European Scholar Journal (ESJ)
Available Online at: https://www.scholarzest.com
Vol. 4 No.08, August 2023
ISSN: 2660-5562

MOLECULAR ASSOCIATION BETWEEN PATHOGENIC *PSEUDMONAS AEROGINOSA* AND TLR4 GENE POLYMORPHISM IN CYSTIC FIBROSIS PATIENTS

Mousa Alkhadim watheq Ali[1],
Wafaa Abdul Wahid Jahil Al Kaabi[2],
[1,2]Department of Medical Biotechnology, College of Biotechnology, University of Al-Qadisiyah, Iraq.
Email: mousawatheq@gmail.com

Article history:

Received: 6th June 2023
Accepted: 6th July 2023
Published: 10th August 2023

Abstract:

The distribution of TLR4 (rs4986790) Polymorphism was detected by ARMS-PCR technique There are three genetic types in this locus; A homozygous wild-type (AA) genotype was shown only A allele at 203 bp of the T-ARMS-PCR product. The homozygous (GG) genotype was shown only as the G allele at 288 bp in the T-ARMS-PCR product, while the heterozygous (AG) genotype was shown as the A and G allele at 203 bp and 288 bp product. T-ARMS-PCR.

The aim of study is to determination the pathogensis of pseudomonas aeruginosa.

The results of the current study revealed that the percentage of males was 48.0%. And the percentage of females was 52.0% for those infected with cystic fibrosis, and there were no clear significant differences on the infection rate.

Keywords: TLR4, ARMS-PCR technique, T-ARMS-PCR product
Key TLR4 is toll like receptor 4, ARMS-PCR technique is Amplification-refractory mutation syste,and T-ARMS-PCR product is Tetra-Primer Amplification

INTRODUCTION

has evolved into one of the most significant approaches in bioscience, diagnostics, and forensic research (Zhu *et al.*,2020). We will go over the history of PCR development and the technologies that have evolved from the original PCR process in this section. PCR is being used in two key areas in bioscience: high-throughput PCR systems and microfluidics-based PCR devices for point-of-care (POC) applications. We also address the commercialization of these techniques before concluding with an examination of their modifications and application in novel areas of biomedicine. Real-time reverse transcription PCR, for example, is the gold standard for SARS-CoV-2 diagnosis. As a major component of the sample-to-answer system, it might also be used for POC applications (Zai *et al.*,2022). The limitations of traditional direct see above and cultivation methodes see below have provided the impetus for the development and validation of new methodologies analysis of nucleic conlained in biological samples (Lenders *et al.*, 2010).

Nowadays,these assays are highly sensitive ,provide rapid results for most microbial agent ,and can be partially automated the unique sequence specificity of DNA from a given species permits the design of assays that are highly specific to a target agent .s performance of the the test is not affected by death of the organism due to antimicrobial therapy ,or to transportation or storge of specimens under suboptimal conditions .DNA is one of the most stable and chemically resistant biological molecules in nature (Sapsford *et al.*,2013) .

Intracellular organism can be detected after a DNA extraction treatment of samples.these techniques can also detected organisms involved in latent infections nucleic acid based assays have been able to demonstrate integration into the human genome of viral DNA(e.g. Hepatitis B Viruse (HBV). These assays can be quantitative or detect microbil mRNA, permitting the analysis of transcriptional activity of amicrobial agent ,such as for cytomegaloviruse (Alvarez-Barrientos *et al.,*2000).

Although the use of direct nucleic acid assays was initially impeded by a lack of sensitivity ,since 1990s genomic amplification techniques have revolutionized diagnostic microbiology .the reliance on radiolabelled probes ,initially to detect specific nucleic acid sequences ,was also an important limitation for diagnostic laboratories. Radioactive probes had a short functional half-life and sometimes necessitated prolonged exposure to photographic plates to reach adequate sensitivity (Davis *et al.*,2003).

Genomic amplification assays have greatly increased the sensitivity of nucleic acid test by extensive amplification of the target nucleic acid sequence before detection .Several amplification technologies have been devised ,of not are those based on thermal cycling amplification ,such as polymerase chain reaction (PCR),and those based on isothermic

amplification ,such as transcription mediated amplification (TMA),nucleic acid sequence based amplification (NASBA)or strand displacement amplification (SDA) (Karami *et al.,*2011).

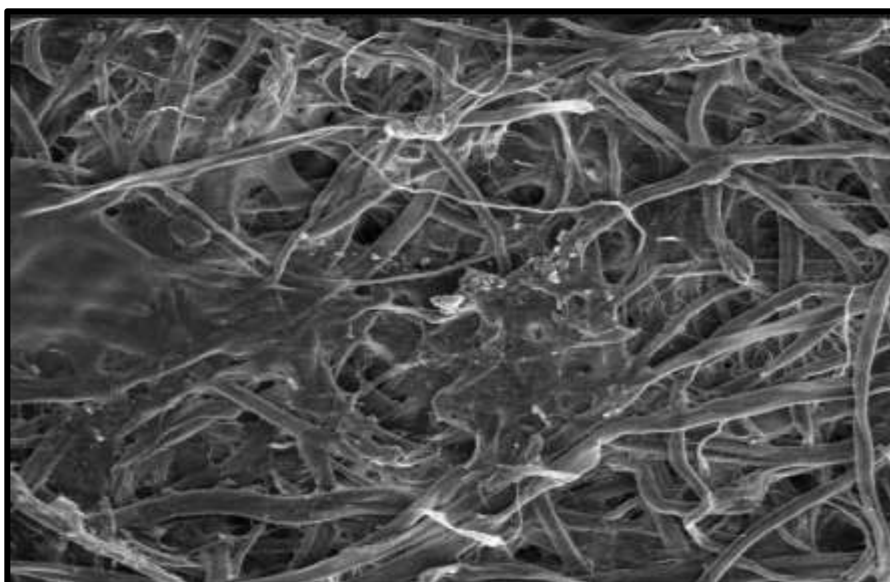
The aim of study is to determination the pathogensis of pseudomonas aeruginosa.

PHENOTYPIC IDENTIFICATION OF BACTERIA

Bacterial infections in people with cystic fibrosis have been the subject of numerous research (May et al., 1972). Thus, in order to detect the bacterial agents (105 CFU/mL), as depicted in figure (4-5), this study cultured samples acquired from individuals suspected of having cystic fibrosis disease on cetrimide agar. Cetrimide agar, commonly known as pseudosel agar, is used to isolate and presumptively identify *Pseudomonas aeruginosa*.

Figure (4-2): Growth of *Pseudomonas arogenosia* on cetrimide agar.

Also, some virulence genes were investigated, and produce biofilm as a gene and algc gene responsible for biofilm, and all isolates showed positive, as in the following figures



Figure(4-3): Electron micrograph showed the bacterial biofilm.... magnification 200micometer and WD 10.6mm and KV 20.00kv

We notice from the figure (4-3) a scanning electron microscope image of the scanning type when growing *Pseudomonas aeruginosa* spores on Konko Agar medium containing a percentage of sucrose (0.02) which is considered a catalyst for the formation of a biofilm. We also note the presence of water passages through which the nutritional exchange is controlled within the biofilm. This The structure, in turn, generates interstitial spaces between the bacterial colonies that are formed

Or in order for these bacteria to form a biofilm, suitable environmental conditions must be available, represented by the presence of a semi-submerged solid surface, sufficient nutrients and a high concentration of water (Relucenti *et al.*,2021).

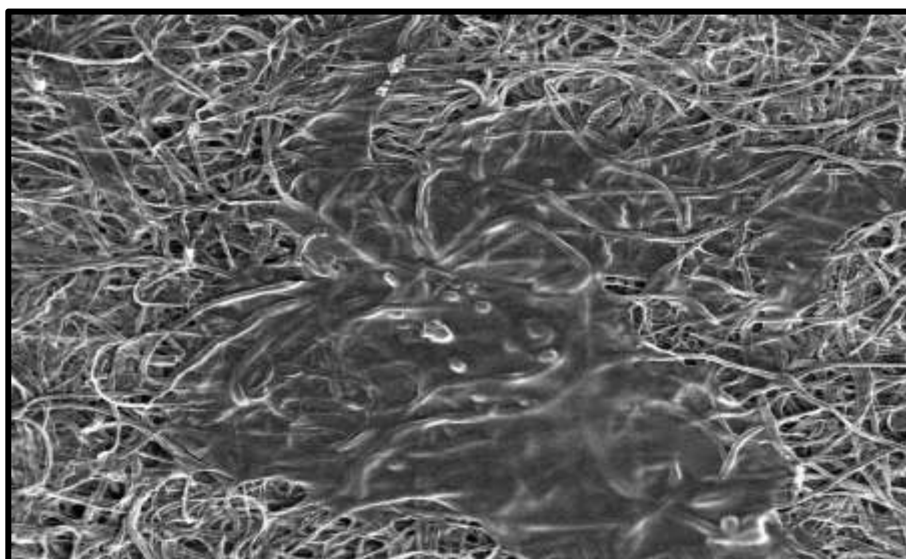


Figure (4-4): Electron micrograph showed the bacterial biofilm.... magnification 500micometer and WD 10.5 mm and KV 20.00kv.

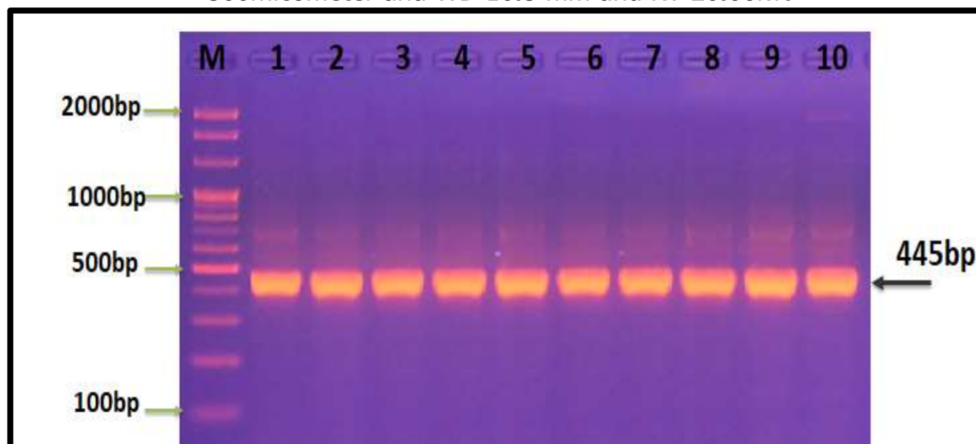


Figure (4-5): Agarose gel electrophoresis image that showed the PCR product analysis of exotoxin S gene in Pseudomonas aeruginosa isolates. Where Marker ladder (2000-100bp), Lane (1-10) showed positive *Pseudomonas aeruginosa exotoxin S gene* at 445bp PCR product size.

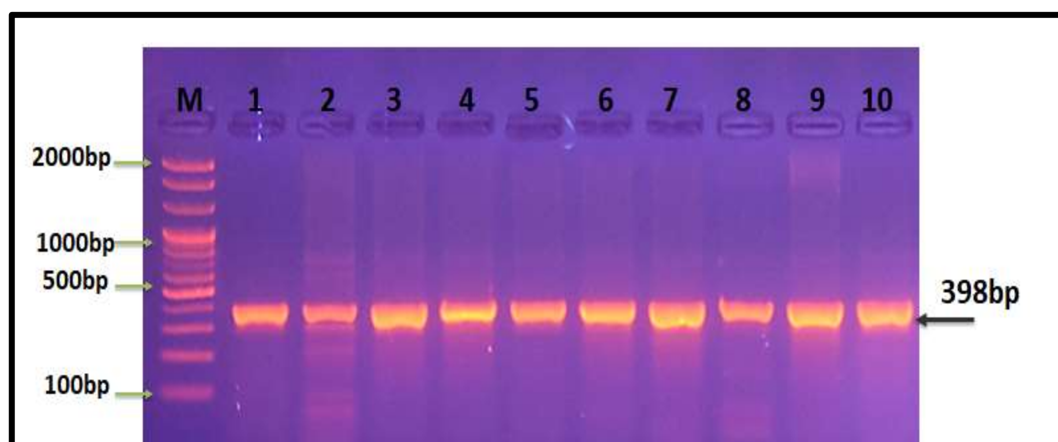
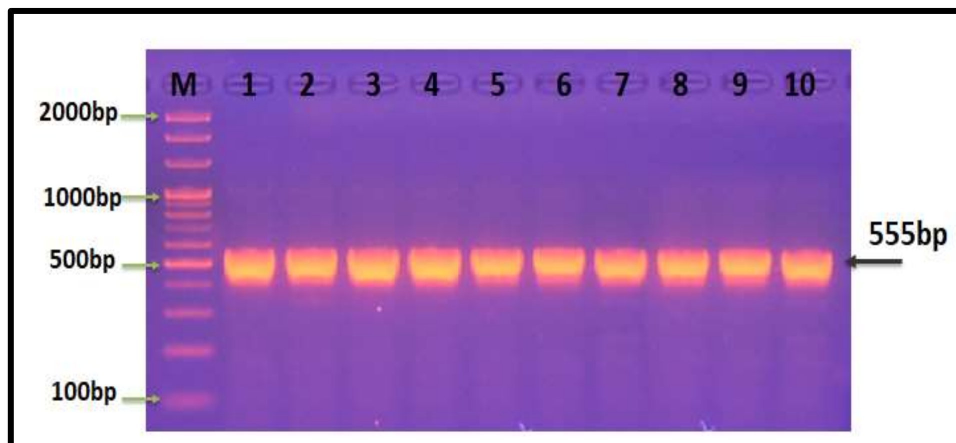


Figure (4-6): Agarose gel electrophoresis image that showed the PCR product analysis of biofilm formation algC gene in *Pseudomonas aeruginosa* isolates. Where Marker ladder (2000-100bp), Lane (1-10) showed positive *Pseudomonas aeruginosa* biofilm formation algC gene at 398bp PCR product size

Infections with bacteria are a major consequence of CF, with *Pseudomonas aeruginosa* and Staphylococcus aureus being the most common [(Camus *et al.*, 2021),(Orazi and O'Toole, 2017)]. In agreement with previous studies [(Malhotra *et al.*, 2019),(Hiby and Koch, 1990)], we found that *Pseudomonas arogenosia* could be isolated from CF

patients using PCR to analyze the 16s rRNA gene. However, no *Staphylococcus aureus* was isolated from CF patients in our investigation.

Therefore, environmental elements including temperature and seasons, community habits, genetic factors, and sample sizes could affect the prevalence of bacterial infections in patients. Studies with people who have cystic fibrosis should be continued.



Figure(4-7): Agarose gel electrophoresis image that showed the PCR product analysis of 16S ribosomal RNA gene for detection *Pseudomonas aeruginosa* isolates. Where Marker ladder (2000-100bp), Lane (1-10) showed positive *Pseudmonas aeruginosa* 16S ribosomal RNA gene at 555bp PCR product size.

3.2.Methods

3.2.1.Samples collection

A 50 sample from blood and sputum of patients suffer from bacterial infection were collected and the blood preserved in EDTA tube for DNA extraction purpose. The sputum was stored by conveying media and transferred to the laboratory directly. It is cultured on different culture media for cultivation. Patients age ranged 16_72 years. At the same time a 40 blood samples of healthy people were collected for the purpose of comparison and their age ranged 18-54 years. Sample collection date from 2/2/2023 to 1/5/2023, Samples were collected from people with cystic fibrosis, taking into account excluded samples such as cancer patients and chronic diseases, and adherence to research ethics.

Bacterial Polymerase Chain Reaction (PCR)

The PCR technique was performed for direct detection of *Pseudomonas aeruginosa* based 16S ribosomal RNA gene as well as detection some their some virulence factors genes (Toxin –exoS gene and Biofilm algC gene). This methods was carried out according to following steps:

PCR master mix preparation

The PCR master mix for each gene was prepared by using (**GoTaq® G2 Green Master Mix kit**) and this master mix done according to company instructions as following table

Standard PCR master mix

Table(3-7): Standard PCR master mix

PCR Master mix	Volume
DNA template 5-50ng	5 µL
Forward primer (10pmol)	2µL
Reverse primer (10pmol)	2µL
Green Master Mix kit	12.5µL
PCR water	3.5µL
Total volume	25 µL

After that, these PCR master mix components that mentioned in table above placed in standard PCR tubes transferred into Exispin vortex centrifuge at 3000rpm for 3 minutes, and then placed in PCR Thermocycler (BioRad-USA).

Primers

Bacteria PCR Primers

The PCR primer for detection *Pseudomonas aeruginosa* based on 16SrRNA gene and virulence factors Toxin -exoS gene and Biofilm algC gene were designed in this study by using NCBI-GenBank Data base and primer 3 plus online. These primers were synthesized by (Scientific Researcher Co. Ltd, Iraq) as following table:

Table (3-5): The *Pseudomonas aeruginosa* PCR primer with their sequence and product size

Gene	Sequence (5′-3′)		Product size	NCBI Reference code
16SrRNA gene	F	TACCTGGCCTTGACATGCTG	555bp	JQ968459.1
	R	TGGGGCCCCTTTTTCTTGTT		
Toxin -exoS gene	F	AGAGCGAGGTCAGCAGAGTA	445bp	AY029250.1
	R	ATCCCGCTGACATCGATTCC		
Biofilm algC gene	F	CATCTATCCGGACCGTCTGC	398bp	M60873.1
	R	CGATGATGGCGAACTTGCTG		

CONCLUSIONS

1. The absence of a risk factor in the dominant genotype of patients AA,GG.
2. The presence of a risk factor for the recessive genetic pattern A/G ,AA.
3. For alleles, the genetic allele G is responsible for genetic predisposition.
4. One of the results of polymorphism genetic heterogeneity is the presence of a genetic predisposition factor and a risk factor for patients in the genotype of cystic fibrosis genotype patients GC,AG.

REFERENCES

1. Relucenti, M., Familiari, G., Donfrancesco, O., Taurino, M., Li, X., Chen, R., Artini, M., Papa, R. and Selan, L., (2021). Microscopy methods for biofilm imaging: focus on SEM and VP-SEM pros and cons. Biology, 10(1), p.51.
2. Camus, L., Briaud, P., Vandenesch, F. and Moreau, K., (2021). How bacterial adaptation to cystic fibrosis environment shapes interactions between Pseudomonas aeruginosa and Staphylococcus aureus. Frontiers in microbiology, 12, p.617784.
3. Orazi, G. and O'Toole, G.A., (2017). Pseudomonas aeruginosa alters Staphylococcus aureus sensitivity to vancomycin in a biofilm model of cystic fibrosis infection. *MBio*, *8*(4), pp.10-1128.
4. Malhotra, S., Hayes Jr, D. and Wozniak, D.J., (2019). Cystic fibrosis and Pseudomonas aeruginosa: the host-microbe interface. Clinical microbiology reviews, 32(3), pp.10-1128.
5. Høiby, N. and Koch, C., (1990). Cystic fibrosis. 1. Pseudomonas aeruginosa infection in cystic fibrosis and its management. *Thorax*, *45*(11), p.881.
6. May, J.R., Herrick, N.C. and Thompson, D., (1972). Bacterial infection in cystic fibrosis. Archives of disease in childhood, 47(256), pp.908-913.
7. Zhu, H., Zhang, H., Xu, Y., Laššáková, S., Korabečná, M. and Neužil, P., (2020). PCR past, present and future. Biotechniques, 69(4), pp.317-325.
8. Zai, Y., Min, C., Wang, Z., Ding, Y., Zhao, H., Su, E. and He, N., (2022). A sample-to-answer, quantitative real-time PCR system with low-cost, gravity-driven microfluidic cartridge for rapid detection of SARS-CoV-2, influenza A/B, and human papillomavirus 16/18. Lab on a Chip, 22(18), pp.3436-3452.
9. Lenders, L.M., (2010). Utility of newer and novel technologies for the diagnosis and treatment-monitoring of tuberculosis using different biological fluids (Master's thesis, University of Cape Town).
10. Sapsford, K.E., Algar, W.R., Berti, L., Gemmill, K.B., Casey, B.J., Oh, E., Stewart, M.H. and Medintz, I.L., (2013). Functionalizing nanoparticles with biological molecules: developing chemistries that facilitate nanotechnology. Chemical reviews, 113(3), pp.1904-2074.
11. Alvarez-Barrientos, A., Arroyo, J., Cantón, R., Nombela, C. and Sánchez-Pérez, M., (2000). Applications of flow cytometry to clinical microbiology. Clinical microbiology reviews, 13(2), pp.167-195.
12. Davis, D.W., Krogh, T.E. and Williams, I.S., (2003). Historical development of zircon geochronology. Reviews in mineralogy and geochemistry, 53(1), pp.145-181.
13. Karami, A., Gill, P., Motamedi, M.H.K. and Saghafinia, M., (2011). A review of the current isothermal amplification techniques: applications, advantages and disadvantages. J Global Infect Dis, 3(3), pp.293-302.