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Type 3 Secretion System Virulotypes in Clinical Isolates of Multidrug Resistant *Pseudomonas aeruginosa*

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Abstract

Background: Multidrug-resistant *Pseudomonas aeruginosa* has epidemiological impact on human health. It poses a threat to the health systems of the world including Iraq. Type 3 Secretion System effectors are among the several virulence factors that this bacterium possess. Determining the virulence profile is essential in prevention of infection. This study investigates the frequency of the four classical Type 3 Secretion System effectors in multidrug resistant *Pseudomonas aeruginosa*.

Patients and Methods: This study initially included 120 bacterial isolates from different clinical samples which were preliminary identified as *P. aeruginosa.* Out of those, 80 isolates were confirmed to be *P. aeruginosa*. Antibiotic susceptibility profile was studied and the existence of *exoY, exoT, exoS and exoU* was investigated by PCR.

Results: 95%, 3.75%, and 1.25% of the isolates were classified as multidrug resistant, extensive drug resistant, and pan drug resistant, respectively. Among the selected isolates, *exoT* was found in 86.7%, *exoY* in 76.7%, *exoS* in 50% and, *exoU* in 30%.

Conclusion: This study highlights an increase in the emergence of multidrug resistant profiles in clinical isolates of *P. aeruginosa*, besides the co-existence of the four classical Type 3 Secretion System effectors in variable frequencies (86.7%, 76.7%, 50%, and 30%, respectively).

Keywords: *P. aeruginosa*, Clinical isolates, T3SS, MDR.

Introduction

Pseudomonas aeruginosa (P. aeruginosa) is an opportunistic bacterium known for its ability to overcome the host immune response and thereby cause severe cellular damage. *P. aeruginosa* is extremely challenging to treat due to the frequent occurrence of antibiotic resistance and persistent colonisation on humid surfaces. *P. aeruginosa* employs a range of intrinsic and acquired resistance mechanisms, including antibiotic inactivation, drug target modification, attenuation of membrane permeability, expression of efflux systems, biofilm formation, and quorum sensing, to achieve a remarkably high level of antibiotic resistance (1). Infections caused by *P. aeruginosa* include pneumonia, burns, urinary tract infection, sepsis, nephrotic syndrome and wound infection (2-8). Nine different secretion systems (T1SS to T9SS) have been identified in bacteria, most of which are distributed in Gram-negative bacteria (9). These systems can either inject molecules into bacterial

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cells or transport molecules from inside bacteria to the extracellular environment. These systems play a pivotal role in bacterial survival in harsh environments and contribute significantly to evasion of the host's immune system (10). The type III secretion system (T3SS), one of *P. aeruginosa's* several secreted virulence factors (toxins, siderophores, proteases, and polysaccharides) (11), contributes to host cell damage. The T3SS's potential to attack the host immune response is demonstrated by its use of a needle-like structure to identify eukaryotic cells and inject toxins directly into their cytoplasm (12). Although several of the nine secretion systems have been identified in this microbe, the T3SS is the most characterized one in human infections. The T3SS plays a prominent role in bacterial pathogenesis by injecting several products known as effectors, which alter the host's signal transduction and actin cytoskeletal pathways, thereby contributing to colonization and replication in host cells (13). T3SS constitute of five functional parts; needle structure (which connects the bacterium with hosts cells), a structure responsible for the injection of effectors into host cells known as the translocation apparatus; 25 genes involved regulation known as the regulatory system; a group of small proteins (chaperones) whose function is to interact with secretions of the needle structure and prevent premature aggregation with bacterial cytoplasm and effectors. Four effectors (ExoS, ExoT, ExoU, and ExoY) have been described in *P. aeruginosa* (14). However, Burstein et al. (2015) found two additional effector proteins (PemA and PemB) in *P. aeruginosa* (15). Nevertheless, the exact role of these two newly described T3SS effectors has yet to be fully elucidated. Nolasco-Romero et al. (2024) identified 11 virulotypes in *P. aeruginosa* based on the presence or absence of T3SS, suggesting that these virulotypes can be linked to the sample type, in addition to playing a role in predicting patients' prognosis (16).

Due to the relevance of T3SS in *P. aeruginosa* clinical infections, this study aimed to investigate the prevalence and virulotypes of the four classical T3SS effectors in *P. aeruginosa* isolated from various clinical samples. In addition, this is the first study to investigate all four classical T3SS effectors in Thi-qar city, Iraq.

Patients and Methods

Study design: A total of 120 bacterial isolates whose preliminary diagnosis referred to *Pseudomonas* species were obtained from different clinical samples (sputum, wound swabs, ear swabs, burn swabs, and bronchoalveolar lavage (BAL)) in Al-Nasiriyah Teaching Hospital, Al-Haboubi Teaching Hospital, and Mohammed Al-Moussawi Children's Hospital, during the period from January 2023 to June 2023. Inclusion criteria included patients whose preliminary cultural results referred to the identification of Pseudomonas species and had not yet been administered antibiotics, while exclusion criteria included patients who had already been administered antibiotics.

Identification of bacterial isolates: Colony morphology, fruity odor, gram staining, inability to ferment lactose, catalase test (positive), oxidase test (positive), indole, methyl red, Voges-Proskauer, citrate utilization, and the ability to grow at 42°C were used for primary diagnosis. Subsequently, the isolates were sub-cultured on a selective medium (cetrimide agar, HiMedia) to confirm the diagnosis of the target microbe.

Antibiotic sensitivity tests: To classify the isolates included in the study according to the type of drug resistance, *P. aeruginosa* isolates were tested on Muller-Hinton agar for their sensitivity to 10 commonly used antibiotics by HiMedia-India; Amikacin (10µg), Gentamicin (10µg), Meropenem (10µg), Imipenem (10µg), Ceftazidime (30µg), Cefepime (10µg), Ciprofloxacin (5µg), Piperacillin (100 µg),

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Aztreonem (30 µg) and Colistin Sulphate (10µg) using the Kirby bauer method. The results were recorded by measuring the inhibition zone (in millimeters) and interpreted in accordance with the Clinical and Laboratory Standards Institute document (13). This bacterium was considered multidrug resistant (MDR), extensively drug resistant (XDR), and pan-drug resistant (PDR) based on the criteria previously described (14).

Genomic DNA extraction and polymerase chain reaction: The genomic DNA of *P. aeruginosa* was extracted from the bacterial growth of thirty randomly selected MDR isolates according to the protocol of FavorPrep Total DNA Mini Kit (FAVORGEN / Korea). Then, the presence of the four classical T3SS genes was investigated using conventional PCR with the Applied Biosystems ProFlex PCR System (Fisher Scientific, USA) and a set of previously published primers (15) listed in Table 1, along with the GoTaq Green Master Mix (Promega, USA).

Table 1. The primers used in this study.

Primer	Primer sequence 5'- - 3'		Size of Product (bp)
exoT	F	AATCGCCGTCCAACTGCATGCG	152
	R	TGTTCGCCGAGGTACTGCTC	
exoY	F	CGGATTCTATGGCAGGGAGG	289
	R	GCCCTTGATGCACTCGACCA	
exoU	F	CCGTTGTGGTGCCGTTGAAG	134
	R	CCAGATGTTCACCGACTCGC	
exoS	F	GCGAGGTCAGCAGAGTATCG	118
	R	TTCGGCGTCACTGTGGATGC	

The cycling conditions used for *exoT* and *exoY* were: 1 cycle of Initial denaturation at 95 °C, then 35 cycles of denaturation at 95 °C (30 seconds), annealing at 55 °C (30 seconds), and extension at 72°C (1 minute). Finally, seven minutes of final extension at 72°C. While the cycling conditions for *exoS* were one cycle of Initial denaturation at 95 °C, followed by 35 cycles of denaturation at 95 °C (45 seconds), annealing at 60 °C (45 seconds), and extension at 72°C (1 minute), finally, 7 minutes of final extension at 72°C. The cycling conditions of *exoU* were the same as those for *exoT* and *exoY*, except that the annealing was at 58°C. Confirmation of the presence of the PCR product was by running an agarose gel electrophoresis (Clarivate /UK) at 80V, 65 Amp for 1 hour. The DNA was visualized under a UV transilluminator (Vilber Lourmat Sté, France).

Statistical analysis

The statistical software IBM SPSS-29 (IBM Statistical Packages for Social Sciences, version 29, Chicago, IL, USA), was used to analyse the data. Simple frequency and percentage measures were used to display the data. The Pearson Chi-square test (x2-test) or Fisher Exact test, as appropriate, were used to assess the significance of differences in various percentages (qualitative data). The results were considered non-statistically significant when the p-value was greater than 0.05, while those with a p-value less than 0.05 were regarded as statistically significant, and those with a p-value less than 0.01 were considered highly significant.

Results

Demographic data and samples: This study initially involved 120 bacterial isolates, which were preliminarily identified as *P. aeruginosa* based on cultural characteristics, colony morphology, and conventional microbiological methods; subsequently, 80 were confirmed to be

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P. aeruginosa.

The demographic and sample characteristics of isolates included in the study are listed in Table 2. This table shows that males were more than females (76.25 vs 23.75%). The patient's age ranged from 17 to 70 years, with a mean age of 33.42±11.73 years. Out of the 80 *P. aeruginosa* isolates, 32 (40%) were isolated from wounds, 27(33.75%) from burns, 11(13.75%) from sputum, 7(8.75%) from ear swabs and 3 (3.75%) from BAL.

Distribution of *P. aeruginosa isolates* according to sex, with the source of clinical sample, showed that out of the 11 sputum samples included in the study, nine were isolated from males and two from females. Twenty-two isolates out of the 32 wound swabs included in the study were isolated from males and 10 from females. Out of the seven ear swabs, six isolates were collected from males and one from females. Regarding burn patients from whom *P. aeruginosa* were isolated, 22 were males and 5 were females.

Table 2. Demographic and sample characteristics of the patients included in the study.

Character		N	%
Sex N (%)	Male	61	76.25
	Female	19	23.75
Age (years)	Mean	33.42±11.73	
	Range	12-70	
Sample N (%)	Sputum	11	13.75
	wound swab	32	40
	Ear swab	7	8.75
	burn swab	27	33.75
	Bronchoalveolar lavage	3	3.75

Finally, out of the three of *P.aeruginosa* isolates identified in BAL samples, two were males and only one was a female. Statistical analysis revealed no significant association (p = 0.71) between sex and the type of clinical sample, as illustrated in Table 3.

Table 3. Distribution of *P.aeruginosa* isolates according to sex with the type of clinical samples. N.S. =Non- statistically significant (Chi-square).

Sex		Clinical Sample type					Total	P-value
		Sputum	wound swab	Ear swab	burn swab	BAL		
Male	N	9	22	6	22	2	61	0.71 (N.S)
	%	11.3%	27.5%	7.5%	27.5%	2.5%	76.3%	
Female	N	2	10	1	5	1	19	
	%	2.5%	12.5%	1.3%	6.3%	1.3%	23.8%	
Total	N	11	32	7	27	3	80	
	%	13.8%	40.0%	8.8%	33.8%	3.8%	100.0%	

Antibiotic resistance profile: Classifying the type of drug resistance in the 80 *P. aeruginosa* isolates showed that 76(95%), 3(3.75%), and 1 (1.25%) isolates were MDR, XDR, and PDR, respectively (Figure 1).

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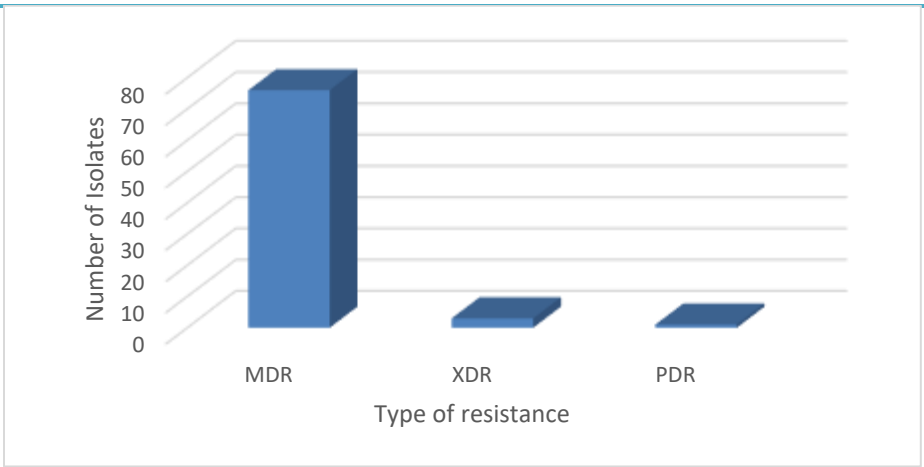

Figure 1. Type of drug resistance in *P. aeruginosa* isolates included in the study. MDR=Multidrug resistant, XDR=extensive drug resistant, PDR=Pan Drug Resistant.

Prevalence of *exoT, exoY, exoS* and *exoU* in *P.aeruginosa*: Figures 2, 3, 4, and 5 show images of PCR products for exoT, exoY, exoS, and exoU of *P. aeruginosa*, fractionated by 1.5% agarose gel and visualized under UV light after staining with a red dye.

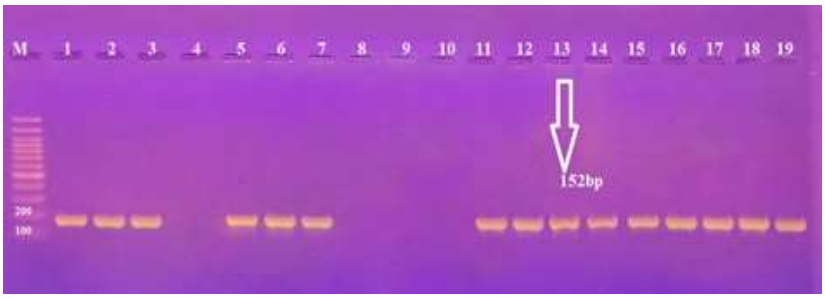

Figure 2. Gel electrophoresis (1.5%) of amplified exoT in P.aeruginosa. Agarose gel electrophoresis analysis shows the amplified exoT of P.aeruginosa. M: (100 ng/5 µl) of 100bp plus DNA ladder (Transgen/China). Lanes 1-3, 5-7 and 11-19: P.aeruginosa harboring exoT (152bp).

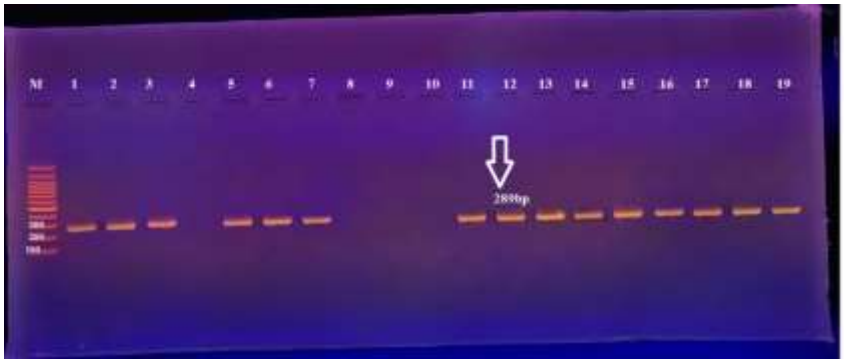

Figure 3. Gel electrophoresis (1.5%) of amplified exoY in P.aeruginosa. Agarose gel electrophoresis analysis shows the amplified exoY of P.aeruginosa. M: (100 ng/5 µl) of 100bp plus DNA ladder, (Transgen/China). Lanes 1-3, 5-7, 11-19: P.aeruginosa isolates harboring exoY (289bp).

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Figure 4. Gel electrophoresis (1.5%) of amplified exoS in P.aeruginosa. Agarose gel electrophoresis analysis shows the amplified exoS of P.aeruginosa. M: (100 ng/5 µl) of 100bp plus DNA ladder, (Transgen/China). Lanes 1-2, 5, 7, 16-19: P.aeruginosa isolates harboring exoS (118bp).

Figure 5. Gel electrophoresis (1.5%) of amplified exoU in P.aeruginosa. Agarose gel electrophoresis analysis shows the amplified exoU of P.aeruginosa M: (100 ng/5 µl) of 100bp plus DNA ladder, (Transgen/China). Lanes 1, 2, 5-7: P.aeruginosa isolates harboring exoU (1348bp).

The distribution of T3SS genes among the studied *P. aeruginosa* isolates revealed that the gene with the highest prevalence (86.7%) was *exoT*, followed by *exoY* (76.7%) and *exoS* (50%). The lowest prevalence was for exoU (30%), as illustrated in Table 4.

Table 4. Distribution of T3SS genes among studied *P. aeruginosa* isolates.

***T3SS* gene**	***Pseudomonas aeruginosa* Isolates N (%)**
exoT	26 (86.7%)
exoY	23 (76.7%)
exoS	15 (50%)
exoU	9 (30%)

The distribution of T3SS effector genes according to the origin of the sample (Table 5) revealed no significant association between the origin of the sample and the prevalence of exoS and exoU. On the other hand, *exoT* and *exoY* were mainly associated with burn patients (p = 0.04 and 0.007).

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Table 5. Distribution *of T3SS* genes in *P. aeruginosa* according to origin of sample**.**

Name of gene	Status	Type of samples				Total	P-value
		Sputum	Wound	Burn	BAL		
exoS	+Ve	3 (10.0%)	4 (13.3%)	8 (26.7%)	0 (0.0%)	15 (50.0%)	0.06
	-Ve	0 (0.0%)	8 (26.7%)	6 (20.0%)	1 (3.3%)	15 (50.0%)	
Total		3 (10.0%)	12 (40.0%)	14 (46.7%)	1 (3.3%)	30 (100.0%)	
exoT	+Ve	3 (10.0%)	8 (26.7%)	14 (46.7%)	1 (3.3%)	26 (86.7%)	0.04
	-Ve	0 (0.0%)	4 (13.3%)	0 (0.0%)	0 (0.0%)	4 (13.3%)	
Total		3 (10.0%)	12 (40.0%)	14 (46.7%)	1 (3.3%)	30 (100.0%)	
exoY	+Ve	2 (6.7%)	6 (20.0%)	14 (46.7%)	1 (3.3%)	23 (76.7%)	0.007
	-Ve	1 (3.3%)	6 (20.0%)	0 (0.0%)	0 (0.0%)	7 (23.3%)	
Total		3 (10.0%)	12 (40.0%)	14 (46.7%)	1 (3.3%)	30 (100.0%)	
exoU	+Ve	0 (0.0%)	5 (16.7%)	3 (10.0%)	1 (3.3%)	9 (30.0%)	0.12
	-Ve	3 (10.0%)	7 (23.3%)	11 (36.7%)	0 (0.0%)	21 (70.0%)	
Total		3 (10.0%)	12 (40.0%)	14 (46.7%)	1 (3.3%)	30 (100.0%)	

***T3SS* virulotypes:** The presence of different combinations of Exotoxins was analyzed according to the combinations published by Nolasco-Romero and co-workers (16) as illustrated in Table 6. The most abundant virulotypes were V3, V5, and V9 (23.3% each) followed by V6 (13.3%), V1 (6.7%), V4 (6.7%), and V7 (3.3%). Whereas, virulotypes V2, V10, and V11 did not exist in any of the *P. aeruginosa* clinical isolates included in the study.

Table 6. T3SS virulotypes in P. aeruginosa clinical isolates included in the study. (+ indicates presence of gene, - Indicates absence of gene).

Virulotype		N	%
V1	exoU+/exoS−/exoT+/exoY+	2	6.7
V2	exoU+/exoS−/exoT+/exoY−	0	0
V3	exoU−/exoS+/exoT+/exoY+	7	23.3
V4	exoU−/exoS−/exoT+/exoY−	2	6.7
V5	exoU−/exoS−/exoT+/exoY+	7	23.3
V6	exoU−/exoS−/exoT−/exoY−	4	13.3
V7	exoU−/exoS+/exoT+/exoY−	1	3.3
V8	exoU−/exoS−/exoT−/exoY+	0	0
V9	exoU+/exoS+/exoT+/exoY+	7	23.3
V10	exoU−/exoS+/exoT−/exoY+	0	0
V11	exoU+/exoS+/exoT+/exoY−	0	0

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Discussion

The WHO has listed antibiotic resistant *P. aeruginosa* among the "critical" group of pathogens necessitating urgent novel antibiotics (17).

Type III secretion system is among the many *P. aeruginosa* virulence factors that have been associated with host cell pathogenicity via activating the immune response and promoting the development of *P. aeruginosa* infections (12) posing as an additional risk factor in hospitals particularly in immune compromised patients (13).

In line with the current findings, the predominance of male patients in infections caused by *P. aeruginosa* has been previously reported (18, 19). The average age of patients included in the study was quite similar to what has been reported in a cross-sectional study isolating *P. aeruginosa* from infectious hospital departments (20). Regarding the source of isolates, most were from wound and burn swabs, coinciding with previous studies in Iraq (21), Saudi Arabia (22), and Pakistan (23). On the other hand, this isolation rate in wounds and burns was considerably higher than those reported in other studies (24, 25) which may be due to different inclusion criteria and different sampling protocols. The reported isolation rate of *P. aeruginosa* from sputum and BAL is 0% to 23%, which is in line with the current study (26). Despite the male predominance, no significant association was noted between sex and the type of clinical sample from which *P. aeruginosa* was isolated.

The current study showed a high prevalence of multidrug-resistance in clinical isolates of *P. aeruginosa* which is consistent with other studies in Iraq (27, 28) and abroad (29, 30).

The increase in the emergence of MDR *P. aeruginosa* is a global problem affecting many countries. The prevalence of MDR *P. aeruginosa* was higher (95 vs 72.63% and 85.49%) than the percentage previously reported in the cities of Basrah (27) and Babylon (28) in Iraq. In contrast to the 95% of MDR reported in the current study, an Iraqi study previously published in 2020 (31) reported that only 42% of *P. aeruginosa* isolates included in their study were MDR highlighting a sharp increase in the emergence of multidrug resistance in *P. aeruginosa* in Iraq. The increased prevalence of multidrug resistance in *P. aeruginosa* may be due to the selection of resistant strains which have emerged due to high consumption of antibiotics used to treat Covid-19 associated secondary bacterial infections (18).

Among the four classical T3SS effector genes, *exoT* was the most prevalent one which agrees with what has been previously published (32). The current result disagrees with the study of Waham and Naser in the city of Misan Iraq who found that the most prevalent exoenzyme was *exoY*. This difference may be attributed to the source of isolate as all of their isolates were from ear swabs (33). Both genes are part of the core genome of the bacterium (34). Statistical analysis has linked the existence of *exoY* and *exoT* with burn patients. Elnagar *et al.* (2022) reported that all *P. aeruginosa* strains isolated from burn sites harbored *exoY and exoT* (35). The second most prevalent gene in the current study was *exoY* followed by *exoS* and *exoU*. This comes in line with previous studies which have documented that *exoS* is more prevalent compared to *exoU* (35, 36). A previous study in the city of Wasit in Iraq has reported a frequency rate of the *exoU* and *exoS* genes of 60.31%, 90.47% in *P. aeruginosa* (37). It has been reported that exoU and exoS were found in 42.22% and 62.22% of *P. aeruginosa* clinical isolates, respectively (35). It is thought that *exoS+/exoU+ P. aeruginosa* strains have increased pathogenicity (38). Published data have reported that the potent A2-family phospholipase encoding *exoU* gene is the most virulent among the T3SS and can result in undesirable outcome such as multidrug

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resistance and death when over-regulated (39).

An *in vivo* study has shown that deletion of *exoU* in *P. aeruginosa* resulted in significant reduction in cytotoxicity and virulence highlighting its major role in pathogenesis (40). *ExoU* is the only *P. aeruginosa* T3SS effector encoded within a Genomic Island environment and Jaun and co-workers have linked its presence with an invasive phenotype (41). On the other hand, *exoS* has a cytotoxic phenotype (42-45). The most frequent virulotypes in the current study were V3, V5 and V9 in the current study. Similarly, a previous study has identified V3 as the most abundant virulotype followed by V1 and V7. The coexistence of the four T3SS genes (V9) was identified in 23.3% of the isolates included in the current study which is more than what has been previously reported (16).

Conclusions

This study highlights an increase in the emergence of multidrug-resistant *P. aeruginosa* in various clinical samples, as well as the presence of the four classical Type 3 Secretion System effectors at variable frequencies, underscoring their significant role in pathogenicity. *exoT* was the most prevalent among the other three Type 3 Secretion System effectors, suggesting that it plays a vital role in the virulence and survival of this pathogen. Furthermore, it was recommended to determine the profile of T3SS effector cases and precisely detect antibiotic susceptibility patterns, which are strongly essential for creating effective measures to prevent *P. aeruginosa* infections. Future studies with a larger sample size that focus on a specific infection site are required to reach a comprehensive conclusion.

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Conflict of interest: None.

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References

1. Starkey M, Lepine F, Maura D, Bandyopadhaya A, Lesic B, He J, Kitao T, Righi V, Milot S, Tzika A, Rahme L. Identification of anti-virulence compounds that disrupt quorum-sensing regulated acute and persistent pathogenicity. PLoS pathogens. 2014Aug21;10(8):e1004321. https://doi.org/10.1371/journal.ppat.1004321
2. Alharbi AS, Sanyi RH, Azhar EI. Bacteria and host: what does this mean for sepsis bottleneck?. World Journal of Emergency Medicine. 2025;16(1):10. https://doi.org/10.5847/wjem.j.1920-8642.2025.001
3. Sathe N, Beech P, Croft L, Suphioglu C, Kapat A, Athan E. Pseudomonas aeruginosa: Infections and novel approaches to treatment "Knowing the enemy" the threat of Pseudomonas aeruginosa and exploring novel approaches to treatment. Infectious medicine. 2023 Sep 1;2(3):178-94. https://doi.org/10.1016/j.imj.2023.05.003
4. Newman JN, Floyd RV, Fothergill JL. Invasion and diversity in Pseudomonas aeruginosa urinary tract infections. Journal of medical microbiology. 2022 Mar 11;71(3):001458. https://doi.org/10.1099/jmm.0.001458
5. Amer Hatem Z, Ali Hammad A, Jasim SA. Virulence gene expression of Pseudomonas aeruginosa isolated from children with nephrotic syndrome. Iranian Journal of Ichthyology. 2021;8:133-7.
6. Tariq TS, Jasim Kzar A, Sami Awayid H. Antimicrobial Activity of Silver Nanoparticles (AgNPs) on Biofilm Formation for Pathogenic Bacteria Isolated from UTI of Iraqi Patients. Journal of Nanostructures. 2024 Oct 1;14(4):1067-76.

ORIGINAL RESEARCH
Published: 25 June 2025
DOI: 10.26505/djm.v28i2.1367

https://doi.org/10.22052/JNS.2024.04.008
7. Jawed HM, Jabur MS. The Effect of Gram Negative Bacteria on Semen Parameters of a Sample of Infertile Men and Antibiotic Susceptibility Tests. Journal of Techniques. 2022 Jun 30;4(2):38-44. https://doi.org/10.51173/jt.v4i2.463
8. Saleh RM, Motib AS. Molecular detection of OprD and ExoA in Pseudomonas aeruginosa and antibiotics resistance. InAIP Conference Proceedings 2023 Mar 31 (Vol. 2475, No. 1). AIP Publishing. https://doi.org/10.1063/5.0103074
9. Abby SS, Cury J, Guglielmini J, Néron B, Touchon M, Rocha EP. Identification of protein secretion systems in bacterial genomes. Scientific reports. 2016 Mar 16;6(1):23080. https://doi.org/10.1038/srep23080
10. Deng W, Marshall NC, Rowland JL, McCoy JM, Worrall LJ, Santos AS, Strynadka NC, Finlay BB. Assembly, structure, function and regulation of type III secretion systems. Nature Reviews Microbiology. 2017 Jun;15(6):323-37. https://doi.org/10.1038/nrmicro.2017.20
11. Liao C, Huang X, Wang Q, Yao D, Lu W. Virulence factors of Pseudomonas aeruginosa and antivirulence strategies to combat its drug resistance. Frontiers in cellular and infection microbiology. 2022 Jul 6;12:926758. https://doi.org/10.3389/fcimb.2022.926758
12. Jouault A, Saliba AM, Touqui L. Modulation of the immune response by the Pseudomonas aeruginosa type-III secretion system. Front Cell Infect Microbiol. 2022;12:1064010. https://doi.org/10.3389/fcimb.2022.1064010
13. Horna G, Ruiz J. Type 3 secretion system of Pseudomonas aeruginosa. Microbiological Research. 2021 May 1;246:126719. https://doi.org/10.1016/j.micres.2021.126719
14. Hauser AR. The type III secretion system of Pseudomonas aeruginosa: infection by injection. Nature Reviews Microbiology. 2009 Sep;7(9):654-65. https://doi.org/10.1038/nrmicro2199
15. Burstein D, Satanower S, Simovitch M, Belnik Y, Zehavi M, Yerushalmi G, Ben-Aroya S, Pupko T, Banin E. Novel type III effectors in Pseudomonas aeruginosa. MBio. 2015 May 1;6(2):10-128. https://doi.org/10.1128/mBio.00161-15
16. Nolasco-Romero CG, Prado-Galbarro FJ, Jimenez-Juarez RN, Gomez-Ramirez U, Cancino-Díaz JC, López-Marceliano B, Apodaca MR, Aguayo-Romero MA, Rodea GE, Pichardo-Villalon L, Parra-Ortega I. The exoS, exoT, exoU and exoY Virulotypes of the Type 3 Secretion System in Multidrug Resistant Pseudomonas aeruginosa as a Death Risk Factor in Pediatric Patients. Pathogens. 2024 Nov 22;13(12):1030. https://doi.org/10.3390/pathogens13121030
17. Daikos GL, da Cunha CA, Rossolini GM, Stone GG, Baillon-Plot N, Tawadrous M, Irani P. Review of ceftazidime-avibactam for the treatment of infections caused by Pseudomonas aeruginosa. Antibiotics. 2021 Sep 18;10(9):1126. https://doi.org/10.3390/antibiotics10091126
18. Hafiz TA, Bin Essa EA, Alharbi SR, Alyami AS, Alkudmani ZS, Mubaraki MA, Alturki NA, Alotaibi F. Epidemiological, microbiological, and clinical characteristics of multi-resistant Pseudomonas aeruginosa isolates in King Fahad Medical City, Riyadh, Saudi Arabia. Tropical Medicine and Infectious Disease. 2023 Mar 30;8(4):205. https://doi.org/10.3390/tropicalmed8040205
19. Qiang C, Liu X, Qin P, Wen H, Li Z, Yang J, Niu Y, Wang W, Ouyang Z, Zhao M, Li J. Multicenter Surveillance of Pseudomonas aeruginosa Isolates From Blood: Clinical Distribution Characteristics and Antibiotic Resistance Trends in Hebei Province, China (2016–2021). Infection and Drug Resistance. 2025 Dec 31:703-13. https://doi.org/10.2147/IDR.S489527
20. Masoumi N, Keshavarzi F. The pattern of antibiotic resistance and distribution of the biofilm-

ORIGINAL RESEARCH
Published: 25 June 2025
DOI: 10.26505/djm.v28i2.1367

producing Pseudomonas aeruginosa (PelD, PslB) isolated from infectious hospital departments. SAGE Open Medicine. 2024 Nov;12:20503121241298826 . https://doi.org/10.1177/20503121241298826

21. Othman N, Babakir-Mina M, Noori CK, Rashid PY. Pseudomonas aeruginosa infection in burn patients in Sulaimaniyah, Iraq: risk factors and antibiotic resistance rates. The Journal of Infection in Developing Countries. 2014 Nov 13;8(11):1498-502. https://doi.org/10.3855/jidc.4707

22. Alshammari HO, Somily A, Qattan MY, Alsubki RA, Moussa IM. Susceptibility pattern of multi-drug resistance Pseudomonas aeruginosa isolates from tertiary care hospital in Riyadh, KSA. Journal of King Saud University-Science. 2023 Jul 1;35(5):102702. https://doi.org/10.1016/j.jksus.2023.102702

23. Chaudhary NA, Munawar MD, Khan MT, Rehan K, Sadiq A, Bhatti HW, Rizvi ZA. Epidemiology, bacteriological profile, and antibiotic sensitivity pattern of burn wounds in the burn unit of a tertiary care hospital. Cureus. 2019 Jun 1;11(6). https://doi.org/10.7759/cureus.4794

24. Abdi FA, Motumma AN, Kalayu AA, Abegaz WE. Prevalence and antimicrobial-resistant patterns of Pseudomonas aeruginosa among burn patients attending Yekatit 12 Hospital Medical College in Addis Ababa, Ethiopia. PloS one. 2024 Mar 7;19(3):e0289586. https://doi.org/10.1371/journal.pone.0289586

25. Coetzee E, Rode H, Kahn D. Pseudomonas aeruginosa burn wound infection in a dedicated paediatric burns unit. South African Journal of Surgery. 2013 May 21;51(2):50-3. https://doi.org/10.7196/sajs.1134

26. Restrepo MI, Babu BL, Reyes LF, Chalmers JD, Soni NJ, Sibila O, Faverio P, Cilloniz C, Rodriguez-Cintron W, Aliberti S, Aruj PK. Burden and risk factors for Pseudomonas aeruginosa community-acquired pneumonia: a multinational point prevalence study of hospitalised patients. European Respiratory Journal. 2018 Aug 9;52(2). https://doi.org/10.1183/13993003.01190-2017

27. Alkhulaifi ZM, Mohammed KA. Prevalence and molecular analysis of antibiotic resistance of Pseudomonas aeruginosa isolated from clinical and environmental specimens in Basra, Iraq. Iranian Journal of Microbiology. 2023 Feb;15(1):45. https://doi.org/10.18502/ijm.v15i1.11917

28. Almuttairi AA, Abdulla AA. Biofilm formation and virulence factors among multidrug resistant Pseudomonas aeruginosa isolated from patients in Babylon province. Medical Journal of Babylon. 2023 Apr 1;20(2):368-74. https://doi.org/10.4103/MJBL.MJBL_135_23

29. Akrami S, Ekrami A, Jahangirimehr F, Yousefi Avarvand A. High prevalence of multidrug-resistant Pseudomonas aeruginosa carrying integron and exoA, exoS, and exoU genes isolated from burn patients in Ahvaz, southwest Iran: A retrospective study. Health Science Reports. 2024 Jun;7(6):e2164. https://doi.org/10.1002/hsr2.2164

30. Dawadi P, Khadka C, Shyaula M, Syangtan G, Joshi TP, Pepper SH, Kanel SR, Pokhrel LR. Prevalence of metallo-β-lactamases as a correlate of multidrug resistance among clinical Pseudomonas aeruginosa isolates in Nepal. Science of the Total Environment. 2022 Dec 1;850:157975. https://doi.org/10.1016/j.scitotenv.2022.157975

31. Sulaiman SD, Abdulhasan GA. Curcumin as efflux pump inhibitor agent for enhancement treatment against multidrug resistant Pseudomonas aeruginosa isolates. Iraqi Journal of Science. 2020 Jan 26:59-67.67. https://doi.org/10.24996/ijs.2020.61.1.6

32. Ullah R, Amir M, Anjum S, Rehman MU, Hasan TN, Naqvi SS, Faryal R, Khan HA, Khadija B, Arshad N, Rashid Z. Presence of T3SS (exoS, exoT, exoU and exoY), susceptibility pattern and MIC of MDR-Pseudomonas aeruginosa from burn wounds. The Journal of Infection in Developing Countries. 2023 Aug 31;17(08):1130-7. https://doi.org/10.3855/jidc.17580

33. Waham AA, Naser LA. Detection of Exoenzyme Effectors and Determination The MIC of Antibiotics for Pseudomonas Aeruginosa Isolated from Ear Infections Patients in Basrah Province, Iraq. (Humanities, social and applied sciences) Misan Journal of Academic Studies. 2024 Jun 30;23(50):1-4. https://doi.org/10.54633/2333-023-050-001

34. Horna G, Ruiz J. Type 3 secretion system of

ORIGINAL RESEARCH
Published: 25 June 2025
DOI: 10.26505/djm.v28i2.1367

Pseudomonas aeruginosa. Microbiological Research. 2021 May 1;246:126719. https://doi.org/10.1016/j.micres.2021.126719
35. Elnagar RM, Elshaer M, Osama Shouman O, Sabry El-Kazzaz S. Type III secretion system (exoenzymes) as a virulence determinant in Pseudomonas aeruginosa isolated from burn patients in Mansoura University hospitals, Egypt. Iranian Journal of Medical Microbiology. 2022 Oct 10;16(6):520-7. https://doi.org/10.30699/ijmm.16.6.520
36. Fazeli N, Momtaz H. Virulence gene profiles of multidrug-resistant Pseudomonas aeruginosa isolated from Iranian hospital infections. Iranian Red Crescent Medical Journal. 2014 Oct 5;16(10):e15722. https://doi.org/10.5812/ircmj.15722
37. AL-Mayyahi AW, AL-Hashimy AB, AL-Awady KR. Molecular detection of exoU and exoS among Pseudomonas aeruginosa isolates from Baghdad and Wasit, Iraq. Iraqi journal of biotechnology. 2018 Nov 12;17(1).
38. Song Y, Mu Y, Wong NK, Yue Z, Li J, Yuan M, Zhu X, Hu J, Zhang G, Wei D, Wang C. Emergence of hypervirulent Pseudomonas aeruginosa pathotypically armed with co-expressed T3SS effectors ExoS and ExoU. Hlife. 2023 Nov 1;1(1):44-56. https://doi.org/10.1016/j.hlife.2023.02.001
39. Kothari A, Kherdekar R, Mago V, Uniyal M, Mamgain G, Kalia RB, Kumar S, Jain N, Pandey A, Omar BJ. Age of antibiotic resistance in MDR/XDR clinical pathogen of Pseudomonas aeruginosa. Pharmaceuticals. 2023 Aug 30;16(9):1230. https://doi.org/10.3390/ph16091230
40. Antimicrobial Susceptibility Patterns of Aerobic Bacterial Species of Wound Infections in Baquba General Teaching Hospital-Diyala.
41. Wu T, Zhang Z, Li T, Dong X, Wu D, Zhu L, Xu K, Zhang Y. The type III secretion system facilitates systemic infections of Pseudomonas aeruginosa in the clinic. Microbiology Spectrum. 2024 Jan 11;12(1):e02224-23. https://doi.org/10.1128/spectrum.02224-23
42. Harrison EM, Carter ME, Luck S, Ou HY, He X, Deng Z, O'Callaghan C, Kadioglu
A, Rajakumar K. Pathogenicity islands PAPI-1 and PAPI-2 contribute individually and synergistically to the virulence of Pseudomonas aeruginosa strain PA14. Infection and immunity. 2010 Apr;78(4):1437-46. https://doi.org/10.1128/IAI.00621-09
43. Juan C, Peña C, Oliver A. Host and pathogen biomarkers for severe Pseudomonas aeruginosa infections. The Journal of infectious diseases. 2017 Feb 15;215(suppl_1):S44-51. https://doi.org/10.1093/infdis/jiw299
44. Reuven AD, Katzenell S, Mwaura BW, Bliska JB. ExoS effector in Pseudomonas aeruginosa Hyperactive Type III secretion system mutant promotes enhanced Plasma Membrane Rupture in Neutrophils. PLoS pathogens. 2025 Apr 2;21(4):e1013021. https://doi.org/10.1371/journal.ppat.1013021
45. Akter T, Stapleton F, Willcox M. Differences in antimicrobial resistance between exoU and exoS isolates of Pseudomonas aeruginosa. European Journal of Clinical Microbiology & Infectious Diseases. 2025 Apr 22:1-3. https://doi.org/10.1007/s10096-025-05132-6

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نمط الضراوة لنظام الإفراز من النوع الثالث في العزلات السريرية لبكتيريا الزائفة الزنجارية المقاومة للأدوية المتعددة

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الملخص

الخلفية: الخلفية: لبكتيريا الزائفة الزنجارية المقاومة للأدوية المتعددة تأثير وبائي على صحة الإنسان. فهي تُشكل تهديدًا للأنظمة الصحية في العالم، بما في ذلك العراق. تُعد مُفعِّلات نظام الإفراز من النوع الثالث من بين عوامل الضراوة العديدة التي تمتلكها هذه البكتيريا. يُعد تحديد نمط الضراوة أمرًا أساسيًا للوقاية من العدوى.

الأهداف: تبحث هذه الدراسة في تكرار مُفعِّلات نظام الإفراز من النوع الثالث الأربعة الكلاسيكية في بكتيريا الزائفة الزنجارية المقاومة للأدوية المتعددة.

المرضى والطرق: شملت هذه الدراسة في البداية ١٢٠ عزلة بكتيرية من عينات سريرية مختلفة، والتي تم تحديدها مبدئيًا على أنها الزائفة الزنجارية. من بين هذه العزلات، تم تأكيد أن ٨٠ عزلة هي الزائفة الزنجارية. تمت دراسة نمط حساسية المضادات الحيوية، وتم التحقق من وجود exoY وexoT وexoS وexoU بواسطة تفاعل البوليميراز المتسلسل (PCR).

النتائج: صُنفت ٩٥٪ و٣,٧٥٪ و١,٢٥٪ من العزلات على أنها مقاومة للأدوية المتعددة، ومقاومة واسعة للأدوية، ومقاومة لجميع الأدوية على التوالي. من بين العزلات المختارة، وُجد exoT في ٨٦,٧٪، وexoY في ٧٦,٧٪، وexoS في ٥٠٪، وexoU في ٣٠٪ على التوالي.P=0.0001.

الاستنتاج: تُبرز هذه الدراسة زيادة في ظهور أنماط مقاومة الأدوية المتعددة في العزلات السريرية لبكتيريا الزائفة الزنجارية، بالإضافة إلى تواجد مُفعِّلات نظام الإفراز من النوع الثالث بنسب متفاوتة (٨٦,٧٪، و٧٦,٧٪، و٥٠٪، و٣٠٪ على التوالي).

الكلمات المفتاحية: الزائفة الزنجارية, المقاومة للأدوية المتعددة, نظام الإفراز من النوع الثالث.

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