Lactose fermentation	-
Hemolysin test	- γ hemolysis
Oxidase production test	-
Catalase production	+
Indol test	-
Vosges-proskauretes test	-
Methyl red test	+
Urease production test	+,-
Simmon citrate test	+
Kliglar iron agar	-

Antibiotic susceptibility testing:

A.baumannii antibiotic isolation sensitivity has been determined by theKirby-Bauer method)

The results showed that all clinical *A.baumannii* isolations have a very high level of antibiotic resistance and 100%anti-Tetracyclineand this result was agreed with the findings of others Al-Sehlawi *et al*[19]: resistance to its study was95.6 % and 84% trimethoprim, where this study

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converged with the findings of Sohail and others [20], with the antibiotic resistance rate of91.2%. And 92%of the Ceftazidime where these results were agreed with the results of the researcher Khafaji [21]) in her study of this antibiotic where it reached97.5%.and 92% Piperacillin where agreed with the findings of the researcher Grochowalska *et al;*[22]The resistance rate for this antibiotic was 94% and 56% &76% for anti-Meropenem and Imipenem, where it converged with the results of the researcher (Kadom et *al;*[23] reached 75%. The resistance rate was 81.70% and 88% for the Cefepime, where our results were agreed with the results of the local study of the researcher (Al-Sehlawi et [*al;*[24] In Najaf, where the resistance rate of these antibiotics % was 100%,84% to the Ciprofloxacin where I agreed with researcher Hashim [25] found that the rate of resistance to anti-Ciprofloxacin was82.5%, and 68% for colistin % 68% converged the results of our study with the findings Tewari et *al;*[26]Isolations are highly sensitive to this antibiotic.

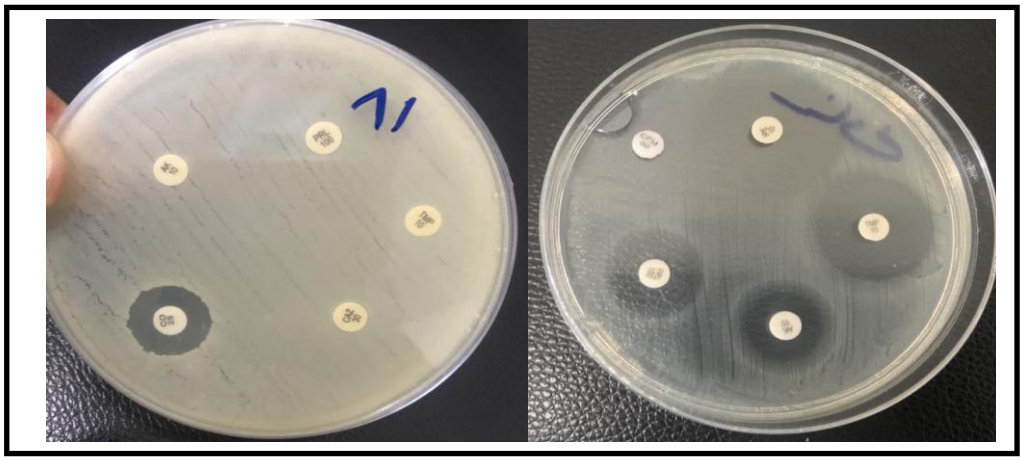

Figure (1): showingAntibiotic susceptibility testing

Table 2 :The percentages of antibiotic resistance of *A. baumannii* bacteria

Isolation resistanse		Isolation intermedia		Isolation sensitive		Antibiotic
%	NO.(25)	%	NO.(25)	%	NO.(25)	
100	25	0.0	0	0.0	0	**Tetracycline(10)**
84	21	0.8	2	0.8	2	**Trimethoprim(10)**
92	23	0.96	1	0.96	1	**Ceftazidime(30)**
92	23	0.0	0	0.0	0	**Piperacillin(100)**
76	19	0.0	0	0.24	6	**Meropenem(10)**
56	14	0.16	4	0.28	7	**Imipenem(10)**
84	21	0.0	0	0.16	4	**Amikacin(10)**
88	22	0.0	0	0.12	3	**Cefepime(30)**
88	22	0.0	0	0.0	0	**Ciprofloxacin(10)**
68	17	0.12	3	0.32	8	**Colistin(10)**

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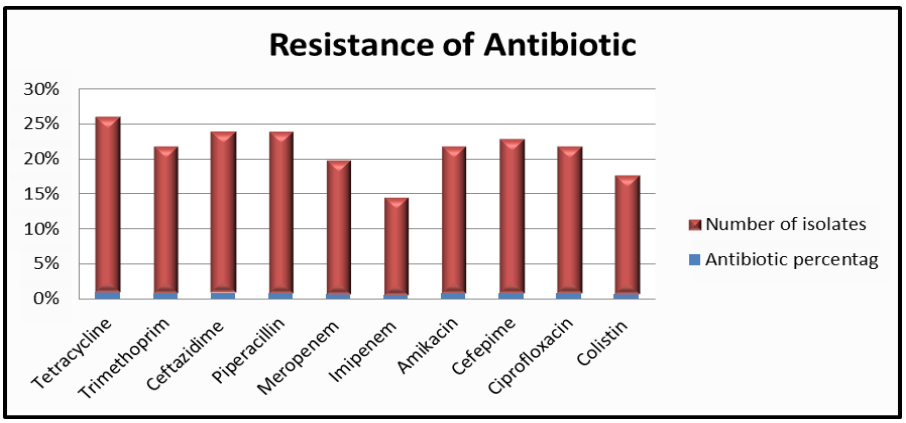

Fig 2: the percentage of antibiotic resistance of *A.baumannii* bacteria

Genetic Contrast:

The results of thegenetic contrast study were revealed using RAPD DNA and the molecular sequence of the gene 16S rRNA, where it gave interesting information about the presence of 10 distinct sequences as shown in the picture [27] , were obtainedand 10 of the 25 genetic patterns of *A.baumnnii* bacteria were obtained, and with based molecular from(2000-100 base pairs)

RAPD-PCR

Although genetic methods of characterizing bacterial isolations differed, RAPD technique was successfully used with *A.baumannii* in this study as molecular-based technique based on PCR technology in an attempt to detect genetic variation and differences and identify DNA fingerprints of 10 isolated *A.baumannii* isolates from different sources. RAPD is a less expensive and time-consuming technique than other techniques and is clear and direct and does not require prior knowledge of the sequence of nucleotids targeted object. Furthermore, RAPD-PCR is distinctive because it analyzes the entire genome. However, due to its sensitivity and reproduction potential, it can be affected by small changes in reaction mixture and temperature cycles [28] [29] .

RAPD-PCR gives different information, since then analyzing different sequences and discovering different types of differences in bacterial DNA. RAPD-PCR detects differences along the entire bacterial genome, not only in a particular sequence.

RAPD was analyzed as described in (shape3 and table3) for differences in molecular weights of multiform bands reflecting the number of targets for each pre-position position within the DNA in question [30,31] , such as a single rule changing the location of the pre-lysis in the genome that prevents amplification by inserting a mismatch into 3"end of the DNA piece [32] . Other sources may include multiple forms deleting the priming site, the insertion that makes preparation sites too far from supporting amplification, or they may change the size of the DNA piece without preventing it from being inflated. The failure of many initiators to amplify DNA may be due to their need for special requirements for amplification in terms of PCR reagents or profile temperature, as all interaction transactions were identical for alprimers.

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Moreover, differences in banding patterns are likely due to specific requirements of a given primer.[33]

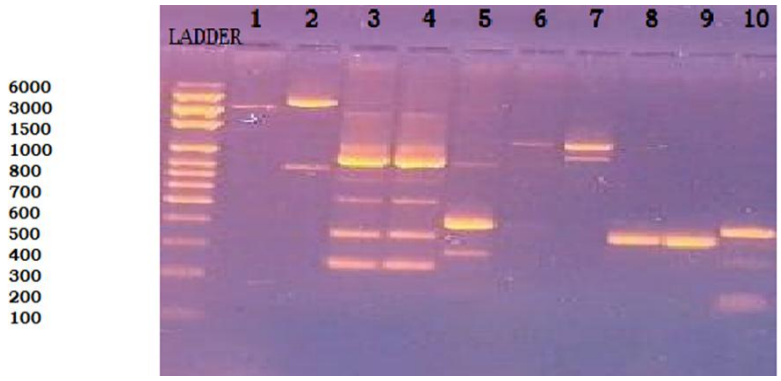

Fig 3:between the molecular detection of RAPD DNA-PCR dramer on acarose gel

RAPD-PCR was used to distinguish between strains of different species, different chapel pattern within species, and different subtypes within the serological pattern [34] .Previous studies [35.36] , in which RAPD-PCR markers help analyze diversity as well as isolated *A.baumannii* fingerprinting.

Table 3: shows the genetic distances of *A.baumannii* bacteria isolations according to RAPD marker

Similarity matrix

	1	2	3	4	5	6	7	8	9	10
1	1.00									
2	0.00	1.00								
3	0.33	0.00	1.00							
4	0.33	0.00	1.00	1.00						
5	0.00	0.00	0.25	0.25	1.00					
6	0.00	0.00	0.00	0.00	0.00	1.00				
7	0.00	0.00	0.00	0.00	0.00	0.67	1.00			
8	0.00	0.00	0.33	0.33	0.00	0.00	0.00	1.00		
9	0.00	0.00	0.33	0.33	0.00	0.00	0.00	1.00	1.00	
10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00

Genetic distance values range from 1.00 to 0.25. The lowest genetic distance found (0.25) was clearly between isolations 3 (wound). And 4 (burn), while the highest genetic distance found (1.00) in the first package of all insulation

10 out of 25 isolates were placed to test the genetic variance of bacteria divided on the basis of the number of bandas, their number of bands, and their molecular weights, as shown in Table (4)

Table 4 :Genotypes of *A.baumannii*

NO.Isolates	10	9	8	7	6	5	4	3	2	1	NO. bands
1	0	0	0	0	0	0	0	0	1	0	**2000**
1	0	0	0	0	0	0	0	0	0	1	**1700**

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2	0	0	0	0	0	0	1	1	0	0	**1500**
2	0	0	0	1	1	0	0	0	0	0	**900**
2	0	0	0	0	0	0	1	1	0	0	**800**
4	0	0	0	1	0	1	1	1	0	0	**700**
1	0	0	0	0	0	0	0	0	1	0	**750**
2	0	0	0	0	0	0	1	1	0	0	**500**
1	0	0	0	0	0	1	0	0	0	0	**400**
4	0	1	1	0	0	0	1	1	0	0	**300**
2	1	0	0	0	0	1	0	0	0	0	**350**
3	1	0	0	0	0	0	1	1	0	0	**200**
1	1	0	0	0	0	0	0	0	0	0	**100**

The 10 isolates of *A.baumannii* were classified according to the RAPD typing system according to the excel program and on the basis of the number of bundles, they were classified into two main groups (Polymorphic band, Unique band) depending on their similarity in the number of beams and molecular weights as shown in Table (5).

Table 5:Recurrence of genotypes between *A.baumannii* isolates and the number of shared bands using RAPD

Groups	**Isolates**	**NO.of shared packages**
Polymorphic band	3,4 8,9,7,2 10,5 6	6 bands 2 bands 3 bands 1bands
Unique band	1	1 bands

Where the polymorphic band that includes isolates (3,4,9,8,5,10) was similar in molecular weight, molecular weight and number of joint bands as shown in Table (3) , As for isolate 7, it includes two bands, but with a portion weight of 900 bp, 700 bp Isolate 6 contains one band with a molecular weight of 900bp, and isolate 2 contains two bands with a molecular weight of 750bp and 2000bp, and the Unique band contains one isolate 1 band with a molecular weight of 1700 bp.

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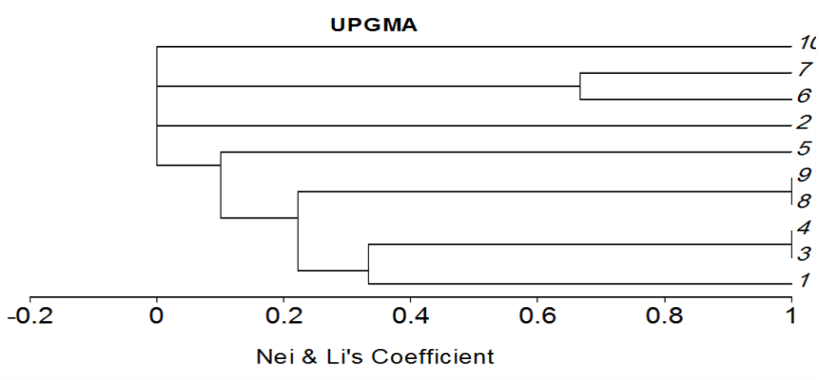

Fig 3: shows the tree of evolution and evolution based on the genetic distances of RAPD

This comforts the fact that there is certainly a high rate of genetic reynthesis or mutations leading to a high level of genetic diversity among

*A.baumannii*isolates the population [37]. A high level of genetic diversity in *A.baumannii*isolates the population also reported as the basis for the multidrug-resistant *A.baumannii* species and high perseverance in environmental adaptation [38.39.40] .

. PCR technology-(RAPD) used random prefixes to amplify a range of randomly distributed sites in any genome and thus to detect the development of genetic markers [41].

Four out of 10isolations showed 300bp molecular weight packs as they converged with what He et al. [42]In his study of the presence of all his isolations with a package containing molecular weight 300bp.

The results of our study were also close to having two 500bp molecular-weight isolations with hussein, [43] with 18 isolations containing 501bp molecular-weight packs.

The results of our study were lower than that of researcher Ahmed[44] in Baghdad in 2017, with the number of genetic patterns of its isolations amounting to 18 genetic patterns of *A.baumannii* bacteria, where the weight of molecular beams ranged from(4000-100)bp.

Molecular sequence of gene 16S rRNA

The results obtained using MEGAX methodanalyzed thetype of middle Neighbor-goining and to find the similarity between these bacterial isolations, where the evolution tree for *a.baumannii* bacteria was created for five isolations and was highly identical to Genbank NCBI based on a 3000 bp alignment part of 16S rRNA, in preparation for1000 bp, [45,46] .

Bacterial isolations were classified in different classification groups based on the similarity and difference between breed sequences.

The partial sequence of the 16S rRNA gene gave interesting information about the existence of 5 distinct sequences separately branched into the tree of origin, including MW599779, MW599780, MW599781, MW599782 and MW5999783.Through NCBI BLAST, it showed

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that there is an almost identical relationship between them(MW282035, MW307348, MT793124, MT277459, MK719821)which were isolated from China, India and Sri Lanka respectively (NCBI base).

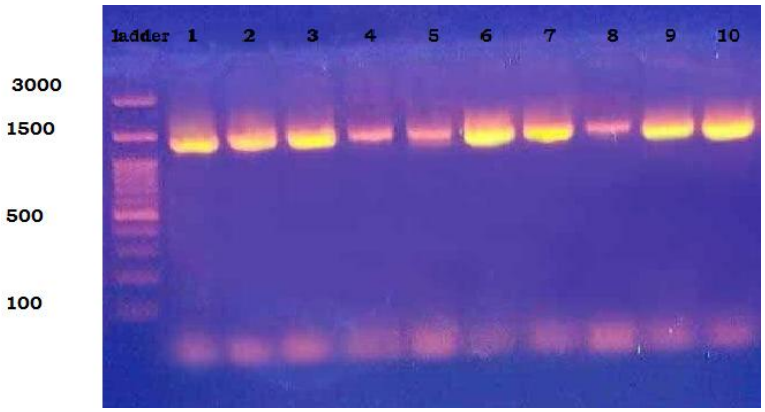

Fig5: shows molecular detection of 16S rRNA genes by PCR. L: ladder with 3000bp. On acarose gel

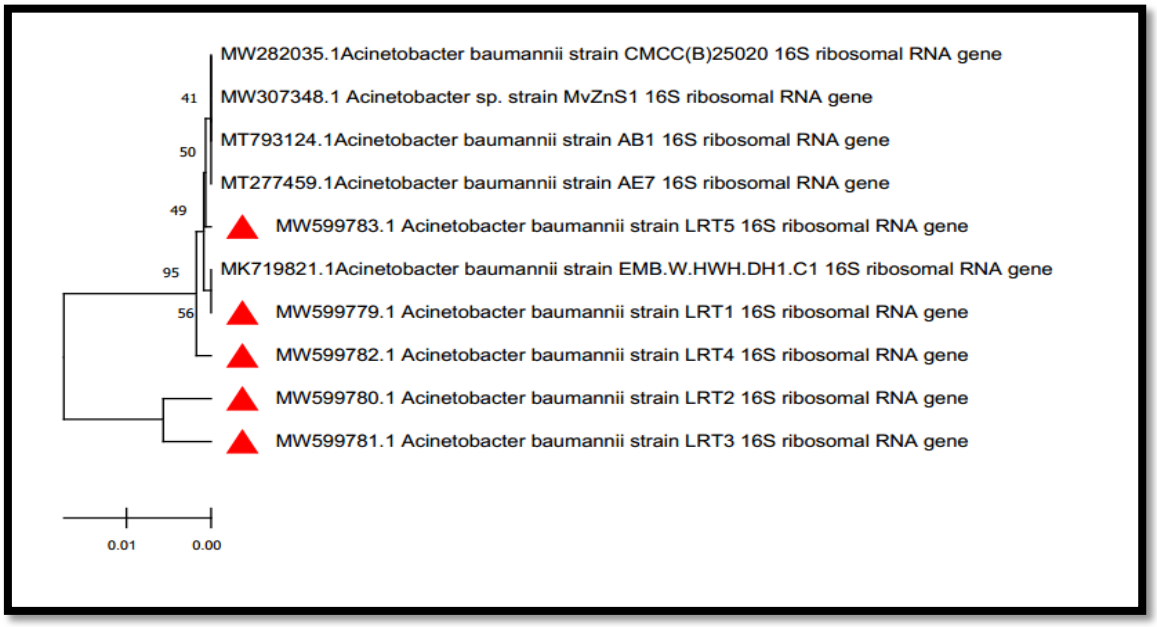

Fig 6: tree emergence and development based on the gene sequence 16s rRNA for five locally isolated strains of *A.baumannii* bacteria the tree was created using megax medium type Neighbor-goining

It is clear from figure 6 the extent of convergence and spacing between isolations, where the insulation MW599783was isolated from the source of the spinal cord fluid CSF and MW599779 isolated from the source of the urine and MW599782 isolated from the source of blood perch and these isolations were close With global isolations, MW599780 isolated from Burns and MW599781 isolated from upper respiratory tract infection sputum are converging

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but far from the isolations MW599783, MW599779 and MW599782 .

The discovery of PCR and DNA sequences, and comparisons of the genetic sequence of bacterial species revealed that the 16S rRNA gene is highly preserved within species and among same-sex species, and therefore can be used as a new "golden standard" for species - determining the level of bacteria [45] .

These differences in similarity ratios between strains of the same type of bacteria are due (to different sources of isolation and to mutations that cause genetic heterogeneity over time[46] .

Differences in the genetic patterns of our local isolationindicate variations in strains of *A.baumnnii* bacteria, and this difference between isolations may be due to where it was taken, diversity in sample taking, hospital variation, period and location of isolation, and the wide geographical distribution of these bacteria in the hospital environment, due to Cross-transmission among hospital patients.

This is what maleki et *al* agreed; [47]Where there is a genetic heterogeneity between strains of pomo-stagnant bacteria among Iranian hospitals.

The difference in genetic patterns indicates clonal diffusion birth prevalence due to prolonged survival of*A.baumannii* bacteria in the hospital environment and their transmission among hospital patients, as well as the horizontal transition of factors that determine resistance between isolations in their various sources of isolation, Termi *et al;*[48]

REFERENCES

1. Moulana, Z; Babazadeh, A; Eslamdost, Z., Shokri, M. and Ebrahimpour, S. (2020). Phenotypic and genotypic detection of metallo-beta-lactamases in Carbapenem resistant *Acinetobacter baumannii*. *Caspian Journal of Internal Medicine, 11*(2):171.
2. Lal, B; Vijayakumar, S., Anandan, S. and Veeraraghavan,B.(2019). Specimen Collection, Processing, Culture, and Biochemical Identification of Acinetobacter spp. InAcinetobacter baumanniiHumana Press, New York, NY.,:1-15.
3. Yadav, S.K; Bhujel, R; Hamal, P; Mishra, S.K; Sharma, S. and Sherchand, J.B. (2020). Burden of Multidrug-Resistant *Acinetobacter baumannii* Infection in Hospitalized Patients in a Tertiary Care Hospital of Nepal. *Infection and Drug Resistance, 13*:725.
4. Howard, A; O'Donoghue, M; Feeney, A; and Sleator, R. D. (2012). Acinetobacter baumannii: an emerging opportunistic pathogen. Virulence, 3(3): 243-250.
5. Da Silva, G.J. and Domingues, S. (2016). Insights on the horizontal gene transfer of carbapenemase determinants in the opportunistic pathogen *Acinetobacter baumannii. Microorganisms, 4*(3):29.
6. Lee, C.R; Lee, J.H; Park, M; Park, K.S., Bae, I.K; Kim, Y.B; Cha, C.J; Jeong, B.C. and Lee, S.H. (2017). Biology of Acinetobacter baumannii: pathogenesis, antibiotic resistance mechanisms, and prospective treatment options. *Frontiers in cellular and infection microbiology, 7*:55.
7. Megeed, A. A; Hayssam, M. A; Salem, M. Z; El-Shikh, M. S; Talea, I. A; and Alogaibi, Y. A. (2016). Investigation of the virulence factors and molecular characterization of the clonal relations of multidrug-resistant *Acinetobacter baumannii* isolates. J. AOAC Int. 16-0139.

8. DeafG. B., Peter M. Oakes, Tim Alexander(2014). The Journal of Deaf Education, Volume 19, Issue 3, July 2014, Pages 285–302.
9. Chen, W. and Kuo, T. (1993). A simple and rapid method for the preparation of gramnegative bacterial genomic DNA. Nucleic Acid Research. 21: 9.
10. Sambrok, J.A.(2001). Molecular Cloning: A Laboratory Manual. pp: 2100. 3rd ed. Cold Spring Harbor Laboratory Press, New York.
11. Willams, J.G.K., Kublik, A.R., Livake, K.J., Rafalski, J.A., and Tingey, S.V., (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18: 6531-6535.
12. Maniatis, T; Fritsch, E.F., and Sambrook, J. (1982). Molecular cloning: A laboratory manual. Cold spring harbor laboratory, New York, U.S.A.
13. Weigand, F; Baum, M. and Udupa, S. (1993). DNA molecular marker techniques. Technical manual No.20. ICARDA. Aleppo, Syria.
14. Rohlif, F. J. (1993). Numerical taxonomy and multivariate analysis system. Version 1. 80. Zxeter. Software. Setauket. N.Y.
15. Sneath, P. H., and Sokal, R. R. (1973). Numerical taxonomy. P:345. Freemon, San Francisco.
16. Nei, M. (1972). Genetic distance between populations. Am. Nat. 106: 283-292.
17. Al-Saffar, Ahmed Alshanon, Shymaa L. Al-Brazanchi, Fatimah A. Sabry(2015)Asian Journal of Biochemistry 12 (1), 16-26.
18. Al-Sehlawi, Z. S; Alshara, J. M; Hadi, Z. J; and Almohana, A. M. (2015). First report of the blaoxa-23 gene in a clinical isolates of *Acinetobacter baumannii* in Najaf hospitals-Iraq. Research Journal of Microbiology, 10(10): 494-501.
19. Sohail , M. ; Rashid, A.; Aslam, B.; Waseem, M.; Shahid, M. ; Akram, M.; Khurshid, M. and Rasool, M. H. (2016). Antimicrobial susceptibility of *Acinetobacter* clinical isolates and emerging antibiogram trends for nosocomial infection management. Revista da Sociedade Brasileira de Medicina Tropical, 49(3): 300-304.
20. Al-Khafaji, Sinai Mahdi Shaker (2006). A study on the bacterial *Acinetobacter baumannii* capsule.
21. and its effect on the immune response. Doctoral thesis, Faculty of Science, UniversityAl-Mustansiriya: Page 134.
22. Grochowalska, A; Kozioł-Montewka, M; and Sobieszczańska, A. (2017). Analysis of *Acinetobacter baumannii* resistance patterns in patients with chronic obstructive pulmonary disease (COPD) in terms of choice of effective empiric antibiotic therapy. Annals of Agricultural and Environmental Medicine, 24(2): 307-311.
23. Kadom, S.M. and ABID, I.N. (2020). Detection of bla OXA-51-like and bla VIM Carbapenemase Genes in Acinetobacter baumannii isolated from burn patients. *International Journal of Pharmaceutical Research*, *12*(2).
24. Al-Sehlawi, Z. S; Alshara, J. M; Hadi, Z. J; and Almohana, A. M. (2015). First report of the blaoxa-23 gene in a clinical isolates of *Acinetobacter baumannii* in Najaf hospitals-Iraq. Research Journal of Microbiology, 10(10): 494-501.
25. *Hashem, Rawnaq Mahdi (2014). Production and purification of Lignin from bacteria Acinetobacter baumannii isolated from sputum and its effect in some microorganisms outside the vivo.*
26. *Master Thesis, Faculty of Science, Al-Mustansiriya University: Page 145.*

27. Tewari, R., Chopra, D., Wazahat, R., Dhingra, S., and Dudeja, M. (2018).Antimicrobial Susceptibility Patterns of an Emerging Multidrug Resistant Nosocomial Pathogen: *Acinetobacter baumannii*. MALAYSIAN JOURNAL OF MEDICAL SCIENCES, 25(3): 129-134.
28. Santhanam,Amutha ,Marialouis,Xavier Alexander(2016*)*.antibiotic Resistance, RAPD- PCR Typing of Multiple Drug Resistant Strains of Escherichia Coli From Urinary Tract Infection (UTI).
29. Graham, J. and McNicol, R. J. (1995).An examination of the ability of RAPD markers to determine the relationship within and between Rubus spp. Theoretical and Applied Genetics. 90: 1128-1132.
30. Ogunseilan, O. (2005). Diagnostic Bacteriology Protocols. P: 256. 2ed. Humana Press Totowa, New Jersey.
31. Weeden, N. F; Timmerman, G. M., and Kneecn, B. E. (1992). Inheritance and reliability of RAPD markers. In: Application of RAPD technologies plant breeding. P: 12-17. Crop. Sci. Society of American Madison, Press. U.S.A.
32. Jarullah, B. M; Subramanian, R. B., and Jummanah, M. S. (2005). Phylogeny of certain biocontrol agents with special reference to nematophagous fungi based on RAPd. Commun Agric Appl Biol Sci.70: 4. 897-903.
33. Distinguishes a unique subpopulation of Escherichia coli O157:H7 strains in cattleand biofilm development of Pseudomonas aeruginosa populations in chronic lung infections. J. Bacteriol. 190: 2767-2776.
34. Grandmann, H; Shneider, C; Hartung, D; Dachner, F. D., and Pilt, T. L.; (1995). Discriminatory, power of three DNA-based typing techniques for Pseudomonas aeruginosa. J. Clin. Microbiol. 33:328-334.
35. Menon, W. C. *et al*; (2003). Random amplification of polymorphic DNA based typing of Pseudomonas aeruginosa. MJAFI 59: 25-28.
36. Perron, G.G., Gonzalez, A. and Buckling, A. (2008). The rate of environmental change drives adaptation to an antibiotic. J. Evolut. Biol. 21: 1724-1731.
37. Perron, G.G., Gonzalez, A. and Buckling, A. (2008). The rate of environmental change drives adaptation to an antibiotic. J. Evolut. Biol. 21: 1724-1731.
38. Nazik, H., Ongen, B., Erturan, Z., and Salcioglu, M. (2007). Genotype and antibiotic susceptibility patterns of Pseudomonas aeruginosa and Stenotrophomonas maltophila isolated from cystic fibrosis patients. Japan J. Infect. Dis. 60: 82-86.
39. Mena, A; Smith, E.E; Burns, J.L; Speert, D.P., Moskowitz, S.M; Perez, J.L and Oliver, A. (2008). Genetic adaptation of Pseudomonas aeruginosa to the airways of cystic fibrosis patients is catalyzed by hypermutation. J. Bacteriol. 190: 7910-7917.
40. Speijer, H; Savelkoul, P.H., Bonten, M.J., Stobberingh, E.E., and Tjhie, J.H. (1999). Application of different genotyping methods for Pseudomonas aeruginosa in a setting of endemicity in an intensive care unit. J. Clin. Microbiol. 37: 11. 3654-3661.
41. Anatomy, M.; Rose, B.; and Pegler, M.B; et al.(2002). Genetic analysis of Pseudomonas aeruginosa isolates from the sputa of Australian adult cystic fibrosis patients. J. Clin. Microbiol. 40: 8. 2772-2778.
42. Al-Jubouri, Noor Hussain (2019). Determination of the inhibitory activity of some substances against *Acinetobacter baumannii* bacteria isolated from different sources and the detection of some of their virulence genes. Master Thesis, College of Science, University of

Mosul: pages 98-99.

43. He, X.; Lu, F.; Yuan, F.; Jiang, D.; Zhu, J. A.; Cheng, H.; Cao, J. and Lua, C. G. (2015). Biofilm Formation Caused by Clinical *Acinetobacter baumannii* isolatests Associated with Overexpression of the AdeFGH Efflux Pump. Antimicrobial Agents and Chemotherapy, 59 (8).
44. Ahmed, Rasha Ziyad Tariq (2017) . Study of the phenotypic and genotype of some bacterial virulence factors *Acinetobacter baumannii* isolated from various clinical conditions. Master Thesis,College of Education for Pure Sciences (Ibn Al-Haytham). Baghdad University. Page 99.
45. Felsenstein, Joseph(1985). Phylogenies and the Comparative Method.Published By: The University of Chicago press Vol. 125 ,No. 1 (Jan;1985) ,pp. 1-15 .
46. Kumar, A. ; Kumar, A. ; Lata, C. ; Kumar, S. ; Mangalassery, S. ; Singh, J. P. ; Mishra, A. K. ; Dayal, D., 2018. Effect of salinity and alkalinity on responses of halophytic grasses *Sporobolus marginatus* and *Urochondra setulosa*. Indian J. Agric. Sci., 88 (8): 149-157
47. OLSEN ,Gary, R, WOESE.R.(1993). Ribosornal RNA: a key to phylogeny. Department of Microbiology University of Illinois, Urbana, Illinois 61801, USA.
48. Ece, G; Erac, B; Cetin, H. Y; Ece, C; & Baysak, A. (2015). Antimicrobial susceptibility and clonal relation between *Acinetobacter baumannii* strains at a Tertiary Care Center in Turkey. Jundishapur journal of microbiology, 8(2):3-15.
49. Maleki, A.; Vandyousefi, J.; Mirzaie, Z.; Ghafourian, S.; Kazemian H. and Sadeghifard, N. (2016). Molecular Analysis of the isolates of *Acinetobacter baumannii* isolated from Tehran Hospitals Using RAPD-PCR Method. Modern Medical of Laboratory, 1(1): 16-1.
50. Hammoudi, D; Ayoub, D.; Moubareck, C.; Hakime, N.; Houmani, M.; Barakat, H.; Najjar, Z.; Suleiman, M.; Fayad, N.; Sarraf, R. and Karam- Sarkis, D. (2015). International Journal of Infectious Diseases, 36: 56–6.
51. Ubaoji, K., Nwosu, O., Agu, K., Nwozor, K., Ifedilichukwu, N., & Okaka, A. (2020). Gas Chromatographic Analysis of the Phyto-Constituents and the Assessment of the Anti-Microbial Properties of the Leave Extracts of Nigeria-Grown Gingko biloba. Journal of Scientific Research in Medical and Biological Sciences, 1(2), 45-56. https://doi.org/10.47631/jsrmbs.v1i2.57