Pakistan J. Zool., pp 1-7, 2024. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20231124100835

Genetic Diversity and Molecular Characterization of Virulence Determinants and Integrons in *Pseudomonas aeruginosa* Isolated from Canal Water in Peshawar Pakistan

Urooj Amjad[1], Kafeel Ahmad[1]*, Hifsa Saadia[1] and Imran Muhammad Khan[2]

[1]*Centre of Biotechnology and Microbiology, University of Peshawar, Peshawar, Pakistan*
[2]*Burns and Plastic Surgery Centre, Hayatabad, Peshawar, Pakistan*

Article Information
Received 24 November 2023
Revised 25 December 2024
Accepted 11 January 2024
Available online 27 May 2024
(early access)

Authors' Contribution
UA performed samples collection, experimental work, data analysis and manuscript writing. KA conceived the idea, contributed to experimental work, conducted analysis, contributed to manuscript writing and proofreading. HS helped in data analysis and manuscript writing. IMK contributed to data analysis and proofreading. All the authors approved the final version of the manuscript.

Key words
***Pseudomonas aeruginosa*, Virulence genes, Integrons, RAPD, Antibiotics, Genetic diversity**

ABSTRACT

Pseudomonas aeruginosa is a clinically significant Gram-negative bacillus with remarkable ability to thrive in diverse environments that could be attributed to its minimal nutritional demands allowing it to exist in settings like wastewater, surface water, and moist conditions and even on inert surfaces. This adaptability makes *P. aeruginosa* noteworthy for its environmental resilience and clinical importance. This study aimed to examine the genetic determinants of virulence (*toxA, lasB, plcH, exoS*), occurrence of integrons (*int1, int2*) and molecular diversity among *P. aeruginosa* isolates recovered from canal water in Peshawar, Pakistan. A total of fifty samples were collected and processed for isolation of *P. aeruginosa.* Antibiotic susceptibility was conducted against eight different classes of antibiotics i.e. Norfloxacin (10µg), Ticarcillin (75µg), Doripenem (10µg), Azithromycin (10µg), Ciprofloxacin (5µg), Levofloxacin (10µg), Polymyxin (300U) and Colistin (10µg). Isolated *P. aeruginosa* were analyzed for the presence of *lasB, toxA, exoS, and plcH, int1*and *int2* genes. Genetic diversity was analyzed through RAPD PCR. Results showed 98%, 74%, 100%, 26%, 70%, 98%, 100%, 100% susceptibility towards Norfloxacin, Ticarcillin, Doripenem, Azithromycin, Ciprofloxacin, Levofloxacin, Polymyxin and Colistin respectively. The occurrence of *lasB, toxA, exoS and plcH* was confirmed in 98% (n=49), 86% (n=43), 88% (n=44) and 96% (n=48) isolates. *Int1* gene was detected in 90% (n=45) isolates whereas *int2* gene was not detected. Among the 50 isolates, 26 were clustered into 11 distinct clones whereas 24 isolates showed distinct RAPD profiling. The presence of highly pathogenic strains of *P. aeruginosa* highlights the requirement for efficient strategies for control and prevention.

INTRODUCTION

Pseudomonas aeruginosa could lead to community-acquired infections like keratitis, folliculitis, and ear infections primarily due to exposure to recreational water containing this bacterium (Moore *et al*., 2011). Presence of *P. aeruginosa* in various water bodies has been reported from different parts of the world. Individuals living in proximity to the river could experience a high incidence of skin diseases, urinary tract infections (UTIs) and gastroenteritis infections (GTIs) (Chakraborti *et al*., 2016). The virulence factors of *P. aeruginosa* enable it to adhere to host cells, disrupt signaling and invade the host leading to persistent infections (Diggle and Whiteley, 2020). The virulence factor *ExoS* serves as the principal cytotoxin essential for multiple functions including invasion, colonization and bacterial dissemination during infection. In *P. aeruginosa* the *toxA* gene encodes the key virulence factor, exotoxin A, which is responsible for inhibiting protein synthesis. *P. aeruginosa* produces the hemolysin encoded by *plcH* gene which leads to the degradation of lipids and lecithin (Hofmann *et al*., 2021). Within the human body, collagen is a crucial protein found in numerous tissues. The product of *LasB* gene is responsible for breaking down type III and type IV collagens. An Iranian study revealed a notably higher

* Corresponding author: kafeelpbg@gmail.com
0030-9923/2024/0001-0001 $ 9.00/0

presence of *lasB*, *exoS*, and *toxA* genes in *P. aeruginosa* isolated from children with cystic fibrosis (Camiade *et al*., 2020). Various other studies have reported the occurrence of various virulence factors in *P. aeruginosa* of clinical and environmental origin (Bhasin, 2020; Ali and Al-Kenanei, 2020; Diggle and Whiteley, 2020; Bahador *et al*., 2019).

Microbial typing plays a crucial role in establishing connections between different microbial strains (Suárez *et al*., 2020). Randomly Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) is commonly used for estimating genetic diversity among bacteria (Goudarzi *et al*., 2016). RAPD offers a rapid and efficient way to assess DNA polymorphism at multiple loci in an organism's genome. It is a simple, cost-effective technique that doesn't require prior knowledge of the target DNA sequence (Suárez *et al*., 2020; Nanvazadeh *et al*., 2013). Horizontal gene transfer mechanisms, facilitated by mobile genetic elements (MGE) are pivotal in the acquisition of resistance genes in *P. aeruginosa*. Resistance genes dissemination in the environment is notably influenced by plasmids or transposons carrying integrons. Among various Integrons, the class 1 integrons are most prevalent in *P. aeruginosa* and are primarily associated with β-lactamase-mediated resistance (Goudarzi *et al*., 2016). Class 1 integrons are widespread among Gram-negative bacteria in diverse environments including wastewater. However, Class 2 integrons are seldom reported in *P. aeruginosa* (Bhasin *et al*., 2020; Bahador *et al*.,, 2019). This study aimed to address the lack of data regarding molecular characteristics of *P. aeruginosa* prevailing in canal water by investigating the presence of virulence genes, integrons and genetic diversity of *P. aeruginosa* isolates recovered from canal water in Peshawar, Pakistan.

MATERIALS AND METHODS

Isolation, culturing and identification

Fifty water samples were collected from various canals in the Peshawar district of Khyber Pakhtunkhwa and transferred to laboratory in cold conditions for further processing. For isolation of bacteria, water samples (100 µl) were inoculated onto MacConkey agar plates (Oxoid, UK) followed by incubation at 37 °C for 24 h. Standard procedures including Gram staining and a series of biochemical tests including catalase, oxidase, motility, citrate, indole, triple sugar iron, methyl red (MR), Voges-Proskauer (VP), gelatin hydrolysis, casein hydrolysis, urease and phenotypic biofilm formation tests were performed to identify *P. aeruginosa* (Al-Bayati *et al*., 2021).

Antimicrobial susceptibility

Antibiotic susceptibility testing was conducted using the Kirby-Bauer method. A fresh bacterial culture was evenly spread on Muller Hinton agar medium and antibiotic discs Levofloxacin (LEV) 5µg; Doripenem (DOR) 10µg; Norfloxacin (NOR) 10µg; Azithromycin (AZM) 15µg; Ticarcillin (TIC) 75µg; Cefaperazone (CFP) 75µg; Polymyxin (PB) 300 U; Colistin (CT) 10µg were placed at specified intervals in accordance with CLSI guidelines and reported procedures (Mohamed *et al*., 2020). Following incubation, zones of inhibition were measured to determine resistance, intermediate sensitivity or sensitivity.

Detection of virulence genes

Bacterial DNA was extracted using bacterial genomic DNA extraction kit (solar bio, D1600). DNA extraction was performed according to the kit instructions. The quality of extracted DNA was checked through gel electrophoresis using 1% agarose.

Presence of four virulence genes i.e. *exoS, toxA, lasB* and *plcH* were analyzed. PCR reaction mix consisted of 4 µl master mix (FIREpol Cat. No. 04-12-00115), 1 µl reverse primer, 1 µl forward primer, 13 µl water and 1 µl DNA template to make the final volume up to 20 µl. PCR products were checked through gel electrophoresis using 1% agarose. Primer sequences for different virulence genes are given in Table I.

Table I. Primer sequence for detection of virulence genes.

Gene	Sequence of primers 5′ → 3′	Size of products in bps
Virulence genes		
toxA	CTGCGCGGGTCTATGTGCC	270
	GATGCTGGACGGGTCGAG	
exoS	CGTCGTGTTCAAGCAGATGGTGCTG	444
	CCGAACCGCTTCACCAGGC	
lasB	GGAATGAACGAAGCGTTCTCCGAC	284
	TTGGCGTCGACGAACACCTCG	
plcH	GCACGTGGTCATCCTGATGC	608
	TCCGTAGGCGTCGACGTAC	
Integrons		
Int1	CAGTGGACATAAGCCTGTTC	160
	CCCGAGGCATAGACTGTA	
Int2	CACGGATATGCGACAAAAAGGT	789
	GTAGCAAACGAGTGACGAAATG	

The PCR thermal cycle for *toxA, exoS, lasB* and *plcH* comprise initial denaturation at 95°C for 5 min followed by 30 cycles each of denaturation at 94 °C for 30 sec, anncaling at 63 °C for 1 min, and extension at 72 °C for 1 min. The final extension was done at 72 °C for 5 min, with the following minor deviations: (1) the number of thermal cycles was 35 for *exoS* instead of 30. (2) annealing was done at 61 °C for *exoS* and 55 °C for *lasB* and *plcH*.

Analysis of integrons

Primer sequences used for the analysis of integrons are shown in Table I. For amplification, the reaction mixture consisted of: 4ul master mix (FIREpol Cat. No. 04-12-00115), 1µl reverse primer, 1µl forward primer, 13 µl water and 1µl DNA template to make the final volume up to 20 µl. The DNA fragments that were amplified were subjected to gel electrophoresis using 1% agarose gel. PCR conditions for *int1* and *int2* genes were initial denaturation at 94 °C for 5 min, followed by 30 cycles, each of initial denaturation at 94 °C for one min, annealing at 59 °C for 1 min, extension at 72 °C for 1 min. The final extension was done at 72 °C for 5 min.

Genetic diversity analysis

For analysis of genetic diversity, previously documented random decamer primer known as RAPD 272 was utilized (Ranjbar *et al*., 2014). The amplified RAPD fragments were separated through gel electrophoresis using 1% agarose gel. A 100 base pair DNA ladder (Solis BioDyne no.07-11-00005) was employed for size comparison.

Statistical analysis

To create bivariate data for statistical analysis, the bands obtained from RAPD-PCR were coded as either 0 (indicating absence) or 1 (indicating presence). This binary data was then used to calculate genetic diversity among *P. aeruginosa* isolates. Genetic distance among isolates was estimated as reported (Ali *et al*., 2021). The software MEGA X was employed to construct a dendrogram using the bivariate data obtained from the RAPD-PCR analysis (Ranjbar *et al*., 2014).

RESULTS

Antibiotics susceptibility

The results of antibiotic sensitivity are shown in Table II. All the isolates (n=50) showed susceptibility towards Doripenem, Polymyxin and Colistin. 98% (n=49) isolates showed susceptibility towards Levofloxacin and Norfloxacin while only 2% (n=1) isolates were resistant. 70% (n=35) of the samples showed susceptibility towards ciprofloxacin, 14% (n=7) isolates were resistant whereas 16% (n=8) isolates were intermediate. For Ticarcillin antibiotic 74% (n=34) isolates were susceptible, 14% (n=7) were resistant while 12% (n=6) were intermediate. Out of 50 samples, 26% (n=13) of them showed susceptibility towards Azithromycin, 56% (n=28) were resistant while 14% (n=7) showed intermediate resistance.

Table II. Antibiotic susceptibility of *Pseudomonas aeruginosa* isolates.

S. No	Name of antibiotics	Sensitive (S) % (no)	Intermediate (I) % (no)	Resistant (R) % (no)
1	Ticarcillin	74% (n=37)	12% (n=6)	14% (n=7)
2	Norfloxacin	98% (n=49)	0% (n=0)	2% (n=1)
3	Levofloxacin	98% (n=49)	0% (n=0)	2% (n=1)
4	Ciprofloxacin	70% (n=35)	16% (n=8)	14% (n=7)
5	Doripenem	100% (n=50)	0% (n=0)	0% (n=0)
6	Polymyxin	100% (n=50)	0% (n=0)	0% (n=0)
7	Colistin	100% (n=50)	0% (n=0)	0% (n=0)
8	Azithromycin	26% (n=13)	14% (n=7)	56% (n=28)

Detection of virulence genes

Result on the occurrence of virulence genes among *P. aeruginosa* isolates are shown in Table III and Supplementary Figure 1. The *lasB* gene was present in 98% isolates, *plcH* gene in 96% isolates, *exoS* gene in 88% isolates and *toxA* in 86% isolates. The highest frequency was observed for the *lasB* gene while the lowest prevalence was seen for the *toxA* gene. The presence of the class 1 integron (*int1* gene) was noted in 90% isolates (Table III, Fig. 1). None of the isolates were found to contain the class 2 Integron genes (*int2* gene).

Genetic diversity

A total of 13 distinct RAPD bands were observed among all the isolates. The highest band size was 1500 bp whereas the lowest band size was 300 bp. A phylogenetic tree based on RAPD genetic diversity data is shown in Figure 1. Among the 50 isolates, 26 isolates were grouped into 11 distinct clones while 24 isolates exhibited unique RAPD profiles based on similarity coefficients of ≥ 80%. The 11 distinct clones were labeled as follows: C1 (isolates 39 and 45), C2 (isolates 28 and 37), C3 (isolates 18, 7, and 48), C4 (isolates 15, 25, and 23), C5 (isolates 6 and 41), C6 (isolates 14 and 32), C7 (isolates 2, 40, and 36), C8 (isolates 1, 50, and 17), C9 (isolates 24 and 43), C10 (isolates 47 and 49), and C11 (isolates 16 and 30).

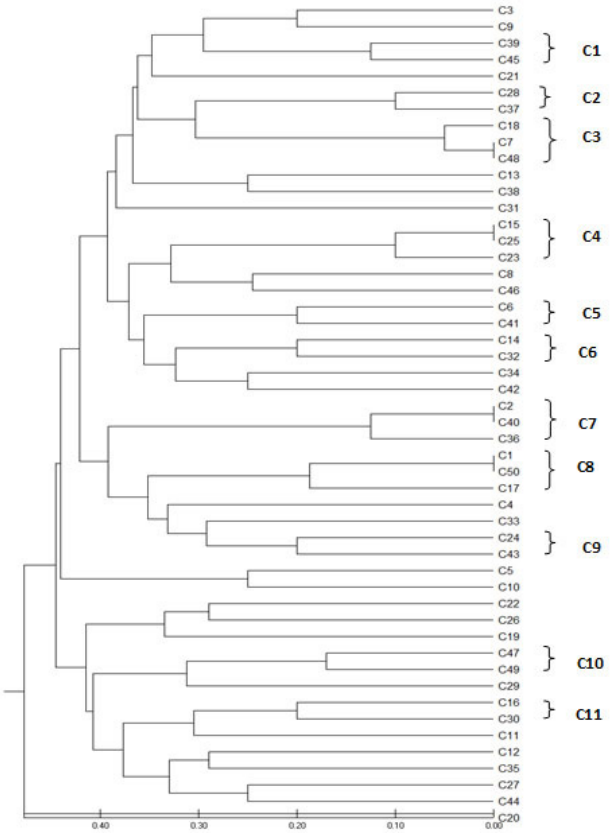


Fig. 1. Phylogenetic tree showing grouping of *P. aeruginosa* isolates. The software MEGA-X was used to generate the phylogenetic tree using the UPGMA method. Thirty-six isolates were grouped into 11 distinct clones: C1 (39, 45) C2 (28, 37) C3 (18, 7, and 48) C4 (15, 25, and 23) C5 (6, 41) C6 (14, 32) C7 (2, 40, and 36) C8 (1, 50, and 17) C9 (24, 43) C10 (47, 49) C11 (16, 30) based on similarity coefficient ≥ 80%. Twenty four isolates showed unique RAPD profiles.

Table III. Occurrence of virulence and integrase genes in *P. aeruginosa* isolates.

Sample	Location	Virulence genes				Integrase genes	
		lasB	*plcH*	*exoS*	*toxA*	*intI1*	*intI2*
C1	Danishabad	+	+	+	+	+	-
C2	Danishabad	+	+	+	+	+	-
C3	Galaxy mart	+	+	+	+	+	-
C4	Galaxy mart	+	+	+	+	+	-
C5	Board bazar	+	+	+	+	+	-
C6	Board bazar	+	+	+	+	+	-
C7	Board bazar	+	+	+	+	+	-
C8	University town	+	+	+	-	+	-
C9	University town	+	+	+	+	+	-
C10	University town	+	+	+	+	+	-
C11	Malakhander	+	+	+	+	+	-
C12	Malakhander	+	+	+	+	+	-
C13	Malakhander	+	+	+	+	+	-
C14	Malakhander	+	+	+	+	+	-
C15	Umer gul road	+	+	+	+	+	-
C16	Umer gul road	+	+	+	+	+	-
C17	Askari 6	+	+	-	-	-	-
C18	Askari 6	+	+	-	-	-	-
C19	DHA gate	+	+	+	+	-	-
C20	DHA gate	+	+	+	-	+	-
C21	Achini	+	+	+	+	+	-
C22	Achini	+	+	+	+	+	-
C23	Shalman phase 2 hayatabad	+	+	+	+	+	-
C24	Shalman phase 2 hayatabad	+	+	+	+	+	-
C25	Shalman phase 2 hayatabad	+	+	+	+	+	-
C26	Jamrud road	+	+	+	+	+	-
C27	Jamrud road	+	+	+	+	+	-
C28	Jamrud road	+	+	+	+	+	-
C29	Regi model town	+	+	+	+	+	-
C30	Regi model town	+	+	+	+	+	-
C31	Regi model town	+	+	+	+	+	-
C32	Shami road	+	+	-	+	-	-
C33	Shami road	+	+	-	-	+	-
C34	Garrison park	+	+	+	+	+	-
C35	Garrison park	+	+	+	+	+	-
C36	Qayyum stadium	+	+	+	+	+	-
C37	Qayyum stadium	+	+	+	+	+	-
C38	Army stadium	+	+	+	+	+	-
C39	Army stadium	+	-	-	-	+	-
C40	Defence colony	-	-	-	-	-	-
C41	Budni canal	+	+	+	+	+	-
C42	Budni canal	+	+	+	+	+	-
C43	Hazarkhwani canal	+	+	+	+	+	-
C44	Hazarkhwani canal	+	+	+	+	+	-
C45	Chamkani	+	+	+	+	+	-
C46	Chamkani	+	+	+	+	+	-
C47	Shoba bazar	+	+	+	+	+	-
C48	Shoba bazar	+	+	+	+	+	-
C49	Yakatoot	+	+	+	+	+	-
C50	Yakatoot	+	+	+	+	+	-

DISCUSSION

In this study a notably high frequency of the *toxA* gene (86%) was detected among *P. aeruginosa* isolates which contrasts with a previous study reporting a low prevalence (2.32%) of the *toxA* gene (Ghorbani *et al*., 2022) isolated from well and spring water which shows that water could potentially serve as a reservoir for *P. aeruginosa* and play a role in disseminating resistance genes within the food chain. Exotoxin A serves as a crucial virulence factor in clinical infections, exerting a cytotoxic impact. Its role involves hindering the biosynthesis of proteins during the elongation factor 2 phase in the polypeptide chain, leading to substantial loss of organs and tissues (Jenkins *et al*., 2004). Significantly high prevalence (98%) of the *lasB* gene was observed in current study whereas a previous study from Eastern Cape, South Africa reported a lower prevalence (75%) of *lasB* gene isolated from hospital wastewater (Mapapi *et al*., 2021). Other studies from Iran showed lower prevalence of *lasB* gene in *P. aeruginosa* isolated from environmental samples such as soil and surface and spring waters (Gholami, 2019; Ghorbani, 2022). These findings showed mutagenic elements within bacteria, inappropriate usage of disinfectants and detergents which find their way to surface water, improper administration of medications in human infections, and unregulated use of drugs in animals and poultry within the context of contemporary industrial practices. The product of *LasB* gene, also recognized as elastase B, facilitates the invasiveness of *P. aeruginosa* and has been demonstrated to exhibit high toxicity to the host. This toxicity arises from its enzymatic activity, which disrupts various mechanisms of both the innate and adaptive immune systems (Reboud *et al*., 2016). A substantially high prevalence (96%) of the *plcH* gene was observed in this study whereas a study conducted in Abidjan, West Africa reported a lower prevalence (72.1%) of the *plcH* gene in *P. aeruginosa* isolated from fresh and smoked fish (Benie *et al*., 2017). These prevalence findings indicate that the isolated strains possess the capability to secrete hemolytic exoenzymes and phospholipase C. Consequently, these strains may play a role in pulmonary infections (Barker *et al*., 2004). Another study from Iran showed a prevalence of 45.9% for the *plcH* gene isolated from burn infections which is considerably less than the findings of current study (Ellappan *et al*., 2018). The *plcH* gene is accountable for provoking pro-inflammatory responses and enhancing virulence, particularly contributing to pulmonary inflammation and suppressing the oxidative burst of neutrophils (Wieland *et al*., 2002). In this study, a high prevalence (88%) of the *exoS* gene was observed. This is in contrast to findings from studies conducted in Iran which showed 62% occurrence of the gene in *P. aeruginosa* isolated from spring water (Gholmai *et al*., 2019). Notably the frequency of *exoS* gene in the study conducted by Firouzi-Dalvand *et al*. (2019) was only 14% in *P. aeruginosa* obtained from clinical samples. The product of *ExoS* gene, with its ADP ribosyltransferase (ADPR) activity and function as a GTPase-activating protein, induces rapid lysis of host cells, showing maximal phospholipase activity (Barbieri *et al*., 2004).

Current study revealed a significantly high prevalence (90%) of class 1 Integrons while prevalence of class 2 Integrons was not detected in *P. aeruginosa* isolates. In contrast, a study from Barcelona, Spain showed 0% presence of class 1 integrons in *P. aeruginosa* isolated from water samples (Ruiz-Martínez *et al*., 2011). Another study from Brazil revealed class 1 and 2 integron in 3.2% isolates of *P. aeruginosa* obtained from lake water (Zanetti *et al*., 2013) which is in contrast with the current study. The findings indicate that *P. aeruginosa* strains in the environment could serve as a potential source for the dissemination of antibiotic resistance genes through mobile genetic elements.

A high genetic diversity among *P. aeruginosa* was observed in the current study. In China, a study reported similar genetic diversity pattern among the isolates of *P. aeruginosa* (Wu *et al*., 2016). Other studies also reported high genetic diversity among clinical isolates of *P. aeruginosa* (Kumari and Thakur, 2014; Zulfahmi *et al*., 2021). A study conducted in Iran reported only nine distinct genotypes indicating a lower genetic diversity among the isolates of *P. aeruginosa* that does not align with the findings of the current study (Kumari and Thakur, 2014). Considerable genetic diversity reflects the fact a wide variety of distinct genetic characteristics are possessed by the isolated *P. aeruginosa* strains to make them better adopted to diverse environments. This diversity suggests that multiple sources or origins may contribute to the population of *P. aeruginosa* in the canal water. The existence of various genetic profiles implies that different strains with diverse genetic makeup are present, and this diversity can influence the bacterium's resistance, adaptability, and potential impact on the environment and human health.

CONCLUSION

The study reveals the occurrence of virulent and Integron possessing *P. aeruginosa* in canal water in Peshawar, Pakistan which is commonly used for irrigation and other human activities. The findings carry substantial clinical implications. These finding show a heightened risk of waterborne infections and the potential dissemination

of antibiotic resistance of these bacteria. The presence of virulence genes in *P. aeruginosa* suggests an increased likelihood of infections upon human exposure to contaminated water. Furthermore, the existence of integrons implies a mechanism for the transfer of antibiotic resistance genes, likely contributing to the emergence of multidrug-resistant strains. The contaminated water poses a threat to public health, particularly for individuals with compromised immune systems, emphasizing the critical importance of robust water quality monitoring, public awareness, and measures to mitigate the spread of both pathogenic and antibiotic resistant bacteria in the community. This highlights the need for taking action by irrigation authorities to maintain cleanliness of canals and educating public about not disposing waste directly into open water bodies.

DECLARATIONS

Acknowledgement

The facilities provided by Centre of Biotechnology and Microbiology, University of Peshawar are greatly acknowledged.

Funding

The study received no external funding.

Supplementary material

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.pjz/20231124100835

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Al-Bayati, S.S., Al-Ahmer, S.D., Shami, A.M.M. and Al-Azawi, A.H., 2021. Isolation and identification of *Pseudomonas aeruginosa* from clinical samples. *Biochem. Cell. Arch.,* **21**: 3931-3935.

Ali, A.M., Al-Kenanei, K.A. and Bdaiwi, Q.O., 2020. Molecular study of some virulence genes of *Pseudomonas aeruginosa* isolated from different infections in hospitals of Baghdad. *Rev. med. Microbiol.*, **31**: 26-41. https://doi.org/10.1097/MRM.0000000000000194

Ali, A., Ahmad, K., Rahat, S. and Ahmad, I., 2021. Genetic diversity and molecular analysis of metallo beta lactamases among imipenem resistant clinical isolates of *Pseudomonas aeruginosa* from Peshawar, Pakistan. *Pak. J. med. Sci.*, **37**: 1865-1870. https://doi.org/10.12669/pjms.37.7.4303

Bahador, N., Shoja, S., Faridi, F., Dozandeh-Mobarrez, B., Qeshmi, F.I., Javadpour, S. and Mokhtary, S., 2019. Molecular detection of virulence factors and biofilm formation in *Pseudomonas aeruginosa* obtained from different clinical specimens in Bandar Abbas. *Iran. J. Microbiol.,* **11**: 25-29. https://doi.org/10.18502/ijm.v11i1.701

Barbieri, J.T. and Sun, J., 2004. *Pseudomonas aeruginosa exos* and *exot*. *Rev. Physiol. Biochem. Pharmacol.*, **6**: 79-92. https://doi.org/10.1007/s10254-004-0031-7

Barker, A.P., Vasil, A.I., Filloux, A., Ball, G., Wilderman, P.J. and Vasil, M.L., 2004. A novel extracellular phospholipase C of *Pseudomonas aeruginosa* is required for phospholipid chemotaxis. *Mol. Microbiol.*, **53**: 1089-1098. https://doi.org/10.1111/j.1365-2958.2004.04189.x

Benie, C.K.D., Dadié, A., Guessennd, N., N'gbesso-Kouadio, N.A., Kouame, N.Z.D., N'golo, D.C. and Dosso, M., 2017. Characterization of virulence potential of *Pseudomonas aeruginosa* isolated from bovine meat, fresh fish, and smoked fish. *Eur. J. Microbiol. Immunol.,* **7**: 55-64. https://doi.org/10.1556/1886.2016.00039

Bhasin, S., Shukla, A. and Shrivastava, S., 2020. Bacterial diversity of river Kshipra with relation to human health. *Environ. Conserv.,* **21**: 63-74. https://doi.org/10.36953/ECJ.2020.211207

Camiade, M., Bodilis, J., Chaftar, N., Riah-Anglet, W., Gardères, J., Buquet, S. and Pawlak, B., 2020. Antibiotic resistance patterns of *Pseudomonas* spp. isolated from feacal wastes in the environment and contaminated surface water. *FEMS Microbiol. Ecol.*, **96**: 8-11. https://doi.org/10.1093/femsec/fiaa008

Chakraborti, A.K., Prasad, K.S.S.V.V. and Bhat, B.S., 2016. Case for integrated development of Urban and Rural Water supply scheme in India and its implementation strategies. *J. Geol. Soc. India*, **5**: 25-33.

Diggle, S.P. and Whiteley, M., 2020. Microbe profile: *Pseudomonas aeruginosa*: Opportunistic pathogen and lab rat. *Microbiology*, **166**: 30-37. https://doi.org/10.1099/mic.0.000860

Ellappan, K., Narasimha, H.B. and Kumar, S., 2018. Coexistence of multidrug resistance mechanisms and virulence genes in carbapenem-resistant *Pseudomonas aeruginosa* strains from a tertiary care hospital in South India. *J. Glob. Antimicrob. Resist.*, **12**: 37-43. https://doi.org/10.1016/j.jgar.2017.08.018

Firouzi-Dalvand, L., Hosseini, F., Moradi D.S. and

Siasi, T.E., 2019. Antibacterial and antibiofilm activity of bismuth oxide nanoparticles produced by *Bacillus subtilis* against clinical *Pseudomonas aeruginosa* isolated from wound infections. *J. Microb. World*, **12**: 172-185.

Gholami, S. and Tabatabaei, M., 2019. The prevalence of virulence factors in human and environmental isolates of *Pseudomonas aeruginosa*. *Avicenna J. clin. Microbiol. Infect.*, **6**: 9-14. https://doi.org/10.34172/ajcmi.2019.03

Ghorbani, G., Rahimi, E. and Shakerian, A., 2022. Antibiotic resistance's genotypic and phenotypic characteristics and the frequency of virulence factors in *P. aeruginosa* isolates isolated from water samples in Iran. *BioMed. Res. Int.*, **22**: 1-13. https://doi.org/10.1155/2022/7076433

Goudarzi, M., Fazeli, M., Azad, M., Seyedjavadi, S.S., Mousavi, R., Rashidan, M. and Azargashb, E., 2016. Carriage of class 1 and class 2 integron in multidrug resistant *Pseudomonas aeruginosa* isolated from burn patients in Tehran hospitals, Iran. *West Indian med. J.*, **65**: 372-379. https://doi.org/10.7727/wimj.2014.315

Hofmann, L., Hirsch, M. and Ruthstein, S., 2021. Advances in understanding of the copper homeostasis in *Pseudomonas aeruginosa*. *Int. J. mol. Sci.*, **22**: 2050-2065. https://doi.org/10.3390/ijms22042050

Jenkins, C.E., Swiatoniowski, A., Issekutz, A.C. and Lin, T.J., 2004. *Pseudomonas aeruginosa* exotoxin A induces human mast cell apoptosis by a caspase-8 and-3-dependent mechanism. *J. biol. Chem.*, **279**: 37201-37207. https://doi.org/10.1074/jbc.M405594200

Kumari, N. and Thakur, S.K., 2014. Randomly amplified polymorphic DNA. A brief review. *Am. J. Anim. Vet.*, **9**: 6-13. https://doi.org/10.3844/ajavsp.2014.6.13

Mapipa, Q., Digban, T.O., Nnolim, N.E. and Nwodo, U.U., 2021. Antibiogram profile and virulence signatures of *Pseudomonas aeruginosa* isolates recovered from selected agrestic hospital effluents. *Sci. Rep.*, **11**: 1180-1187. https://doi.org/10.1038/s41598-021-91280-6

Mohamed, A. and Abdelhamid, F., 2020. Antibiotic susceptibility of *Pseudomonas aeruginosa* isolated from different clinical sources. *Zagazig J. Pharm. Sci.*, **28**: 10-17.

Moore, N.M. and Flaws, M.L., 2011. Introduction: *Pseudomonas aeruginosa*. *Clin. Lab. Sci.*, **24**: 41-45. https://doi.org/10.29074/ascls.24.1.41

Nanvazadeh, F., Khosravi, A.D., Zolfaghari, M.R. and Parhizgari, N., 2013. Genotyping of *Pseudomonas aeruginosa* strains isolated from burn patients by RAPD-PCR. *Burns*, **39**: 1409-1413. https://doi.org/10.1016/j.burns.2013.03.008

Ranjbar, R., Karami, A., Farshad, S., Giammanco, G.M. and Mammina, C., 2014. Typing methods used in the molecular epidemiology of microbial pathogens: A how to guide. *New Microbiol.*, **37**: 1-15.

Reboud, E., Elsen, S., Bouillot, S., Golovkine, G., Basso, P., Jeannot, K. and Huber, P., 2016. Phenotype and toxicity of the recently discovered *exlA*-positive *Pseudomonas aeruginosa* strains collected worldwide. *Environ. Microbiol.*, **18**: 3425-3439. https://doi.org/10.1111/1462-2920.13262

Ruiz-Martínez, L., López-Jiménez, L., Fusté, E., Vinuesa, T., Martínez, J.P. and Viñas, M., 2011. Class 1 integrons in environmental and clinical isolates of *Pseudomonas aeruginosa*. *Int. J. Antimicrob. Agents*, **38**: 398-402. https://doi.org/10.1016/j.ijantimicag.2011.06.016

Suárez, P., Gutiérrez, A.V., Salazar, V., Puche, M.L., Serrano, Y., Martínez, S. and Fernández-Delgado, M., 2020. Virulence properties and antimicrobial resistance of *Pseudomonas aeruginosa* isolated from cave waters at Roraima Tepui, Guayana Highlands. *Lett. appl. Microbiol.*, **70**: 372-379. https://doi.org/10.1111/lam.13283

Wieland, C.W., Siegmund, B., Senaldi, G., Vasil, M.L., Dinarello, C.A. and Fantuzzi, G., 2002. Pulmonary inflammation induced by Pseudomonas aeruginosa lipopolysaccharide, phospholipase C, and exotoxin A: Role of interferon regulatory factor 1. *Infect. Immun.*, **70**: 1352-1358. https://doi.org/10.1128/IAI.70.3.1352-1358.2002

Wu, Q., Ye, Y., Li, F., Zhang, J. and Guo, W., 2016. Prevalence and genetic characterization of *Pseudomonas aeruginosa* in drinking water in Guangdong Province of China. *LWT Fd. Sci. Technol.*, **69**: 24-31. https://doi.org/10.1016/j.lwt.2016.01.014

Zanetti, M.O., Martins, V.V., Pitondo-Silva, A. and Stehling, E.G., 2013. Antimicrobial resistance, plasmids and class 1 and 2 integrons occurring in *Pseudomonas aeruginosa* isolated from Brazilian aquatic environments. *Water Sci. Technol.*, **67**: 1144-1149. https://doi.org/10.2166/wst.2013.676

Zulfahmi, Z., Parjanto, P., Purwanto, E. and Yunus, A., 2021. Genetic diversity and population structure of *Eurycoma apiculata* in Eastern Sumatra, Indonesia. *Biodivers. J.*, **22**: 10-11. https://doi.org/10.13057/biodiv/d221036

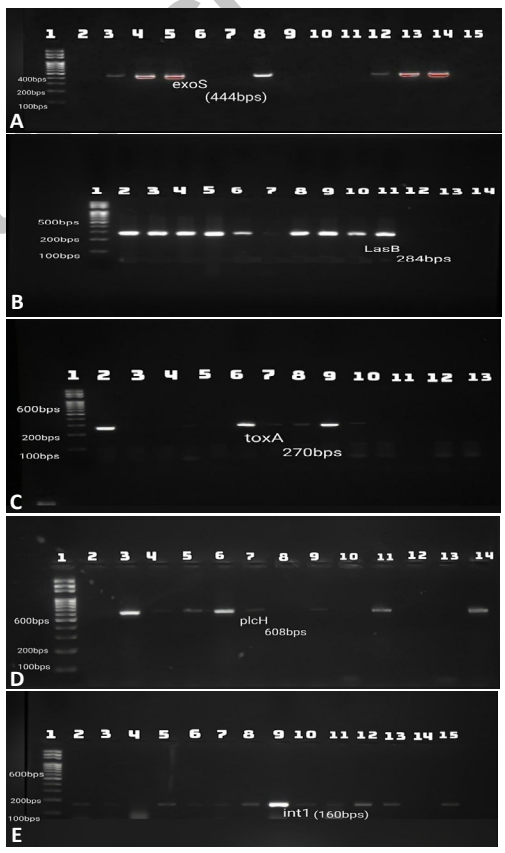
Pakistan J. Zool., pp 1-7, 2024. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20231124100835

Supplementary Material

Genetic Diversity and Molecular Characterization of Virulence Determinants and Integrons in *Pseudomonas aeruginosa* Isolated from Canal Water in Peshawar Pakistan

Urooj Amjad[1], Kafeel Ahmad[1]*, Hifsa Saadia[1] and Imran Muhammad Khan[2]

[1]*Centre of Biotechnology and Microbiology, University of Peshawar, Peshawar, Pakistan*
[2]*Burns and Plastic Surgery Centre, Hayatabad, Peshawar, Pakistan*



Supplementary Fig. 1. Occurrence of *exoS* (A), *lasB* (B), *toxA* (C) and *int1* (D) genes in *P. aeruginosa* isolates: Lane 1 = 100 bps DNA ladder. Lane 3, 4, 5, 8, 12, 13, 14 are positive for *exoS* gene (444 bp) in A, Lane 2-11 are positive for *lasB* gene (284 bp) in B, Lanes 2, 4, 6, 7, 8, 9, 10 are positive for *toxA* gene (270 bp) in C, Lane 3, 4, 5, 6, 7, 9, 11, 14 are positive for *plcH* gene (608 bp) in D, and Lane 2, 3, 5, 6, 7,8, 9, 10, 11, 12, 13 ,15 positive *P. aeruginosa* isolates for *int1* gene(284 bp) in E.

* Corresponding author: kafeelpbg@gmail.com
0030-9923/2024/0001-0001 $ 9.00/0