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Genotyping of *Acinetobacter baumannii* that isolate from different cases by using ERIC

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التنميط الجيني لـ *Acinetobacter baumannii* المعزولة من حالات سريرية مختلفة

باستخدام ERIC

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ABSTRACT

Determination of genetic relatedness typing between isolates has done for *Acinetobacter baumannii* isolates by using Enterobacterial Repetitive Intergenic Consensus-PCR (ERIC-PCR), the results of the current study showed the presence of a genetic relationship between bacterial isolates isolated from different clinical sources. The results of the study showed the presence of 18 patterns, the molecular weight of these bands ranged between (100-4000) bp.

Dendogram analysis of the results showed that there was genetic relatedness between the isolates of *Acinetobacter baumannii* in only one clone while 37 isolated contain different genotyping.

Key words: *Acinetobacter baumannii*, ERIC, Genotyping.

الخلاصة

تم تحديد التتميط الجيني بين العزلات بكتريا *Acinetobacter baumannii* باستخدام Enterobacterial Repetitive Intergenic Consensus-PCR (ERIC-PCR)، وأظهرت نتائج الدراسة الحالية وجود علاقة وراثية بين العزلات البكتيرية المعزولة من مصادر سريرية مختلفة. أوضحت نتائج الدراسة وجود 18 نمطاً تراوح الوزن الجزيئي للحزم بين (100-4000) زوج قاعدة.

أظهر تحليل Dendogram للنتائج وجود علاقة وراثية بين عزلات *Acinetobacter baumannii* في نسيلة واحدة فقط بينما احتوت 37 معزولة على أنماط وراثية مختلفة.

الكلمات المفتاحية :- *Acinetobacter baumannii* ، ERIC ، التتميط الجيني.

1. Introduction

Genotyping distinguishes between bacterial isolates on their genetic content. There are several methods for genotyping that have become important in the field of finding genetic relatedness between bacterial isolates, identifies the source of infection and works to identify strains of high virulence to prevent infection, especially in intensive care units that are a place for the emergence of many antibiotic resistance bacteria [1].

There are several methods of genotyping that used in present time that different in its ability to distinguish between the bacterial isolates in the same species. One of these methods is Restriction Fragment Length Polymorphism (RFLP), the method of gel electrophoresis with a pulsed field Electrophoresis, Repetitive Sequence-PCR [2, 3]. This method easy and fast methods that do not need time to accomplish, compared to other methods. One of the benefits of this method is the ability to find the genetic affinity for bacterial isolates of the same species [4, 5].

The most important types of methods of repeated sequences that was easy, fast, less expensive and less complicated in analyzing the results was Enterobacterial Repetitive Intergenic Consensus (ERIC) method. The sequences of this method were originally discovered in *Escherichia coli* and *Klebsiella pneumoniae* [6]. This methods are across multiple regions of the genome and the length of sequences was 126 bp. and the location sequences are different from one strain to another and this method has been increasingly used to identify the genetic related relationship of bacteria [7, 8].

ERIC method is used between bacterial strains and epidemiological studies, as well as to classify many gram negative bacterial [9]. It has shown a high efficiency to determine the epidemiological range of *A. baumannii* bacteria [10]. Another study mentioned that this method is one of the best and most accurate methods for

determining the genetic affinity in *A. baumannii* [11] and in a study of *A. baumannii* that showed this method is useful to determining the epidemiological and studying the diversity between isolates. Isolates were divided into several groups by this technique [12], as shown in Studies that *A. baumannii* isolated have resistance genes from several hospitals in Korea can be identified by ERIC-PCR technique. It has been observed that there are 7 isolates that possess a gene resistance anti-beta-complement OXA-23 and 6 isolates that produce an OXA-23 resistance gene from the same hospital [13].

Amis of this study Genotyping of *A. baumannii* isolates using the Enterobacterial Repetitive Intergenic Consensus (ERIC) method.

2. Material and methods

2.1: Isolates of bacteria

Were obtained from a previous study of various clinical sources (blood sample, wounds infection, burns infection, urinary tract infections, and stool sample).

2.2: DNA extracted of DNA

DNA kit (Geneaid Biotech kit system, UK) was used to extract the DNA of bacterial isolates according to the manufacturer's instructions.

2.3: Genotyping of A. baumannii using ERIC method

The polymerase chain reaction mixture was prepared using ERIC method. The proliferation of the *ERIC* gene with specific primer Prepare the solutions of the Stock according to the instructions of the manufacturer Alpha DNA (Canada) (F: ATGTAAGCTCCTGGGGATTAC3') and (R: 5' AAGTAAGTAAGTGACTGGGGG TGA GCG 3'). The solution concentration of 10 P mol / µl keep the stock solution under - 20° C. The amplification of DNA was performed at a volume of 20µl. The

reaction mixture is a component of 10µl GO Taq green master mix, 1µl F-primer, 1µl R-primer, 2µl template DNA, and 6µl Nuclease Deionized nuclease-free water [[14]]. Then the contents were mixed using the Vortex mixer and then placed in a PCR. Steps of PCR reaction were step 1 (Only one cycle for 1 minutes at a temperature of 94°C for the primary DNA denaturation), step 2 (35 cycle included: A: 45 sec at 94°C for DNA template denaturation, B: 45 sec at 94°C for the primers to bind to DNA template annealing, C: 2 minute at 48°C for the associated primers to be elongated. Step3 (Only one cycle for 10 minute at 72 ° C for the final elongation of the double DNA strip.

2.4: Separation of DNA bands

Five µl of PCR products were separated on a 2% agarose with 5µl Ethidium bromide, at 100 vol. for 80 min. DNA Ladder 100bp. The DNA bands were visualized and photographed under UV light at 300 nanometer by using UV-transilluminator [15]. Measuring DNA Concentration with a Fluorometer quantus.

2.5: Dendogram synthesis to determine the genetic affinity of *A. baumannii* isolates

A synthesis dendogram was found to determine the genetic affinity between the isolates of *A.baumannii* by converting the results that appeared in the gel into a descriptive table, as when a package is present, 1 is placed and when it is absent, 0 is placed and this data is entered into the Past Software **Jaccard / up GMA** program to obtain the Dendogram. The molecular weights of the resulting bands were determined using the Cs Analyzer program.

3. Results and discussion

Genetic typing was found and identified by PatternA. *baumannii* under study by using (ERIC) method, the results showed the presence of a genetic relationship between bacterial isolates. The results of the study showed the presence of 18 group

(ERIC1 to ERIC18) using Past Jaccard / up GMA program, the molecular weight of these bands ranged between (100-4000) bp and the Percentage ranged (5-60)% as shown in Figure (1 (A and B)) Table (1) and this result corresponds to the result obtained by [13] with 17 patterns using the ERIC method to standardize the isolates of *Acinetobacter* from several hospitals in Brazil, and the results in Figure (2) showed the presence of 37 isolates contain different genotypes, While only one clusters contained three isolates which are (32,35, 37), there was a genetic affinity between them. These isolates were isolated from patients at Yarmouk Teaching Hospital and the source from which these isolates were isolated was from stool Our results were close to the study by [11] who found a genetic affinity between the isolates of *A. baumannii* isolated from several hospitals in Turkey and from different sources who showed that using the ERIC-PCR method there was a genetic affinity between three groups of *A. baumannii* isolates. In the study by [10] showed that there were 18 types of *A. baumannii* isolates that were isolated from ICU intensive care units and from several hospitals in Lebanon using the ERIC-PCR method. There was a genetic convergence between four groups of *A. baumannii* isolates. The researchers showed [16] using the ERIC-PCR method and a genetic affinity between four groups of *A. baumannii* isolates isolated from ICUs and researchers [17] four groups were found between isolates of *A. baumannii* using ERIC-PCR technique isolated from different sources and from several hospitals in China.

Figure (1-A): Electrophoresis of the PCR product of gene ERIC (100-1500 bp) for *A. baumannii* isolates on 2% agarose and 100 volts for 80 min. The M ladder (100 bp), the 1-19 was positive isolates. The molecular weights of the resulting bands were determined using the CS Analyzer 3 program.

Figure (1-B): Electrophoresis of the PCR product of gene ERIC (100-1500 bp) for *A. baumannii* isolates on 2% agarose and 100 volts for 80 min. The M ladder (100 bp), the 20-38 was positive isolates. The molecular weights of the resulting bands were determined using the CS Analyzer 3 program.

Table (1): Molecular weights and percentages of the resultant band by ERIC method

Bands	Molecular weight bp	Isolates no.	Percentage %
ERIC1	100	8	20
ERIC2	150	9	22.5
ERIC3	200	2	5
ERIC4	300	3	7.5
ERIC5	400	3	7.5
ERIC6	500	5	12.5
ERIC7	600	18	45
ERIC8	700	4	10
ERIC9	800	9	22.5
ERIC10	850	3	7.5
ERIC11	900	15	37.5
ERIC12	1000	13	32.5
ERIC13	1500	17	42.5
ERIC14	2000	23	57.5
ERIC15	2500	7	17.5
ERIC16	3000	24	60
ERIC17	3500	11	27.5
ERIC18	4000	4	10

Figure (2): Dendrogram Synthesis of *A. baumannii* isolates using Past Jaccard / up GMA program.

4. Conclusions

ERIC method has shown high efficiency in determining the genetic and epidemiological relationship between *A. baumannii* isolates.

Abbreviations

Abbreviation	Meaning
<i>A. baumannii</i>	<i>Acinetobacter baumannii</i>
API20E	Analytical Profile Index
bp	base pair
DNA	Deoxyribonucleic acid
ERIC	Enterobacterial Repetitive Intergenic Consensus
RFLP	Restriction Fragment Length Polymorphism
NCBI	National Center for Biotechnology Information
PCR	Polymerase chain Reaction
U.V.	Ultra Violate

References:

- [1] I. H. Yildirim, S.C. Yildirim, N. Kocak, "Molecular methods for bacterial genotyping and analyzed gene regions", *Journal of Microbiology and Infectious diseases*. 1(1) (2011) 42-46.
- [2] B. Foxman, L. Zhang, J.S. Koopman, S.D. Manning, C.F. Marrs, "Choosing an appropriate bacterial typing technique for epidemiologic studies", *Epidemiologic Perspectives Innovations*. 2 (10) (2005) 1-8.
- [3] H. S. Martins, M.R. Bomfim, R.O. Franca, L.M. Farias, M. A. Carvalho, J.C. Serufo, *et al.* "Resistance markers and genetic diversity in *Acinetobacter baumannii* strains recovered from nosocomial blood stream infections", *International Journal of Environ Research Public Health*. 11(2) (2014) 1465-78.
- [4] R. M. Abdullah, , R. Z. T. Ahmed, S. N. A. Al-Azzawi " Genotyping of *Acinetobacter baumannii* by using BOX Method", *Indian Journal of Forensic Medicine and Toxicology* , 13(4)(2019) 934-938
- [5] A. Doleans-Jordheim, B. Cournoyer, E. Bergeron, J. Croize, H. Salord, J. Andre, M. Mazoyer, F. Renaud, J. Freney, " Reliability of *Pseudomonas aeruginosa* semi-automated rep-pcr genotyping in various epidemiological situations", *Journal of Clinical Microbiology Infectious*. 28(9) (2009) 1105-1111.
- [6] S.H. Jeong, I.K. Bae, K.O. Park, Y.J. An, S.G. Sohn, S. J. Jang, K.H. Sung, K. S. Yang, K. Lee, D. Young, S.H. Lee, "Outbreaks of imipenem- resistant *Acinetobacter baumannii* producing carbapenemases in Korea", *Journal of Microbiology Seoul, Korea*. 44(4) (2006) 423-431.
- [7] F. Al-Zahraa, M. Gomaa, Z.H. Helal, "Phenotypic Characterization and ERIC-PCR Typing of MDR *Acinetobacter* species in Egypt", *Egyptian Journal of Medical Microbiology*. 20(1) (2011). 1-5.
- [8] H. Goudarzi, F. Karimi, F. A. Amoli, Z. Abedinyfar, F. Doustdar, F. Mehrnejad, "Genetic fingerprinting and antimicrobial susceptibility profiles of *Pseudomonas aeruginosa* isolates from eye infections", *Journal of clinical infection diseases*. 6(1) (2011) 41-46.
- [9] A. F. Mehdi, "Study of Genotyping and some virulence factor of *Pseudomonas aeruginosa*", Master Thesis, College of Education for pure science, University of Baghdad, (2015) 4-20.
- [10] D. Hammoudi, D. Ayoub, C. Moubareck, N. Hakime, M. Houmani, H. Barakat, Z. Najjar, M. Suleiman, N. Fayad, R. Sarraf, D. Karam- Sarkis, *International Journal of Infectious Diseases*. 36 (2015) 56–6.
- [11] G. Ece, B. Erac, H.Y. Cetin, C. Ece, A. Baysak, " Antimicrobial Susceptibility and Clone Relation between *Acinetobacter baumannii* Strains at a Tertiary care center in Turkey", *Microbiology* 8(2) (2015).e15612:1-5.
- [12] M. Fouad, A. S. Attia, W.M. Tawakkol, A. Hashem, "Emergence of Carbapenem-resistant *Acinetobacter baumannii* harboring the OXA-23 carbapenemase in intensive care unit of Egyptian hospitals", *International Journal of Infectious Diseases*. 11 (2013). e1252-4.
- [13] A.E. Ferreira, D.P. Marchetti, G.R. da Cunha, L.M. de Oliveira, D. B. Fuentefria, A.G. Dall Bello, G. Corcao, "Molecular characterization of clinical multi resistant isolates of *Acinetobacter* sp. from hospitals in Porto Allegre, State of Rio Grande do Sul, Brazil", *Revista da Sociedade Brasileira de Medicina Tropical*. 44 (2011) 725–730.

- [14] S.A. Mansour, O. Eldaly, A. Jiman-Fatani, M.L. Mohamed, E.M. Ibrahim, "Epidemiological characterization of *Pseudomonas aeruginosa* isolates of intensive care units in Egypt and Saud Arabia ", *Eastern Mediterranean Health Journal*. 19(1) (2013) 71-80.
- [15] J. Sambrook, D. Russell, "Molecular Cloning Laboratory Manual", 3rd ed. Cold Spring Harbor, New York. USA. (2001).
- [16] J.C. Medina-presentado, V. Seija, R. Viognoli, J. Pontet, L. Robino, N. F. Cordeiro, I. Bado, G. Fulgueriras, M. Bero, C. Bazet, E. Savio, G. Reppi, "Polyclonal endemicity of *Acinetobacter baumannii* in Ventilated Patient in an intensive care unit in Uruguay", *International Journal of Infectious Diseases*. (2013)17. e422- e427.
- [17] X, He, F. Lu, F. Yuan, D. Jiang, J. A. Zhu, H. Cheng, J. Cao, C.G. Lua, "Biofilm Formation Caused by Clinical *Acinetobacter baumannii* isolates Associated with Overexpression of the AdeFGH Efflux Pump", *Antimicrobial Agents and Chemotherapy*. 59 (8) (2015). 4817-4825.