IHJPAS. 37 (2) 2024
Ibn Al-Haitham Journal for Pure and Applied Sciences
Journal homepage: jih.uobaghdad.edu.iq
PISSN: 1609-4042, EISSN: 2521-3407

Detection of (*pslA*, and *PA-SS)* Genes in *Pseudomonas aeruginosa* Isolated from Clinical Cases

Tara Fouad Raouf Raouf [1], Melda Dölarslan[2] and Rana Mujahid Abdullah[3]
[1,2]Department of Biology, Graduate School of Natural and Applied Sciences, Çankiri Karatekin University, Türkiye.
[3]Department of Biology, College of Education for Pure Science (Ibn Al-Haitham), University of Baghdad, Baghdad, Iraq.
*Corresponding Author.

Received: 12 April 2023 Accepted: 7 June 2023 Published: 20 April 2024
doi.org/10.30526/37.2.3402

Abstract

In the current study, 100 different clinical samples were collected in Baghdad hospitals, including Teaching Laboratories Hospital, Medical City, Baghdad Teaching Hospital, and Martyr Ghazi Hariri Hospital, for a period from May 2022 to September 2022. After diagnosis, we obtained 19 isolates of *Pseudomonas aeruginosa* from different clinical sources, including burns, wounds, urinary tract infections, otitis media, and blood. Cultured all samples on MacConkey, cetrimide agar. Then it underwent several microscopic and biochemical tests. Included are: Oxidase, Citrate utilization, Triple sugar-iron (TSI), catalase, Gelatinase, Motility and Ornithine decarboxylation tests. *Pseudomonas aeruginosa* susceptibility to 11 antibiotics was tested using the disc diffusion method. It has been shown that *Pseudomonas aeruginosa* has multi-antibiotic resistance. It showed that all isolates were resistant to Penicillin 98%, Cefuroxime Sodium 98%, Amoxicillin/clavulanic Acid 98%, Erythromycin 98%, Oxacillin 98%, Cefotaxime 76%, Ofloxacin 58%, Cefipime 22%, Ceftazidime 16%, and Imipenem 16%. Amikacin was the most effective in isolates, as it was found that 8% are resistant to it. The bacteria's ability to produce biofilm was assessed using a crystal violet (CV) assay, and it was found that all isolates by 100% had the ability to produce biofilm. 15 isolates were 84% strong-forming, 2 (8%) were moderate-forming, and 2 (8%) were weak-biofilm-forming. The biofilm genes pslA, and PA-SS were studied. The results of this study showed that all isolates of *Pseudomonas aeruginosa* possess these genes by 100%.

Keywords: *pslA gene, PA-SS gene, Pseudomonas aeruginosa* .

1. Introduction

Pseudomonas aeruginosa is ubiquitous and gram-negative and causes nosocomial infections in immune-compromised patients with cancer, post-surgery burns infections, and human immunodeficiency virus (HIV) [1, 2]. In 2017, *Pseudomonas aeruginosa* was recognized as one of the most life-threatening bacteria and listed as a priority pathogen for research and development of new antibiotics by the World Health Organization [3]. Common antimicrobial agents frequently exhibit limited efficacy due to the adaptability and high intrinsic antibiotic resistance of *Pseudomonas aeruginosa* [4]. Additionally, treatment of these infections is also hindered by *Pseudomonas aeruginosa's* ability to form biofilms, which protect from surrounding

environmental stresses through phagocytosis and thereby confer the capacity for colonization and long-term persistence [5, 6].

Pseudomonas aeruginosa uses multiple interconnected signal transduction pathways for quorum sensing (QS), enabling the bacteria to communicate between cells and orchestrate collective behavior, which is essential for the adaptation and survival of whole communities [7]. Biofilms have a substantial bearing on over 90% of chronic wound infections. In the United States, approximately 6.5 million patients were affected by chronic wound infections, which resulted in a high health-care burden and economic consequences estimated at over US$25 billion [8]. It is important to diagnose *Pseudomonas aeruginosa* infections at an early stage before biofilm development, which could enhance the susceptibility of this bacteria to antimicrobial treatments. Biofilm is a complex aggregate of bacteria encased in a self-generated matrix of extracellular polymeric substances (EPS) and is one of the species' survival mechanisms during unexpected changes in living conditions such as temperature, fluctuation, and nutrient availability [9]. Bacteria can be saved from immune responses and are resistant to antimicrobials, increasing up to 1000 times more than other organisms. *Pseudomonas aeruginosa* is a well-known biofilm, which makes it an excellent model to study biofilm formation [10]. Biofilm is critical for bacteria to compete, survive, and dominate in the cystic fibrosis lung environment [11]. The aim is to study the detection of the *pslA* and *PA-SS* genes in *Pseudomonas aeruginosa* clinical cases.

2. Materials and Methods

2.1.Sample collection

100 samples were collected from different clinical cases for a period from May 2022 to September 2022 from Teaching Laboratories Hospital/Medical City, Martyr Ghazi Hariri Hospital, and Baghdad Teaching Hospital.

2.2.Identification of bacterial

2.2.1.Morphological examination

Phenotypic characteristics were studied through its growth on Cetrimide agar, MacConkey agar, and color generation on adjusted specialized agar to diagnose characteristics in terms of colony shape, color, size, and smell [12].

2.2.2.Microscope examination

Bacterial isolates were subjected to a microscopic examination by staining with Gram for the purpose of identifying the shape of the cells, their aggregation, and their reaction with Gram [13].

2.2.3.Biochemical tests

Biochemical tests done, which included Oxidase, catalase, Citrate utilization, Triple sugar-iron (TSI), Gelatinase, Motility, and Ornithine decarboxylation [14].

2.3. Antibiotic Susceptibility

A sensitivity test for bacterial isolates carried out using the Kirby-Bauer method After incubating overnight, we measured and recorded the distance across each zone in millimeters, and compared the results with CLSI (2018) [15].

2.4.Biofilm Formation

A microtiter plate measure was utilized for the measurement of the biofilm arrangement of pholates. Tests were performed in pre-sterilized polystyrene, 96-well microtiter plates [16].

2.5.Genomic DNA extraction:

A special kit was used to extract genomic DNA from the Presto ™ Mini g DNA Bacteria Kit. Genomic DNA **extraction** according to ABIO pure extraction.

2.6.Quantitation of DNA

A Quantus Fluorometer was used to detect DNA concentration.

2.7.Primer preparation

Lyophilized primers (Macrogen Company) were dissolved in nuclease-free water at a concentration (100 pmol/µl) as a stock solution. The Primer solution was prepared by adding (10 µl) of stock solution to (90 µl) of nuclease-free water to obtain (10 pmol/µl) (stored at -20 C). Primers (pslA and PA-SS) and conditions of PCR are shown in **Tables 1.** and **2.**

Table 1. Primers of (*pslA and PA-SS)* genes in this study

Primers	Sequence 5`-3`	Annealing (°C)	Product (bp)	References
pslA-F	TGGGTCTTCAAGTTCCGCTC	55	119	Abdulhaq *et al*., 2019 [17]
pslA-R	ATGCTGGTCTTGCGGATGAA			
PA-SSF	GGGGATCTTCGGACCTCA	56	956	Oluborode *et al*., 2018 [18]
PA-SSR	TCCTTAGAGTGCCCACCCG			

Table 2. Optimal conditions for (PCR)

Steps	Temperature		Mint. : sec.	Cycle
Initial Denaturation	95 °C		05:00	1
Denaturation	95°C		00:30	30mu
	Primer	**Temperature**		
Annealing	*pslA*	55°C	00:30	
	PA-SS	56°C		
Extension	72°C		00:30	
Final extension	72°C		07:00	1
Hold	10°C		10:00	

3.Results and Discussions

3.1. Diagnosis of bacteria

100 samples collected from clinical samples, including burns, wounds, urinary tract infection, otitis media, and blood, for a period of May 2022 to September 2022, from hospitals: Teaching Laboratories Hospital/Medical City, Baghdad Teaching Hospital, and Martyr Ghazi Hariri Hospital. After the final diagnosis, 19 isolates were found to belong to *Pseudomonas aeruginosa***.** A microscopic diagnosis was done, and it was found on examination by Gram stain that all isolates showed gram-negative status, and the culture characteristics of colonies of all were observed to be colonies with a characteristic odor that smells like grapes by a compound called 2-aminoacetophenone, as mentioned [19].

The diagnosis and phenotypic characteristics of bacteria were grown on Cetrimide agar, and it appeared that all confines had developed on this medium and created diverse sorts of shades. 10 (66%) created pyocyanin (blue-green); 3 (22%) created pyorubin (yellow-green); 4 (8%) created pyorubin (red-brown); and the final 2 (4%) showed up as unpigmented colonies [20]. On MacConkey agar, it looked non-lactose-fermented and paler [21].

As for the biochemical tests, all isolates were positive for oxidase. This shows that bacteria contain the enzyme cytochrom oxidase and catalase assays. It was positive for the citrate; there were no fermenters on the TSI test; all confines were motile on the motility test; negative for ornithine decarboxylation; and positive for gelatinase action.

Due to its widespread distribution and pathogenic potential, *Pseudomonas aeruginosa* is one of the most significant Pseudomonas species. An important source of infection in the critical care unit, it is an opportunistic pathogen [22]. According to studies, bacteremia (50%), VAP (30%), and bacteremia with *Pseudomonas aeruginosa* infections all had greater global death rates than other infections (20%) [23].

The study of [24] showed only 33 isolates of burn and wound infections from 75 isolates of *Pseudomonas aeruginosa,* while [25] showed (69%) of clinical sources of *P. aeurginosa.* This bacteria is a common cause of burn infection. *P.aeruginosa* was a very common infection in wounds and burns because of the ease of obtaining low-quality and ineffective antibiotics without a prescription. This may be due to the contamination of the hospital environment [26].

3.2.Antimicrobial Susceptibility test

An antibiotic sensitivity assay was tested on 11 antibiotics, and the results showed all isolates were resistant to penicillin, oxacillin, erythromycin, cefuroxime sodium, and amoxicillin/ clavulanic Some isolates appeared to be moderately resistant to Penicillin (98%), Cefuroxime (98%), Amoxicillin (98%), Oxacillin (98%), Erythromycin (98%), Cefotaxime (76%), and Ofloxacin (58%). The general resistance was for cefipime (22%), ceftazidime (16%), and imipenem (16%). Whereas amikacin appeared to have the most elevated strength against all confines, and as it were, 8 percent of the segregates were sensitive, all results as appeared in **Figure** 1.

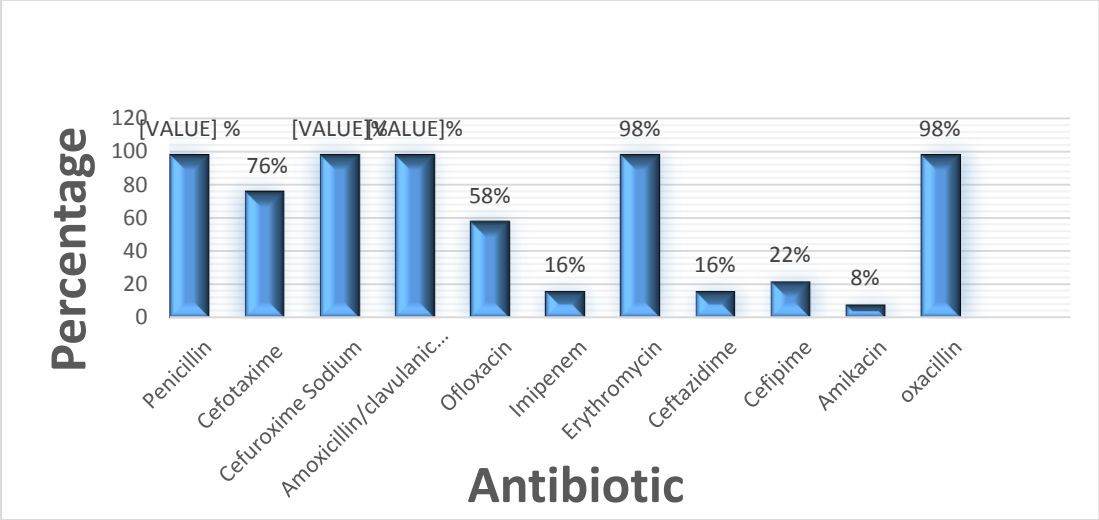

Figure 1. Percentage of resistance *Pseudomonas aeruginosa* to different antimicrobial.

The results showed that *Pseudomonas aeruginosa* is resistant to many antibiotics, including Ceftazidime (16%), which is similar to [27]. It was found that isolates were resistant to Ceftazidime by 16%. While the resistance to Amikacin was 8%, which was the highest resistance to antibiotics, This agrees with [28] that the resistance to Amikacin was 11.7%. As for Amoxicillin, the percentage was 98%, which is similar to [29], where the percentage was 100%. The resistance of Imipenem bacteria was 16%, which is close to the results obtained by [30]. by 23.5%. The rate of resistance to *Pseudomonas aeruginosa* in the current study to Ofloxacin was 58%, and this agrees with [31], as it was shown that the rate of resistance to Ofloxacin was 37%. The researchers [32] from Saudi Arabia reported that the resistance rate of *Pseudomonas aeruginosa* isolates to Imipenem was 35.4%, 43% for each of Ciprofloxacin and Amikacin, 38.5% for Cefepime, and 43% for Ceftazidime. This study is not compatible with the current study. [24] has shown that the isolates of *Pseudomonas aeruginosa have a* high resistance to

Cefotaxime 78% which is similar to what was obtained in this study. The study by Mustafa and Abdullah (2020) [33] shows that the gene encoded for aminoglycoside 6´-N-acyteltransferase type Ib can acetylate aminoglycosides and fluoroquinolones, leading to a reduction in susceptibility to these agents.

In the study by Abdullah (2016) [34], it was shown that Pseudomonas aeruginosa is resistant 100% to Cephalothin؛ Carbencillin؛ Amikacin Amoxicillin/clavulanic Acid, Ciprofloxacin, and Gentamicin.

3.3.Biofilm quantification by CV assay:

The biofilm-shaping capacity of *Pseudomonas aeruginosa* confines was assessed using the CV strategy. The biofilms of isolates were classified as powerless, medium, and solid biofilm makers according to their OD values. All isolates were 100% biofilm-producing, including: 15 isolates (84%) were strong biofilm makers; 2 isolates (8%) were direct biofilm makers; and the final 2 isolates (8%) were destitute biofilm makers, as shown in **Figure 2**.

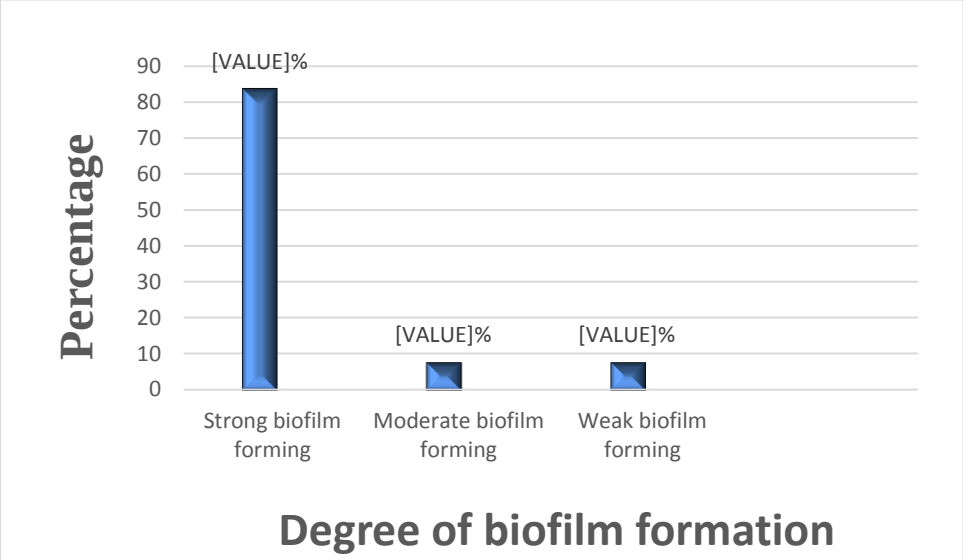

Figure 2. Biofilm formation degree of *Pseudomonas aeruginosa* using CV assay.

Our study, which showed all isolates were biofilm makers using the microtiter plaque strategy utilizing the CV test, this study agreed with [28]. as the ratio was 98.4%, and with [35] What was found was that the ratio was 96%, indicating a high rate of biofilm formation, and [36] showed that 98% of *Pseudomonas aeruginosa* isolates isolated from different clinical samples were biofilm-forming. However, a study conducted in Iran [37] reported that only 24% of Pseudomonas aeruginosa isolates were found to be biofilm-forming, which contradicts the findings of the current study. In Egypt, [38] reported that *Pseudomonas aeruginosa* had a percentage of 27.7% that was strong biofilm-forming, 21.3% that was moderate biofilm-forming, and 51.1% that was weak or non-biofilm-forming.

3.4. DNA extraction

A DNA kit (Promega, USA) was used to extract the DNA of bacterial isolates according to the manufacturer's instructions.

3.5. Measurement of DNA Concentration

After DNA extraction, the concentrations of DNA were measured using the Quantus Fluorometer. The results revealed that the concentrations of extracted DNA ranged between 20 and 25 ng/µl.

3.6. Detection of biofilm genes using polymerase chain reaction (PCR)

The detection of biofilm genes among our *Pseudomonas aeruginosa* was done through PCR using a thermal cycler. The PCR is based on the amplification of biofilm genes with specific primers. These genes included pslA *and PA-SS.* The results showed that isolates of *Pseudomonas aeruginosa* possess these genes in different proportions, as shown in **Table 3**. A polymerase chain reaction was carried out for all *Pseudomonas aeruginosa* using specialized primers for the purpose of detecting these genes. The results showed that 19 isolates at a rate of 100% possess a *pslA* gene. It was found that the gene has a molecular weight of 119 bp, as shown in **Figure 3**. Results showed that 19 isolates (100%) have the *PA-SS* gene. It was found that the molecular weight of this gene was 956 bp, as shown in **Figure 4**.

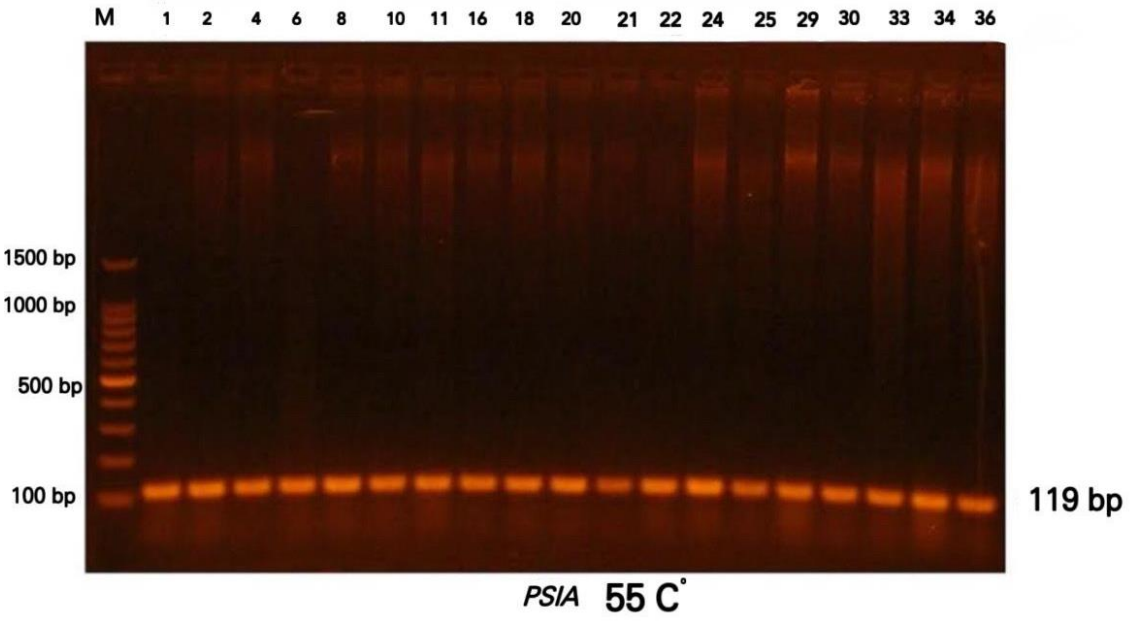

Figure 3. Amplification (*psIA*) in *Pseudomonas aeruginosa* on 1.5% agarose gel , a voltage 100 volts for 45 minutes with Eth.Br. M:100bp ladder. Lanes 1-36 isolate have *psIA* gene (119bp).

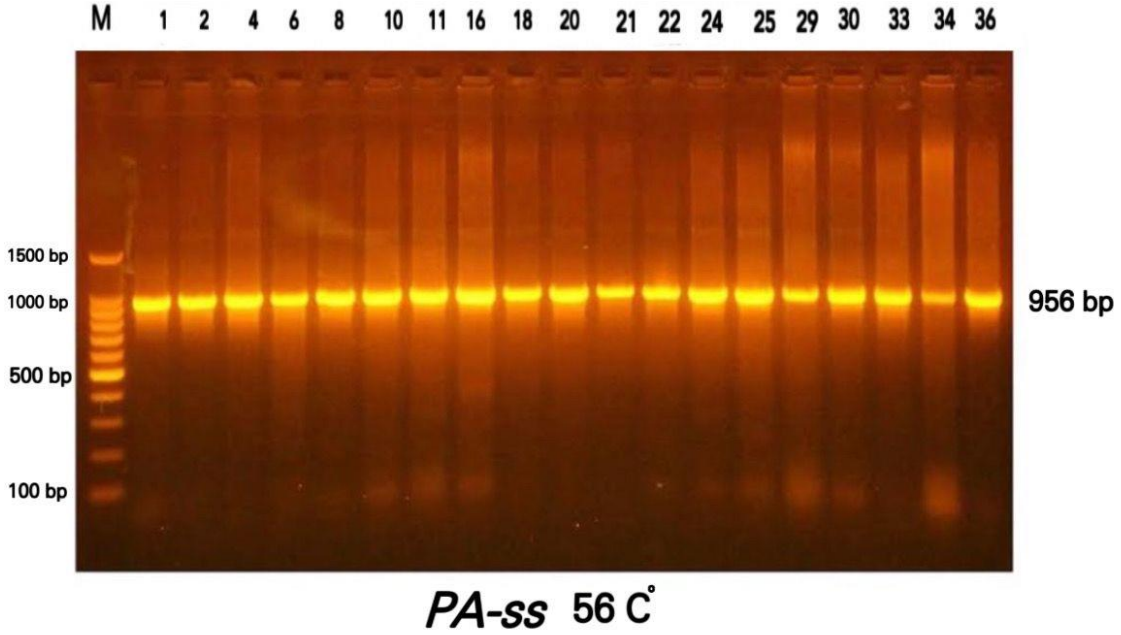

Figure 4. amplification (*Pa-ss*) in *Pseudomonas aeruginosa* on 1.5% agarose gel , a voltage 100 volts for 45 minutes with Eth.Br. M:100bp ladder. Lanes 1-36 isolate have *Pa-ss* gene (956bp).

Table 3. Percentage of Biofilm genes possessed by *Pseudomonas aeruginosa*

Sources	genes	The number of isolates that possess the genes	Percentage of presence of the genes (%)
Various clinical samples	*psIA*	19	100%
	Pa-ss	19	100%

Our research found that all 19 isolates had the *psIA* gene (100%). Previously, very similar results were obtained by [37, 39], showing that there is 100% of the *pslA* gene in *Pseudomonas aeruginosa.* [40] indicated that 90% of (35/39) isolates of *Pseudomonas aeruginosa* possess the *pslA* gene. and [41] found that the bacterial isolates possessed *pslA* by 92.1%. And [42] in Korea indicated that bacterial isolates isolated from different clinical samples possessed the *pslA gene* by 93.4%. And in Egypt, it was found that [38] the presence of the *pslA* gene was 89.4% of *Pseudomonas aeruginosa*.

The presence of the *pslA gene* has been shown to be a good marker for biofilm formation in *Pseudomonas aeruginosa* isolates, as reported in previous results. among the 48 isolates of *Pseudomonas aeruginosa*, primers for *PASS-F* and *PASS-R* confirmed that 40 species of *Pseudomonas aeruginosa* biofilm component by 83.34%, while the current study found (19/19) isolates have biofilm component *Pa-ss* gene in 100%. Biofilm formation by *Pseudomonas aeruginosa* is an important virulence factor as it provides self-protection from the host's phagocytic cell and prevents antibiotic access to it, as well as the persistence of infection and its role in causing Nosocomial infection [43].

4. Conclusions

All isolates of *Pseudomonas aeruginosa* showed high resistance to Penicillin, Cefuroxime Sodium, Amoxicillin/clavulanic acid, Erythromycin, and Oxyacillin. The lowest resistance was to Amikacin. The current study showed that all isolates of *Pseudomonas aeruginosa* are capable of biofilm formation. All isolates contained the biofilm genes *pslA and PA-SS*. at a rate of 100%.

Acknowledgment

The Authors would like to thank all workers at Teaching Laboratories Hospital, Medical City, Baghdad Teaching Hospital, and Martyr Ghazi Hariri Hospital for their assistance in samples collection.

Conflict of Interest

The authors declare that they have no conflicts of interest.

Funding

No funding**.**

References

1. Gomila, A.; Carratalà, J.; Badia, J.M., Camprubí, D.; Piriz, M.; Shaw, E.; Diaz-Brito, V.; Espejo, E.; Nicolás, C.; Brugués, M. Preoperative oral antibiotic prophylaxis reduces *Pseudomonas aeruginosa* surgical site infections after elective colorectal surgery: A multicenter prospective cohort study. *BMC Infect. Dis*. **2018**, *18*, 507. DOI: http://dx.doi.org/10.1186/s12879-018-3413-1.

2. Al-Shwaikh, R.M.A.; AL-Shuwaikh A.M.A.; Alornaaouti, A.F. BOX-PCR Fingerprinting for molecular genotyping of *P. aeruginosa. Asian Jr. Microbiol. Biotech. Env. Sc*. **2018**, *20*(*3*), 738-743.
3. World Health Organization. *Prioritization of Pathogens to Guide Discovery, Research and Development of New Antibiotics for Drug-Resistant Bacterial Infections, Including Tuberculosis*. World Health Organization; Geneva, Switzerland **2017**.
4. Pang, Z.; Raudonis, R.; Glick, B.R.; Lin, T.J.; Cheng, Z. Antibiotic resistance in *Pseudomonas aeruginosa*: Mechanisms and alternative therapeutic strategies. *Biotechnol. Adv.* **2019**, *37*, 177–192. DOI: http://dx.doi.org/10.1016/j.biotechadv.2018.11.013.
5. Moradali, M.F.; Ghods, S.; Rehm, B.H. *Pseudomonas aeruginosa* Lifestyle: A Paradigm for Adaptation, Survival, and Persistence. *Front. Cell Infect. Microbiol*. **2017**, *7*, 39. DOI: http://dx.doi.org/10.3389/fcimb.2017.00039.
6. Al-Shwaikh, R.M.A. DNA Sequences of *LasB* Gene in *Pseudomonas aeruginosa* Isolated from Some Clinical Cases. *Ibn Al-Haitham J. for Pure & Appl. Sci.* **2016**, *29(1),* 366-376.
7. Mukherjee S. and Bassler B.L. Bacterial quorum sensing in complex and dynamically changing environments. *Nat. Rev. Genet.* **2019**, *17*, 371–382. DOI: http://dx.doi.org/10.1038/s41579-019-0186-5.
8. Sen, C.K.; Gordillo, G.M.; Roy, S.; Kirsner, R.; Lambert, L.; Hunt, T.K.; Gottrup F.; Gurtner, G.C.; Longaker, M.T. Human skin wounds: A major and snowballing threat to public health and the economy. *Wound Repair Regen.* **2009**, *17*, 763–771. DOI: http://dx.doi.org/10.1111/j.1524-475X.2009.00543.x.
9. Moradali, M.F.; Rehm, B.H.A. Bacterial biopolymers: From pathogenesis to advanced materials. *Nat. Rev. Microbiol* **2020**, *18*, 195–210. DOI: http://dx.doi.org/10.1038/s41579-019-0313-3.
10. Crespo, A.; Blanco-Cabra, N.; Torrents, E. Aerobic Vitamin B12 Biosynthesis Is Essential for *Pseudomonas aeruginosa* Class II Ribonucleotide Reductase Activity During Planktonic and Biofilm Growth. *Front. Microbiol*. **2018**, *9*, 986. DOI: http://dx.doi.org/10.3389/fmicb.2018.00986.
11. Oluyombo, O.; Penfold, C.N.; Diggle, S.P. Competition in Biofilms between Cystic Fibrosis Isolates of *Pseudomonas aeruginosa* Is Shaped by R-Pyocins. *mBio.* **2019**, 10, e01828-18. DOI: http://dx.doi.org/10.1128/mBio.01828-18.
12. Wanger, A.; Chavez, V.; Huang, R.S.P.; Wahed, A.; Actor, J.K.; Dasgupta, A. *Microbiology and Molecular Diagnosis in Pathology*. Elsevier Inc. All Rights Reserved, 300 pp. **2017**.
13. Levinson, W. *Review of Medical Microbiology and Immunology*. 14th ed. McGraw-Hill education, Inc, 821 pp. **2016**.
14. Tille, P.M. *Bailey and Scott's diagnostic microbiology*. Thirteen edition. Mosby, Inc., an affiliate of Elsevier Inc. 3251 Riverport Lane. St. Louis. Missouri 63043, **2014**.
15. CLSI. Performance standards for antimicrobial susceptibility testing. 28th ed. Clinical and Laboratory Standards Institute, Wayne, PA2018 (CLSI supplement M100) **2018**.
16. Stepanovic´, S.; Vukovic´, D.; Hola, V.; Di Bonaventura, G.; Djukic´, S.; C´ irkovic´, I.; Ruzicka, F. Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. APMIS **2007**, *115*, 891–899. DOI: http://dx.doi.org/10.1111/j.1600-0463.2007.apm_630.x.
17. Abdulhaq, N.; Nawaz, Z.; Zahoor, M.A.; Siddique, A.B. Association of biofilm formation with multi drug resistance in clinical isolates of *Pseudomonas aeruginosa*. *EXCLI J*. **2020**, *18(19),* 201-208. DOI: 10.17179/excli2019-2049.
18. Oluborode, B.; Smith, S.; Seriki, T.; Ajayi, A.; Coker, A. (2018). Antibiotic Susceptibility Pattern and Molecular Typing By PCR-RAPD Analysis of Clinical and Environmental Isolates of *Pseudomonas aeruginosa*. *Microbiology and Biotechnology Letters*. **2018**, *46*, 434-437. DOI: http://dx.doi.org/10.4014/mbl.1805.05007.
19. Sastory, A.S.; Bhat, S. *Essentials of Medical Microbiology*. Jaypee Brothers Medical Publishers. New Delhi.844pp. **2021**.

20. SudhaKar, T.; Karpngam, S.; Premkumer, J. Biosynthesis antibacterial activity of pyocyanin pigment ced produced by *pseudomonas aeruginosa* SU1. *JCPRG5* **2015**, *7(3)*, 921-924.
21. Baron, E.J.; Finegold, S.M.; Peterson, I.L.R. *Bailey and Scotts Diagnostic Microbiology*. 9th ed. Mosby Company. Missouri **2007**.
22. Patil, P.; Joshi, S.; Bharadwaj, R. Aerobic bacterial infections in a burns unit of Sassoon General Hospital, Pune. *International J. Healthcare Biomed. Res*. **2015**, *03*, 106-112.
23. Ceniceros, A.; Peertega, S.; Galeiras, R.; Predicting mortality in burn patients with bacteremia. *Infection*. **2016**, *44(2)*, 215–22. DOI: 10.1007/s15010-015-0847-x.
24. Al-Shwaikh, R.M.A.; Alornaaouti, A.F. Detection of *tox A* gene in *Pseudomonas* aeruginosa that isolates from different clinical cases by using PCR. *Ibn Al-Haitham Journal for Pure and Applied Science* **2017**, 26-30. DOI: http://dx.doi.org/10.30526/2017.IHSCICONF.1767.
25. Al-Azzawi, S.N.; Abdullah R.M. Study of the resistance of *P. aeruginosa* isolated from wounds and burns for some disinfects and antiseptic from some Baghdad hospitals. *J. Pharm. Sci. and Res*. **2018**, *10(6)*, 1481-1484.
26. Obaid, S.A.; Al-Shwaikh, R.M. Evaluation the Efficacy of Bacteriophage against *Pseudomonas aeruginosa* Isolated from Wound and Burn Infections. *Pakistan Journal of Medical & Health Sciences* **2022**, *16(4)*, 440- 444. DOI: http://dx.doi.org/10.53350/pjmhs22164440.
27. Shariati, A.; Asadian, E.; Fallah, F.; Azimi, T.; Hashemi, A.; Yasbolaghi Sharahi, J.; Taati Moghadam, M. Evaluation of Nano-curcumin effects on expression levels of virulence genes and biofilm production of multidrug-resistant *Pseudomonas aeruginosa* isolated from burn wound infection in Tehran, Iran. *Infect Drug Resist*. **2019**, *12*, 2223-2235. DOI: http://dx.doi.org/10.2147/IDR.S213200.
28. Roulová, N.; Mot'ková, P.; Brožková, I.;Pejchalová, M. Antibiotic resistance of *Pseudomonas aeruginosa* isolated from hospital wastewater in the Czech Republic. J Water Health **2022**, *20*(4), 692–701. DOI: http://dx.doi.org/10.2166/wh.2022.101.
29. Al-Shwaikh, R.M. *Production and Characterization of Protease from Pseudomonas aeruginosa Isolated from Some Clinical Cases and its Relation with some Antibiotic Agents*. Ph.D. Thesis. College of Science . AL-Mustansiryia University **2006**.
30. Idrees, E.K.M. Comparison between different phenotypic and genotypic methods for detection of metallo β–lactamases in *Pseudomonas aeruginosa* that has multidrug resistance to antibiotics . College of Science. Abdel Aziz University **2012**.
31. Hassuna, N.A.; Ibrahim, A.H.; Abo Eleuoon, S.M.; Rizk, H.A.W. High prevalence of Multidrug Resistant *Pseudomonas aeruginosa* recovered from Infected Burn Wounds in children. *Archives of Clinical Microbiology* **2015**, *6(4)*, 1-7.
32. Asghar, A.H.; Ahmed, O.B. Prevalence of aminoglycoside resistance genes in *Pseudomonas aeruginosa* isolated from a tertiary care hospital in Makkah, KSA. *Clinical Practice* **2018**, *15(2)*, 541-547**.** DOI: http://dx.doi.org/10.4172/clinical-practice.1000391.
33. Mustafa, S.M.; Abdullah, R.M. Investigation for some Aminoglycosides Modifying Enzymes-Encoding Genes and Co-Resistance to Fluoroquinolones among *Klebsiella pneumoniae* Isolates from Different Clinical Cases. Iraqi Journal of Science **2020**, *61(11)*, 2866-2878. DOI: http://dx.doi.org/10.24996/ijs.2020.61.11.10.
34. Abdullah, R.M. A Study the Effect of TiO2 Nanoparticles Combination with Antibiotics and Plant extracts Against Some Gram Negative Bacteria. *Baghdad Journal of Science* **2016**, *3(13)*, 425- 434. DOI: http://dx.doi.org/10.21123/bsj.2016.13.3.0425.
35. Banar, M.; Emaneini, M.; Satarzadeh, M.; Abdellahi, N.; Beigverdi, R; Leeuwen, W.B. Evaluation of Mannosidase and Trypsin Enzymes Effects on Biofilm Production of *Pseudomonas aeruginosa* Isolated from Burn Wound Infections. *PLoS One* **2016**, *11(10)*, e0164622. DOI: http://dx.doi.org/10.1371/journal.pone.0164622.
36. Namuq, A.O.; Ali, K.O.M. and Al-Ani, A.H. Correlation between biofilm formation, multi-drug resistance and *algD* gene among *Pseudomonas aeruginosa* clinical isolates. *Journal of University of Babylon for Pure and Applied Sciences* **2019**, *27(3)*, 143-150.

37. Elmaraghy, N.; Abbadi, S.; Elhadidi, G.; Hashem, A.; Yousef, A. Virulence genes in *Pseudomonas aeruginosa* strains isolated at Suez canal university hospitals with respect to the Site of infection and antimicrobial resistance. *Int. J. Clin. Microbiol Biochem Technol*. **2019** *2*, 8-19. DOI: https://doi.org/10.29328/journal.ijcmbt.1001006.
38. Abootaleb, M.; Zolfaghari, M.R.; Soleimani, N.A.; Ghorbanmehr, N.; Yazdian, M.R. Biofilm formation with Microliter plate 96 and *pslA* detection of *Pseudomonas aeruginosa* isolates from clinical samples in Iran. *Int J Adv Biol Biomed Res*. **2020**, *8*, 58-66.
39. Heydari, S.; Eftekhar, F. Biofilm formation and β-lactamase production in burn isolates of *Pseudomonas aeruginosa*. *Jundishapur J Microbiol*. **2015**, *8*, 1-5. DOI: http://dx.doi.org/10.5812/jjm.15514.
40. Issa, M.A.; Ghaima, K.K.; Saleh, M.B.; Nader, M.I. Antibiogram study and prevalence of pslA gene among biofilm *pseudomonas aeruginosa* producers isolated from some clinical specimens in Thiqar province. *Pakistan Journal Biotechnol*ogy **2018**, *15*(*3*), 681-688. DOI: https://pjbt.org/index.php/pjbt/article/view/361.
41. Motevasel, M.; Haghkhah, M. Antimicrobial resistance profiles and virulence genes of *Pseudomonas aeruginosa* isolates originated from hospitalized patients in Shiraz, Iran. *Journal of Medical Microbiology and Infectious Diseases* **2018**, *6(2)*, 72-76. DOI: http://dx.doi.org/10.29252/JoMMID.6.2.3.72.
42. Cho, H.H.; Kwon, K.C.; Kim, S.; Park, Y.; Koo, S.H. Association between biofilm formation and antimicrobial resistance in carbapenem-resistance *Pseudomonas aeruginosa*. *Ann. Clin. Lab. Sci*. **2018**, *48(3)*, 363-368.
43. Pedersen, S. S.; Hoiby, N.; Espersen, F.; Koch, C.H. Role of alginate in infection with mucoid *Pseudomonas aeruginosa* in cystic fibrosis. *Thorax* **1992**, *47*(*1*), 6-13. DOI: http://dx.doi.org/10.1136/thx.47.1.6.