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Molecular analysis of clinical isolated *Pseudomonas aeruginosa* by ERIC-PCR

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Abstract*---Pseudomonas aeruginosa*** is a significant mortality factor due to nosocomial infections in humans. P. aeruginosa has been known with severe infections, high incidence, and multiple drug resistance The present study aims to rapidly diagnose and biotype the isolates of P. aeruginosa isolated from human infections in intensive care units, Al-Sadder Medical City, Al-Hakeem General Hospital, and the burn center in Najaf city by ERIC-PCR. From 156 clinical sample 23 *P. aeruginosa* were isolated including burn 17/77 (22%), 2/21wound (9.5%), 3/43 urine (6.9%), 1/15 throat swab(6.6) between August 2021 to February 2022. After phenotypic confirmation, isolates were examined by PCR for molecular confirmation Enterobacterial Repetitive Intergenic Consensus (ERIC) PCR were done on 24 isolates. In ERIC-PCR Genetic analysis of *P. aeruginosa* isolates depending on ≥ 75% similarity revealed the presence of 8 different cluster as figure 2, four of them with single isolate other two clusters with two similar isolates while the two other clusters contained seven identical isolates profile each. The profile that observed within ERIC- PCR showed various band owning different molecular sizes ranging from (1- 8) bands with (100-900) bp, The cluster number two and four showed the highest number of similar isolates the first cluster group comprised (PA9,PA11,PA2,PA10,PA12,PA13 and PA4) isolates whereas the second highestcluster group contained (PA 15, PA 23, PA 3, PA 16, PA 18, PA 17,and PA21) isolates. In conclusion the ERIC PCR technique is a reliable low cost method that can be used to type isolates of *P aeruginosa* isolated from clinical samples.

***Keywords*---**ERIC-PCR, *Pseudomonas aeruginosa,* Molecular analysis.

Introduction

Pseudomonas aeruginosa is a Gram-negative, aerobic rod-shaped bacterium that belongs to the Pseudomonadaceae bacterial family, which is part of the -proteobacteria. *P. aeruginosa* is one of 12 subgroups of the genus *Pseudomonas* (Todar , 2008). Flagella, fimbriae, superficial polysaccharides, and pili-type IV are other key virulence factors in *P. aeruginosa* that are involved in bacterial colonization(Bel Hadj Ahmed *et al.*,2020).

Pseudomonas aeruginosa is a multidrug-resistant (MDR) and extensively drug-resistant bacterial pathogen that infects several organs, including the respiratory tract, vascular system, urinary tract, and central nervous system, causing substantial morbidity and death (XDR) Pseudomonas aeruginosa strains have emerged as the primary source of nosocomial infections in humans, posing a life-threatening threat to immune-compromised individuals in health-care settings. (Abdelrahman,D.N *et al.*,2020; Pang, Z. *et al.*,2018).number of researchers has demonstrated that *Pseudomonas aeruginosa* may be a popular inhabitant of soil, water and vegetation; it's non-fastidious and requires a little amount of nutrition.It mainly found in solutions like disinfectants, soaps, eye drops, also as sinks, hot tubs, respiratory equipment and showerheads (Todar, 2008).

Pseudomonas aeruginosa produces the depository of virulence factors, including pili, flagella exopolysaccharides, proteases, elastase, lipases, iron chelators and variety of various toxins. For example, pyocyanin compound, exotoxin A, as well as Type III Secretion System (T3SS) toxins exoS, exoT, exoY, and exoU (Figure,1,2),(Van delen and Iglewski, 1998; Harper *et al.*,2014).

ERIC is a Gram-negative enteric bacteria-specific repetitive extragenic palindromic PCR (rep-PCR) assay that uses primers that target highly conserved repetitive sequence elements.(Versalovic *et al.*,1991). Enterobacterial repetitive intergenic consensus PCR (ERIC-PCR) enotyping of *P. aeruginosa* isolates has been utilized to research the epidemiology of P. aeruginosa in Australia and Brazil. (Stehling *et al.*,2010; Dawod *et al.*,2011). ERIC-PCR sequences are 127-bp imperfect palindromes found in many copies in the genomes of enteric bacteria and vibrios. (Wilson *et al.*,2006).

In various investigations, ERIC-PCR was found to be a better choice for subtyping *P. aeruginosa* isolates than other PCR-based approaches. (Ojeniyi B Høiby N ,1991; Hematzadeh *et al* .,2021). ERIC-PCR allows researchers to differentiate between bacterial species and strains that have these repeated sequences. (Syrmis *et al.,*2004).Molecular typing is a technique for determining the genetic diversity that underpins essential phenotypic characteristics such as host specificity, pathogenicity, antibiotic resistance, and virulence factors.(Li *et al.*,2009). Because ERIC sequences are longer and hence more helpful in comparative analyses, they may have greater potential as a model for studying the development of bacterial interspersed repetitive sequences. They are also found in a larger range of taxa (Wilson and Sharp .,2006). Antibiotic selection, detection of non-usual phenotypes, identification of some specific features of the isolates, and also identification of a potential cluster in patients with single clone infections all

benefit from typing strains of this bacteria (Ojeniyi and Høiby.,1991; Hematzadeh and Haghkha.,2021).

ERIC PCR uses primers that are specific for enterobacterial repeated intergenic consensus sequences. These primers can be used to match the target DNA to create different-sized DNA finger printing. (Wassenaar and Newell 2000). This is where the bacterial repeated intergenic consensus comes in (ERIC) PCR is a simple, rapid, and cost-effective method of testing.. (Wassenaar and Newell 2000).

Materials and Methods

Specimen collection

The current cross sectional study was conducted From August 2021 to February 2022, a total of 156 clinical specimens were collected from patients at Al- Sadder Medical City, Alfurat Al-Awsat Hospital, and the burn center as in table (4-2).The samples were taken from both genders and ranged in age from 6 to 70 years old. Standard procedures were used to process the clinical samples.

Table (4-2)
Distribution of *P.aurogenosa* isolates collected from medical service utilities in Najaf Province

Hospital's name	No.of samples	No.(%)of *P.auerogenosa*	No. (%) of otherbacterial spp. Isolates	No. (%) of no growth cultures	Chi.square	P.Value
Al-Sader Medical City	28	2	10	15	9.655	0.008
Burn center	96	17	38	41	10.805	0.0045
Alfurat Al- Awsat Hospital	32	4	11	18	9.0024	0.0111
Total	156	23(14.7%)	59(37.8)	74(47.4)		
Chi.square		17.481	25.926	16.9787		
P.Value		0.0002	< 0.0001	0.0003		

Identification of *Pseudomonas aeruginosa* isolates

In a cross-sectional study, a total of 156 clinical samples of hospitalized patients in Najaf City , from August 2021 to February 2022. *Pseudomonas aeruginosa* isolates were identified and confirmed by conventional microbiological and biochemical tests (Sorkh *et al.*,2017). All specimens were cultured on MacConkeys agar and incubated at 37°C for 24 hours in an aerobic state. Bacterial isolates were identified mostly based on the morphologic properties, outline, odor, and

color of the colonies. A sterile loop selects one colony to arrange a purified subgroup on nutrient broth and agar prior to biochemical identification using standard methods (Preethirani *et al.*, 2015), as well as vaitk system identification.

DNA Extraction

Two to three colonies of bacteria were resuspended in a 500-µL of sterile distilled water. Suspensions were heated at 100°C for 15 minutes. Then, they were centrifuged at 3000 g for 10 minutes for the precipitation of cell debris. The supernatant was transferred to a new microtube and stored at -20°C (Hornef *et al.*,2000; Salas *et al.*,2005).

ERIC-PCR Typing

The total 24 clinical isolates were selected for molecular typing by ERIC- PCR. This technique was carried out in a thermocycler (Bio-Rad, Inc. USA) using the primer ERIC (F): 5′-ATG TAA GCT CCT GGG GAT TCAC-3′ and ERIC (R): 5′-AAG TAA GTG ACT GGG GTG AGC G3′ (Zarei, O *et al.*,2018) according to the following protocol: initial denaturation (94 °C for 5 min) followed by 40 cycles of denaturation (91 °C for 1 min), annealing (25 °C for 2 min), extension (72 °C for 2 min), and a final cycle of extension at 72 °C for 5 min. The PCR products were loaded on a 2% agarose gel (Sigma-Aldrich) at 70 V for 1 h, and the banding patterns were visualized on an ultraviolet illumination.

ERIC-PCR results analysis

The ERIC patterns were analyzed by online data analysis service (inslico.ehu.es). ERIC profiles were compared using Dice method and clustered by PAST4 program.

Results

The Clinical isolates of *P. aeruginosa* were isolated from clinical samples including burn 17/77 (22%), 2/21wound (9.5%), 3/43 urine (6.9%), 1/15 throat swab(6.6).Genetic analysis of *P. aeruginosa* isolates depending on ≥ 75% similarity revealed the presence of 8 different cluster as figure 2, four of them with single isolate other two clusters with two similar isolates while the two other clusters contained seven identical isolates profile each. The profile that observed within ERIC PCR showed various band owning different molecular sizes ranging from (1-8) bands with(100-900) bp, The cluster number two and four showed the highest number of similar isolates the first cluster group comprised (PA9,PA11,PA2,PA10,PA12,PA13 and PA4) isolates whereas the second highest cluster group contained (PA 15, PA 23, PA 3, PA 16, PA 18, PA 17,and PA21)isolates figure (1).

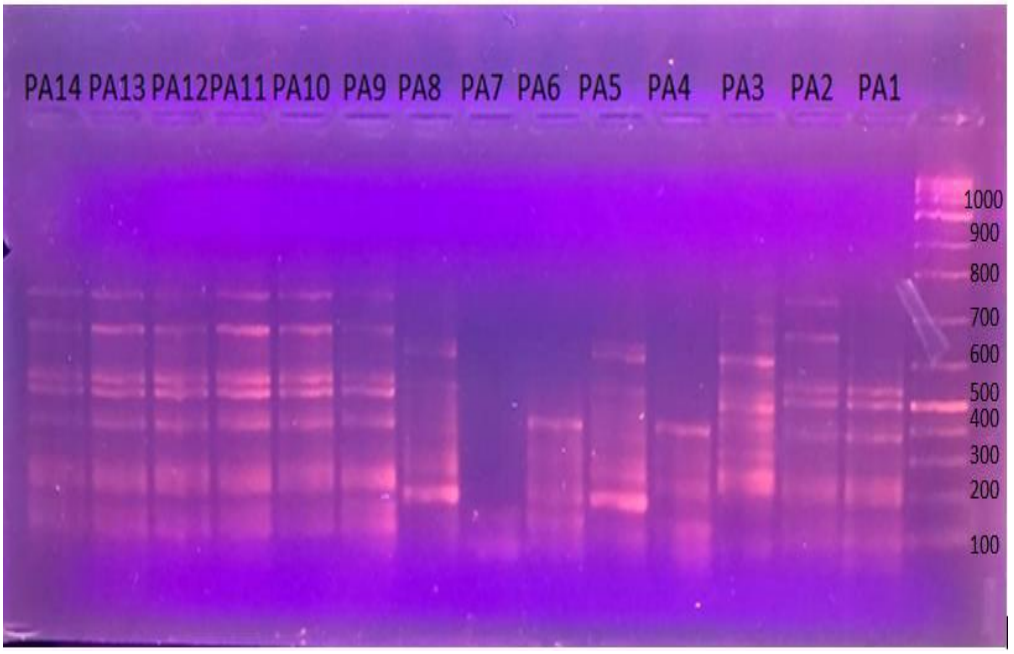

Figure-1 Agarose gel (including agarose powder and TB buffer in appropriate values, in addition to (DSRed Nuclic Acid stain) electrophoresis of *ERIC*. A 1k bp plus ladder (L) was included right side of the gel to estimation the gene size.

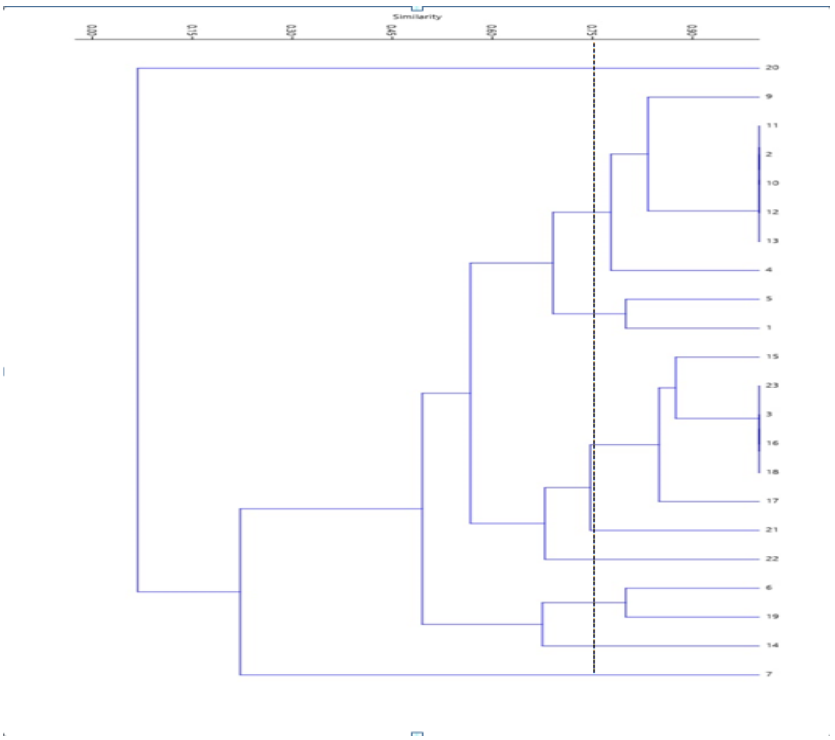

Figure-2 phylogenetic dendrogram analysis of quinolone-resistant Pseudomonas isolates by dice similarity coefficient clustering using ERIC-PCR based on the

unweighted pair group method with arithmetic averages (UPGMA) at a position tolerance at 0.75 The banding pattern generated by ERIC-PCR was analyzed using PAST4 software.

Discussion

as compared with aformer native study on 61 clinical *P.aeruginosa* isolates the result showed the presence of seven clusters with 1-6 band with molecular size from 100-1200bp.(Sallman *et al.*,2017). Other native study conducted in Baghdad aimed to compare between environmental and clinical isolates by ERIC typing reveled the occurrence of high similarity(at 0.5) among each group of isolates and among isolates that obtained from similar source of samples in particular clinical samples in addition to that the environmental isolates possessed distinguished clones. (Auda *et al.*,2017).In study by AL-Badawy out of 92 *P.aeruginosa* 39 of them showed resistance to quinolone that submitted to ERIC typing which reveled 38 different ERIC profile (El-Badawy *et al.*,2019).

A recent study in shiraz, Iran out of 96 *P. aeruginosa* ERIC fingerprinting showed 22 divergent fingerprints ,23 isolates contained similar profile(Hematzadeh *et al.*,2021). An Egyptian study on 44 *P.aeruginosa* isolates showed occurance of 28 cluster with identical profile of four cluster each one contain two-three isolates the obtained pattern of bands range from 200-3000 which is diverse from ours (Abdel- Rhman *et al.*,2021).

Multiple techniques are present and designated to measure the relatedness within different isolates obtained from various sources which reflect the genetic diversity of microorganism. for example PFGE(pulsed field gel electrophoresis),ribotyping ,RAPD (random amplified polymorphic DNA analysis) and ERIC-PCR. Each method has its discriminatory power, type ability, reproducibility and agreement between other typing techniques which make them variable in their convenience. In addition other features should be taken in consideration when choosing one of them, such features include time consumption and cost.

Its worthy to mention that(enterbacterial repetitive intergenic consensus) ERIC-PCR is based on the use of PCR technique that involves the amplification of defined sequence which is in this case is the highly conserved non coding sequence that represent incomplete palindromes found in number of bacteria including *P.aeruginosa.*(Asgarani *et al.*,2015; Ranjbar *et al.*,2017).

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