Genotyping of *Pseudomonas Aeruginosa* Isolated from Different Clinical Samples by Using ERIC Method

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Abstract

This study included 100 clinical samples collected as following: 33, 27, 15, 14 and 11 samples from patients with otitis media, burn infections, urinary tract infections, wound infections, and bacteremia, respectively, during the period from September to December, 2014. Seventy-five *Pseudomonas aeruginosa* isolates were identified. To determine the genetic relatedness of different isolates, *Pseudomonas aeruginosa* has been typed using Enterobacterial Repetitive Intergenic Consensus-PCR (ERIC-PCR) and dendrogram analysis. Results showed that there was a genetic relatedness among *Pseudomonas aeruginosa* isolates in 19 clones, while 8 isolates contained different genotyping. In conclusion. The results inducted that ERIC-PCR is a practical, useful and easy method for typing *Pseudomonas aeruginosa* isolates.

Keywords: *Pseudomonas aeruginosa*, ERIC- PCR, Typing, Dendrogram analysis.

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التنميط الجيني لبكتيريا الزوائف الزنجارية المعزولة من حالات سريرية مختلفة باستخدام طريقة ERIC

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الخلاصة

شملت هذه الدراسة 100 عينة تم جمعها من مصادر سريرية مختلفة شملت 33 عزلة من المرضى الذين يعانون من التهاب الأذن الوسطى و 27 عزلة من المرضى المصابين بالتهابات الحروق و 15 عزلة من مرضى التهابات المسالك البولية و 14 عزلة من المرضى المصابين بالتهابات الجروح و 11عزلة من مرضى تجرثم الدم خلال الفترة من سبتمبر إلى ديسمبر 2014. بعد اجراء تشخيص العينات تم الحصول على 75 عزلة تعود لبكتريا *Pseudomonas aeruginosa* . استخدمت طريقة (ERIC-PCR). لتحديد علاقة الانماط الوراثيه لعزلات بكتريا *Pseudomonas aeruginosa*. أوضحت النتائج وجود علاقة وراثية بين عزلات بكتريا *Pseudomonas aeruginosa* في 19 نسيلة، بينما كانت 8 عزلات تحتوي على أنماط وراثية مختلفة ، بينت النتائج ان طريقة التنميط الوراثي باستخدام طريقة ERIC-PCR هي عملية مفيدة وسهلة لدراسة العلاقات الوراثية بين عزلات *Pseudomonas aeruginosa*.

الكلمات المفتاحية : بكتريا *Pseudomonas aeruginosa*، طريقة ERIC-PCR، التنميط الوراثي، التحليل التجميعي.

Introduction

Pseudomonas aeruginosa is *a* Gram- negative bacillus, non-lactose fermentative, aerobic microbe. *Pseudomonas aeruginosa* is one of the major opportunistic and nosocomial pathogens that causes many severe and often fatal infections, especially in immune compromised patients [1-3]. *Pseudomonas aeruginosa* causes cystic fibrosis, chronic lung infections, bronchiectasis, neoplastic, neutropenia, diabetes, AIDS, burn, urinary or wound infections, bacteremia, endocarditis [4, 5].

Genotyping distinguishes between bacterial isolates on the basis of their genetic content [6] Genotyping methods were important in the development of genetic relatedness among bacterial

isolates, identification of the source, rote of infection and identification of high virulent strains [7]. Infections should be prevented to control their distribution, especially in hospitals, including (intensive care unit (ICU), which is often the site for the emergence of many multidrug resistant pathogens, including *P. aeruginosa*, as well as the epidemiological bacterial pathogens [8].

The methods of Genotyping are of great strategy to classify microorganisms at the level of the strain and to efficiently distinguish among bacterial strains and the other bacteria of the same species [6].

Enterobacterial Repetitive Intergenic Consensus (ERIC), the sequences are found in the *E. coli, K. pneumonia* and other enteric bacteria [9], are dispersed in multiple regions of the genome, the length of ERIC sequences is a 127- base pair.
The number of these sequences is different from one strain to another. This method has been increasingly used to identify genetic relatedness of bacteria. The function of the ERIC sequences is not yet known [10].

Several studies have used ERIC sequences to distinguish bacterial strains and epidemiological studies to classify many bacterial species [11]. At study of [12], the authors compared the ERIC and PFGE methods to determine the genetic relatedness of *P. aeruginosa*. The author found that both methods showed good results in determining the genetic relatedness of these bacteria. The ERIC was characterized as less complicated method for genotyping of bacterial isolates in a faster, easier and less expensive protocol. The aim of this study was to determine the genetic relatedness between *P. aeruginosa* isolates using the ERIC-PCR method.

Material and Methods

Collection of samples

One hundred clinical samples were collected from patients have (otitis media, burn infections, wound infections, urinary tract infections and bacteremia) during the period from September to December 2014.

Identification of bacteria

The samples were inoculated on MacConkey agar, Cetrimide agar, Pseudomonas agar and CHRO Magar Orientation. The isolates were identified by performing biochemical tests based on oxidase and catalase tests and then further confirmed using an API20E system [13].

Isolates of DNA

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

Genotyping by using ERIC method

Genetic relatedness of *P. aeruginosa* isolates was done by using ERIC-PCR method.

The Primer used in this study

gene		Primer sequence Primer sequence (5′–3′)	Product (bp)	Sours
ERIC	F	ATGTAAGCTCCTGGGGATTCAC	Variable Bands	[14]
	R	AAGTAAGTGACTGGGGTGAGCG		

Detection of ERIC gene the solution concentration of 10 P mol / µl (by taking 10 µl from stock solution and addition of 90 µl of a deionized sterile distilled water) keep the stock solution under - 20° C [15].

DNA amplification reactions were performed by using PCR. The reaction mixture consisted (5µl GO Taq Green Master Mix Bioneer (Korea), 5µl DNA template, 2µl F-Primer, 2µl R-Primer, 11µl Deionized sterile D.W. Bioneer (Korea)). The total volume of reaction mixture was 25µl the program of ERIC -PCR typing was execution according to [15]. Shown in the following:

Step	Program	
one	Initial denaturation at 94ºC for 1 min 1 cycle	
Two	35 cycles	
	A	Denaturation DNA template at 94°C for 45 sec.
	B	Annealing at 48ºC for 45 sec
	C	Extension at 72ºC for 2 min
Three	A final extension at 72ºC for 10 min 1 cycle	

Separation of DNA bands

PCR products were separated on a 2% agarose with 5 µl Ethidium bromide, at 50 vol. for 2 hours. The DNA bands were visualized and photographed under UV light [16].

Statistical Analysis

Dendogram analysis was done to determine the genetic relationship between all bacterial isolates.

Result and Discussion

One hundred sample which has been collected from different clinical cases. After identification we obtained 75 isolates confirmed as *P. aeruginosa* including: 28, 23, 10, 8 and 6 isolates from otitis media, burn infections, wound infections, urinary tract infections and blood, respectively. Enterobacterial Repetitive Intergenic Consensus (ERIC) primer sequence was used to detect the relationship among *P. aeruginosa* isolated from different clinical samples. The clusters were shown in 15 band, with molecular weight ranging between (100-1700bp) among the 75 *P. aeruginosa* isolates with fragment table 1 figure 1 (A, B, C, D). This study was similar to [13] who showed that 12, 13 genetic patterns genotypes, containing 4-11 band and 3-9 bands in this isolate from Egypt and Saudi Arabia, respectively, the molecular weight of the band between 110-1535 bp. In this study, Dendogram showed that 19 clones were identified, while 8 isolates had different genotypes: 8, 19, 27, 40, 49, 55, 71, 75. Other bacterial isolates of *P. aeruginosa*, 52 and 63. Were untypeable due to the mutations at the site of repeated sequences. These mutations prevent primer bonding with sequencing, thus not showing beams on the agarose gel [17].

Table 1: Molecular weight and percentage of ERIC Band

Percentage (%)	Molecular Weight (bp)	Band
6.66	100	ERIC 1
57.33	160	ERIC 2
18.66	200	ERIC 3
9.33	250	ERIC 4
9.33	300	ERIC 5
50.66	350	ERIC 6
14.66	400	ERIC 7
69.33	450	ERIC 8
45.33	500	ERIC 9
25.33	600	ERIC 10
56	650	ERIC 11
41.33	760	ERIC 12
45.33	1000	ERIC 13
44	1200	ERIC 14
8	1700	ERIC 15

Dendogram analysis showed two group; Group A contains 16 isolates (21.9%) and consists of 5 clones, while group B contains 57 isolates (78.1%) and is composed of 14 clones. The isolates of Bacteria were obtained from the same hospital showed a genetic relationship among them. The results showed that Nosocomial infections by *pseudomonas aeruginosa* were observed among Patients of the hospital. The bacterial isolates of *P. aeruginosa* from the same source, showed genetically converging among them.

The results were also consistent with the results of [11] who found that there were 31 patterns of *P. aeruginosa* were isolated from urinary tract infection in Egypt. In a study of [18], genetic relatedness was found in *P. aeruginosa* that isolated from hospitals at different periods using ERIC-PCR method.

Several studies have used the ERIC method to distinguish among bacterial strains and epidemiological studies as well as to classify many bacterial species. This method is reflecting less complexity to analysis the results, faster and low cost when compared to many other genetic modeling methods [12].

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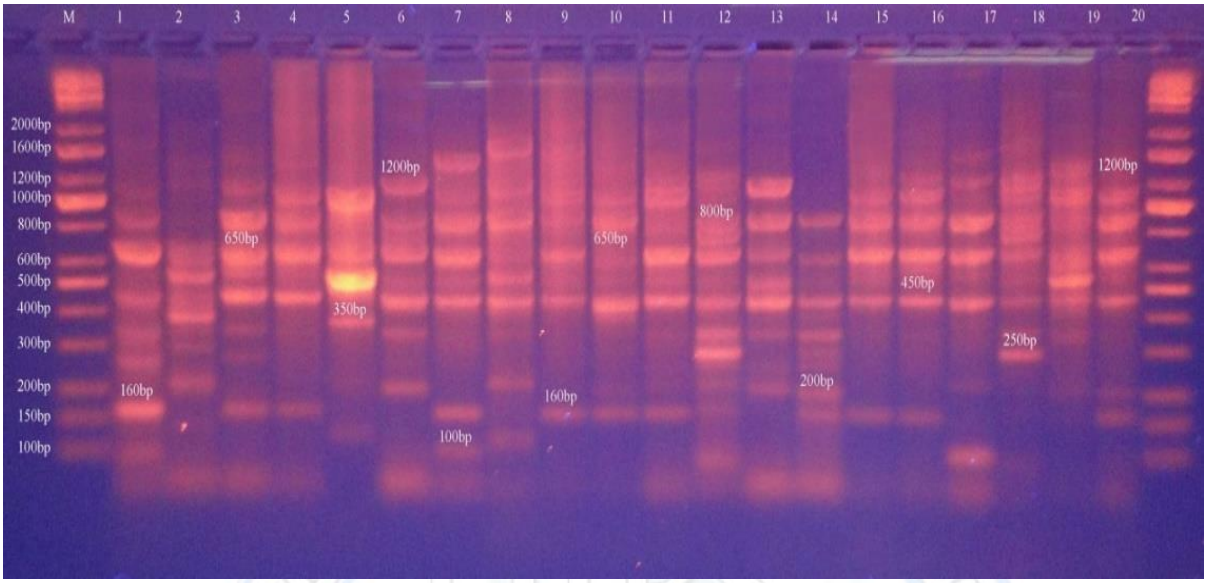

Figure 1A: Gel electrophoreses Genotyping of *P. aeuginosa* by using ERIC method (2% agarose, 50 volte /cm^2 for 2 hours). (Lane M: (MW 100-1700 bp DNA ladder), Lanes: 1-20 *P. aeruginosa*).

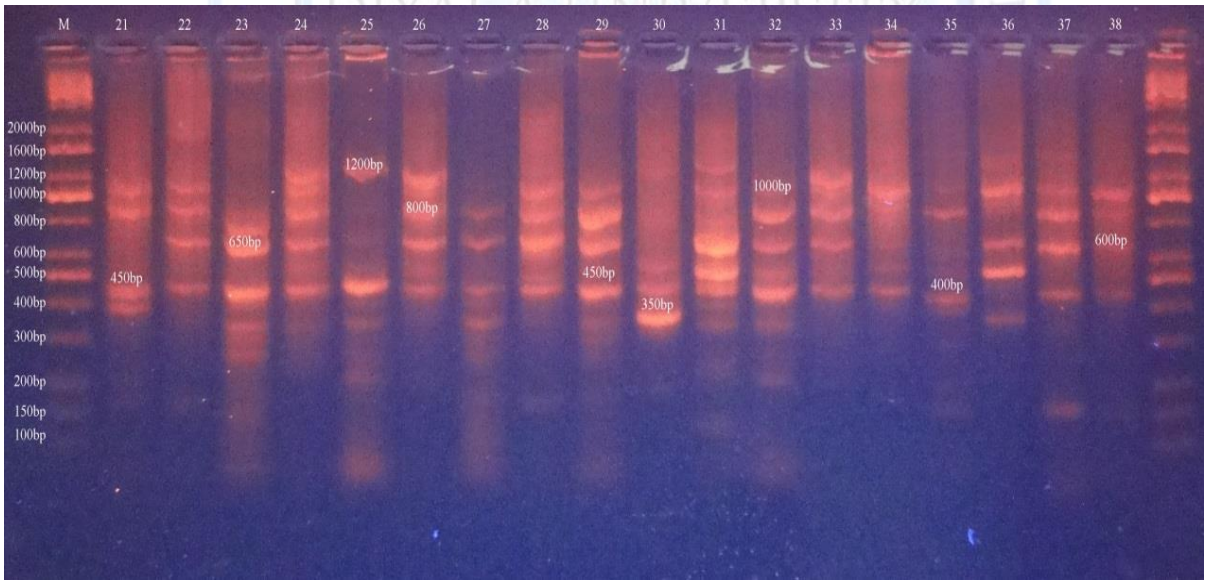

Figure 1B: Gel electrophoreses Genotyping of *P. aeuginosa* by using ERIC method (2% agarose, 50 volte /cm^2 for 2 hours). (Lane M: (MW 100-1700 bp DNA ladder), Lanes: 21-38 *P. aeruginosa*).

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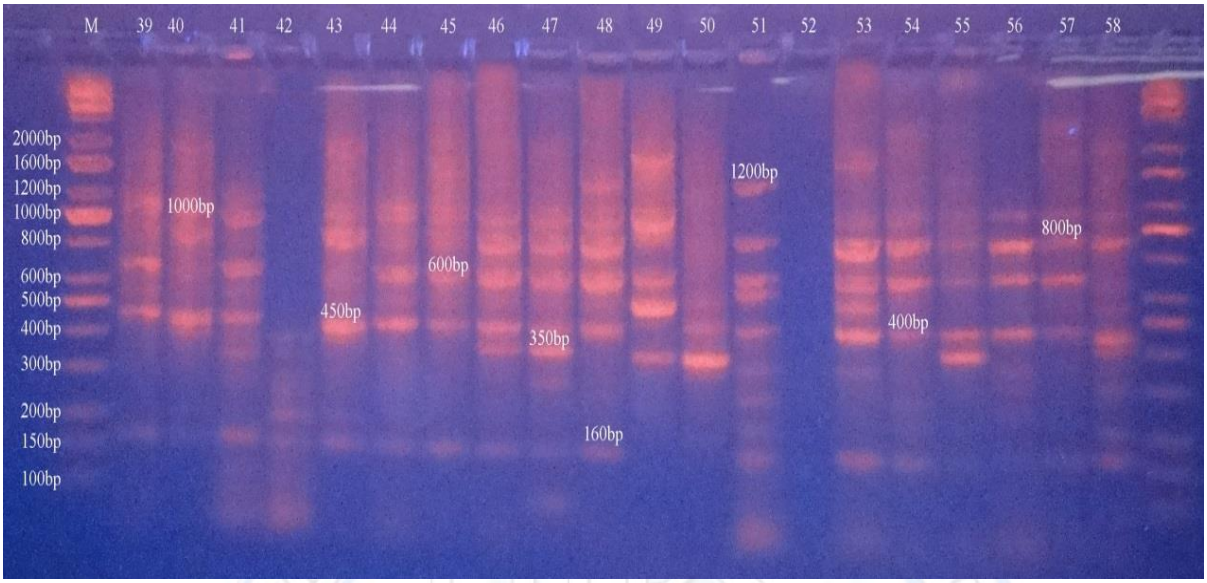

Figure 1C: Gel electrophoreses Genotyping of *P. aeuginosa* by using ERIC method (2% agarose, 50 volte /cm^2 for 2 hours). (Lane M: (MW 100-1700 bp DNA ladder), Lanes: 39-58 *P. aeruginosa*).

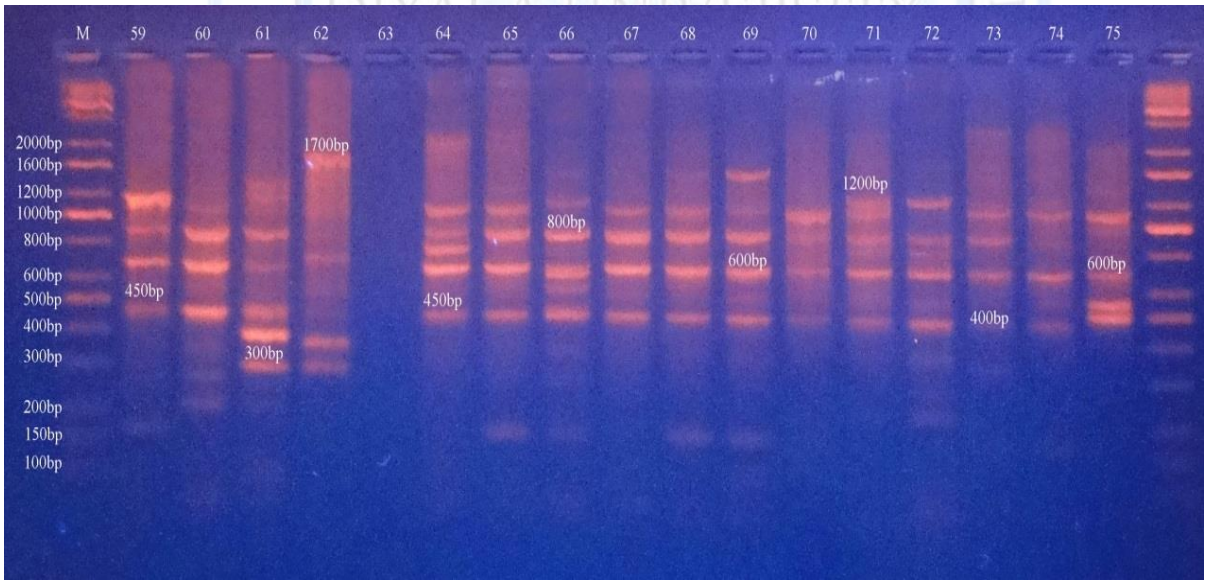

Figure 1D: Gel electrophoreses Genotyping of P. aeuginosa by using ERIC method (2% agarose, 50 volte /cm^2 for 2 hours). (Lane M: (MW 100-1700 bp DNA ladder), Lanes: 59-75 P. aeruginosa).

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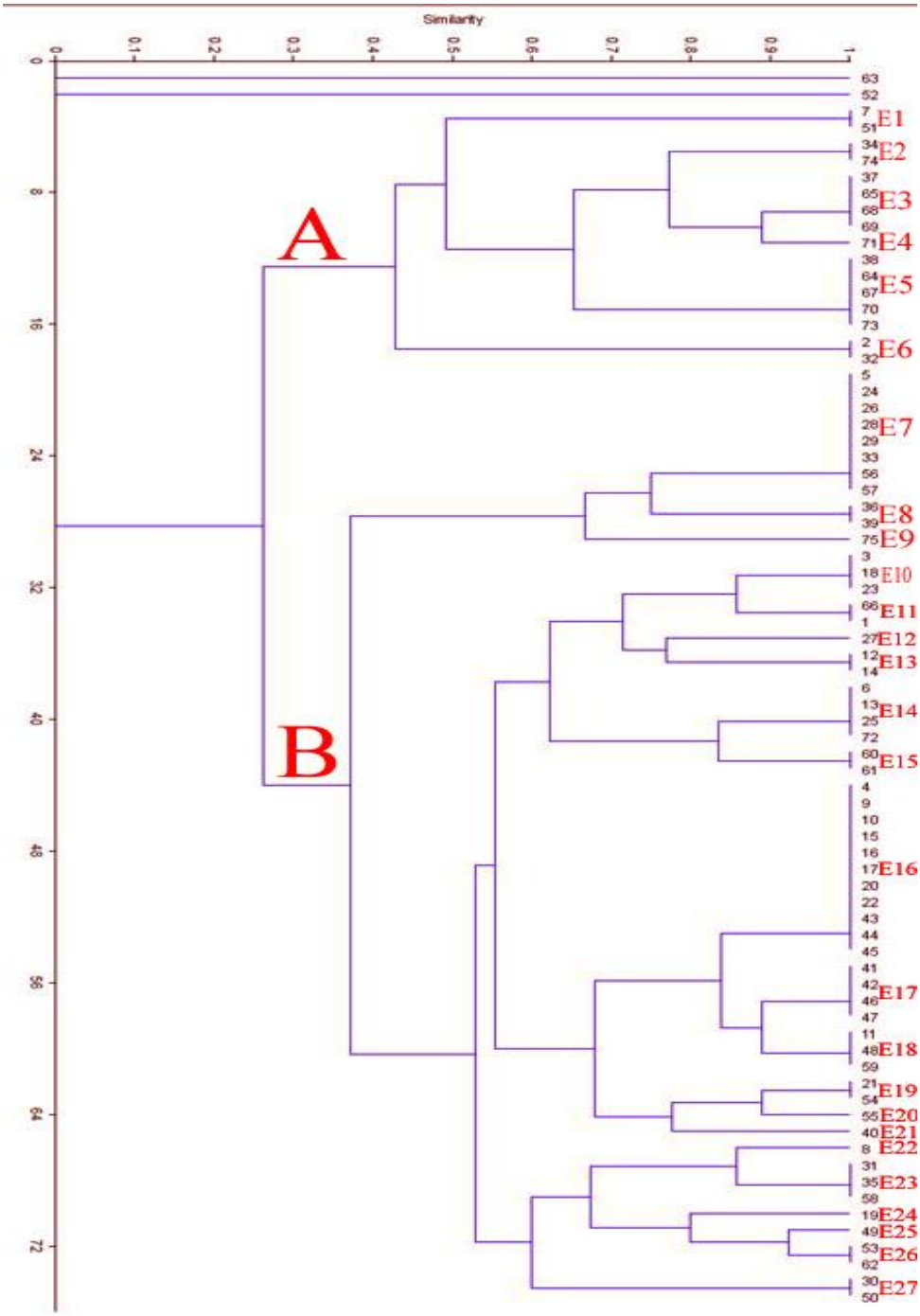

Figure 2: Dendrogram showing the relatedness of *P. aeruginosa* by using past program.

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