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BACTERIOLOGICAL AND MOLECULAR STUDIES ON *PSEUDOMONAS AERUGINOSA* ISOLATED FROM AFRICAN SHARPTOOTH CATFISH AND NILE TILAPIA

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ABSTRACT

Pseudomonas aeruginosa is a Gram-negative bacterium commonly found in aquatic environments. It poses a serious threat to aquaculture due to its opportunistic pathogenicity and causes severe infections, high mortality rates in fish populations. The present investigation involved 250 organ samples isolated from lesions in the muscle, liver, kidney, intestine, and spleen of diseased Nile tilapia and African sharptooth catfish of different sizes. The fish were collected from various fish markets in Qalyubia Governorate, Egypt, between September 2023 and February 2024 for isolation, phenotypic and genotypic characterization of *Pseudomonas aeruginosa* (*Ps. aeruginosa*). The results showed that 52 *Ps. aeruginosa* isolates were recovered from fish organs, and they were isolated from 25 muscle samples (48.1%), followed by intestine 13 (25%), then liver 8 (15.4%), kidney 5 (9.6%), and one spleen sample from African sharptooth catfish (1.9%). The disc diffusion test revealed multidrug resistance (MDR) in all isolates against the used antimicrobial agents. The isolated *Ps. aeruginosa* were highly sensitive to norfloxacin, followed by gentamicin and ciprofloxacin. In contrast, they were highly resistant to ampicillin, then oxacillin, amoxicillin, tetracycline, cefotaxime, and nalidixic acid. The 16s-*Pseudomonas* (genus) and the 16s-*Pseudomonas aeruginosa* (species-specific) genes were detected by PCR in all selected isolates. The virulence genes (*psl*A and *opr*L) were found in eight and five studied *Ps. aeruginosa* isolates, respectively. It is being concluded that the main gene responsible for the virulence of studied *Ps. aeruginosa* isolated from freshwater fishes is *psl*A. So, this study also sheds light on the public health concerns of Pseudomonas for consumers.

Keywords: *Pseudomonas aeruginosa*, bacteriological studies, molecular studies, African sharptooth catfish, Nile tilapia.

INTRODUCTION

Pseudomonas aeruginosa (Ps. aeruginosa) is a Gram-negative, rod-shaped bacterium, strictly aerobic, motile, and non-spore-forming. It is 0.5–1.0 µm in size, straight or slightly curved, and grows readily on common laboratory media. Its nutritional requirements are extremely straightforward. It is a catalase- and oxidase-positive, non-lactose-fermenting bacterium on MacConkey agar with large, pale colonies. It can be

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identified via its characteristic large, flat, spreading, irregular, greenish-blue colonies with a fruity, grape-like odor of aminoacetophenone on nutrient agar (Markey *et al*., 2013; Shahrokhi *et al*., 2022).
Ps. aeruginosa is a significant fish pathogen that causes *Pseudomonas* septicemia and ulcerative syndrome. This condition is characterized by exophthalmia, petechial hemorrhage, detached scales, and abdominal ascites, resulting in high mortalities and significant economic losses (Hanna *et al*., 2014, Abd El-Tawab *et al*., 2016,2019a and Ali *et al*., 2023).

Ps. aeruginosa is resistant to a variety of commercial antibiotics; both innate and acquired resistance to a wide range of antimicrobials, including β-lactams, makes treating infections caused by the bacteria challenging. The primary mechanisms of resistance to these antibiotics include the overexpression of chromosomal cephalon-sporinases and the synthesis of β-lactamases, which are expressed by plasmids for Ambler class A (ESBLs), B (MBLs), and D (oxacillinases) (Esposito and De Simone, 2017; Rou lová *et al*., 2022). Therefore, it is more critical than other foodborne bacteria, as it has the potential to transmit its multidrug-resistant (MDR) plasmids to humans following the consumption of contaminated undercooked fish and fish products containing MDR *Ps. aeruginosa* (Shahrokhi *et al.,* 2022). Furthermore, *Ps. aeruginosa* is regarded as a problematic pathogen due to its extracellular products, including rhamno-lipid, phospholipase C, haemolysin, lecithin-ase, protein exotoxin A, proteases, and other exoenzymes. It also possesses cell-associated virulence factors, including pili, flagella, lipopolysaccharide, and the capacity to form biofilms. All of these factors are implicated in the development of severe and aggressive infections in humans, fish, and animals (Mavrodi *et al*., 2001 and Markey *et al*., 2013).

Additionally, the lipoprotein L (oprL) gene is important for making Ps. aeruginosa resistant to many types of antibiotics and disinfectants (Abdullahi *et al*., 2013; Abdulhaq *et al*., 2020). The ability of the bacterium to form biofilms is linked to its production of harmful exopolysaccharides (EPSs), like psl, which help stick to other cells and surfaces, leading to higher illness and death rates in infected fish (Ghafoor et al., 2011; Yang et al., 2011). The *pslA* gene plays a crucial role in biofilm development and regulates the whole psl operon in both planktonic and biofilm cells (Overhage *et al*., 2005, Nader *et al*., 2017 and Abdulhaq *et al*., 2020). *Ps. aeruginosa* is a major fish pathogen that can infect those who eat it. The importance of fish to Egypt's economy has grown significantly recently. So, this research aimed to shed light on their infection in fishes, Nile tilapia, and African sharptooth catfish at Qalyubia Governorate, Egypt. Additionally, phenotypic and genotypic characterization was conducted. Also, PCR was employed to identify specific virulence and antimicrobial resistance genes in certain isolates.

MATERIALS AND METHODS

Handling of fish and all practical protocols in accordance with the Ethics Committee of the Veterinary Medicine Faculty, Benha University, with ethical no. BUFVTM03-03-24

1. Sample collection:

The present study was conducted on 250 organ samples from fish showing lesions (hemorrhages, erosions, ulcers, and abdominal distension) in the muscles, livers, kidneys, intestines, and spleens of diseased Nile tilapia and African sharptooth catfish. The collected fish were of various sizes (250-500 g) and collected from different fish markets in Qalyubia Governorate, Egypt, during the period from September 2023 to February 2024. The diseased fish were put in sterile plastic bags and sent on ice to the lab rapidly for bacteriological, isolation and phenotypic and genotypic characterization of *Ps. aeruginosa.*

Examination of internal organs was done according to the method described by Roberts

(2012). Disinfection of the skin has been performed using 70 % ethyl alcohol. Fish were dissected using aseptic techniques, and their internal organs were carefully examined for any signs of abnormalities.

2. Isolation and phenotypic identification of *Pseudomonas aeruginosa*:

After cauterizing the lesion surfaces with a hot spatula, a sterile loop was inserted through the burned area and inoculated into Tryptone Soya Broth (TSB) (Oxoid), followed by aerobic incubation at 25°C for 24 hours. A loopful from the incubated TSB was then streaked onto nutrient agar and incubated aerobically at 25°C for another 24 hours. Colonies displaying a greenish-blue coloration with a distinct fruity, grape-like odor characteristic of aminoacetophenone were selected and purified through further subculturing on nutrient agar (Oxoid). Subsequently, oxidase and catalase tests were conducted. Colonies testing positive for both oxidase and catalase were cultured on MacConkey agar, *Pseudomonas* agar, and *Pseudomonas* Cetrimide agar (Oxoid) and incubated at 37°C for 24–48 hours. The suspected colony was then transferred to a semisolid nutrient slope (Oxoid) for further morphological and biochemical identification, including tests such as Triple Sugar Iron (TSI), Lysine Iron (LI), Simmons citrate, urease activity, indole reaction, and Voges-Proskauer according to Markey *et al*. (2013); Austin and Austin (2016).

3. In-vitro anti-microbial sensitivity test for *Ps. aeruginosa* isolates:

The in vitro antimicrobial susceptibility pattern of *Ps. aeruginosa* isolates were evaluated against 12 antibiotics using the Kirby–Bauer disc diffusion method, as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2018). Twelve standardized antimicrobial discs (Oxoid) were used, including amoxicillin (AMX/25 µg), ampicillin (AMP/10 µg), cefotaxime (CTX/30 µg), ciprofloxacin (CIP/5 µg), doxycycline (DO/30 µg), gentamicin (CN/10 µg), nalidixic acid (NA/30 µg), norfloxacin (NOR/10 µg), oxacillin (OX/1 µg), streptomycin (S/10 µg), tetracycline (TE/30 µg), and trimethoprim/sulfamethoxazole (SXT/25 µg). The bacterial cultures were streaked onto Mueller-Hinton agar (Oxoid) plates and incubated at 25°C overnight. The zones of inhibition were then measured and compared with standard reference values.

4. Genotypic identification and detection of both virulence and antimicrobial resistance genes in some *Ps. aeruginosa* isolates by PCR:

Eight selected *Ps. aeruginosa* isolates (four from each fish species) were subjected to genotypic identification through the detection of various genes using conventional PCR. The genes analyzed included the general *Pseudomonas* gene (16S rDNA), the species-specific *P. aeruginosa* gene (16S rDNA), two virulence genes-*pslA* (exopolysaccharide synthesis locus) and *oprL* (outer membrane lipoprotein L)-as well as antimicrobial resistance genes. The resistance genes targeted were *blaTEM* (β-lactam resistance), *blaCTX-M* (extended-spectrum β-lactam resistance, cefotaxime), *sul1* (sulfonamide resistance), *aadA1* (streptomycin resistance), *tetA(A)* (tetracycline A resistance), and *qnrA* (quinolone resistance). The DNA extraction was performed using the QIAamp® DNA Mini Kit (Qiagen, Germany, GmbH; Catalogue no. 51304), while PCR amplification was carried out using EmeraldAmp GT PCR Master Mix (Takara, Japan; Code No. RR310A). The amplified products were analyzed by running on 1.5% agarose gel electrophoresis (Sambrook *et al*, 1989). The primers, target genes, amplicon sizes, and cycling conditions are described in Table (1).

RESULTS

1. Isolation and phenotypic identification of *Ps. aeruginosa:*

All recovered *Ps. aeruginosa* isolates (n=52) exhibited good growth, forming large, flat, spreading, and irregular greenish-blue colonies with a distinct fruity odor on nutrient agar. On MacConkey agar, they appeared as

large, pale colonies, indicating their non-lactose-fermenting nature. They produced bluish-green colonies on *Pseudomonas* agar and small, smooth colonies with blue-green pigmentation on *Pseudomonas* Cetrimide agar. Microscopic analysis confirmed that the isolates were Gram-negative, non-capsulated, non-spore-forming, and appeared as straight or slightly curved rods. Furthermore, all isolates demonstrated motility in semisolid agar. Therefore, all isolates were suspected as *Ps. aeruginosa*. The biochemical identification results revealed that all 52 isolates exhibited characteristic biochemical reactions to *Ps. aeruginosa*. They showed positive results for glucose fermentation, mannitol oxidation, oxidase, catalase, citrate utilization, urease, and lysine decarboxylase, but did not produce hydrogen sulfide (H_2S). However, they were negative for sucrose and lactose fermentation, as well as for the indole, Voges-Proskauer, and methyl red tests.

Table 1: Primer sequences, target genes, and their PCR cycling conditions.

Target agent	Target gene		Primer sequence (5'-3')	Amplified segment (bp.)	Primary Denaturation	Amplification (35 cycles)			Final extension	References
						Secondary denaturation	Annealing	Extension		
*P*seudo mona*s*	16S rDNA	F	GACGGGTGAGTA ATGCCTA	618 bp.	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	**Spilker *et al.*, 2004**
		R	CACTGGTGTTCC TTCCTATA							
Ps. aeruginosa	16S rDNA	F	GGGGGATCTTCG GACCTCA	956 bp.	94˚C 5 min.	94˚C 30 sec.	52˚C 40 sec.	72˚C 50 sec.	72˚C 10 min.	
		R	TCCTTAGAGTGC CCACCCG							
	*psl*A	F	TCCCTACCTCAG CAGCAAGC	656 bp.	94˚C 5 min.	94˚C 30 sec.	60˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	**Ghadaksaz *et al.*, 2015**
		R	TGTTGTAGCCGT AGCGTTTCTG							
	*opr*L	F	ATG GAA ATG CTG AAA TTC GGC	504 bp.	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	**Xu *et al.*, 2004**
		R	CTT CTT CAG CTC GAC GCG ACG							
	*bla*TEM	F	ATCAGCAATAAA CCAGC	516 bp.	94˚C 5 min.	94˚C 30 sec.	54˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	**Colom *et al.*, 2003**
		R	CCCCGAAGAACG TTTTC							
	*bla*CTX-M	F	ATG TGC AGY ACC AGT AAR GTK ATG GC	593 bp.	94˚C 5 min.	94˚C 30 sec.	54˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	**Archambault *et al.*, 2006**
		R	TGG GTR AAR TAR GTS ACC AGA AYC AGC GG							
	*sul*1	F	CGGCGTGGGCTA CCTGAACG	433 bp.	94˚C 5 min.	94˚C 30 sec.	60˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	**Ibekwe *et al.*, 2011**
		R	GCCGATCGCGTG AAGTTCCG							
	*aad*A1	F	TATCAGAGGTAG TTGGCGTCAT	484 bp.	94˚C 5 min.	94˚C 30 sec.	54˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	**Randall *et al.* 2004**
		R	GTTCCATAGCGT TAAGGTTTCATT							
	*tet*A (A)	F	GGTTCACTCGAA CGACGTCA	576 bp.	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	
		R	CTGTCCGACAAG TTGCATGA							
	*qnr*A	F	ATTTCTCACGCC AGGATTTG	516 bp.	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	**Robicsek *et al.*, 2006**
		R	GATCGGCAAAGG TTAGGTCA							

2. Prevalence of *Ps. aeruginosa* in freshwater fish:

The bacteriological examination of 250 organ samples from diseased freshwater fish (Nile tilapia and African sharptooth catfish) resulted in 52 isolates of *P. aeruginosa,* representing a prevalence of 21%. Out of 52 isolates of *Ps. aeruginosa*, 23 (44.2%) were isolated from the Nile tilapia organs and 29 (55.8%) were isolated from African sharptooth catfish. The muscle is the most infected organ, followed by the intestine, liver, and kidney. *Ps. aeruginosa* was isolated from a spleen sample of an African catfish (Table 2).

Table 2: Prevalence of *Pseudomonas aeruginosa* in freshwater fish organs.

lesion samples	Nile tilapia (*O. niloticus*)		African catfish (*C. gariepinus*)		Total	
	No./total (52)	%*	No./total (52)	%*	No.	%*
Muscle	12	23.1	13	25.0	25	48.1
Intestine	6	11.5	7	13.5	13	25.0
Liver	3	5.8	5	9.6	8	15.4
Kidney	2	3.8	3	5.8	5	9.6
Spleen	0	0.0	1	1.9	1	1.9
Total	23	44.2	29	55.8	52	100

*% percentage with total number of isolated *Ps. aeruginosa* (52)

3. The antimicrobial sensitivity tests of *Pseudomonas aeruginosa* isolates:

The obtained results of *in vitro* sensitivity tests for 52 *Ps. aeruginosa* isolates (Table 3) showed high sensitivity to norfloxacin (82.7%), followed by gentamicin (73.1%), and ciprofloxacin (69.2%). Meanwhile, they were intermediately sensitive to doxycycline (69.2%), sulphamethoxazole/ trimethoprim (57.7%), and streptomycin (53.9%). Moreover, they have been highly resistant to ampicillin (94.2%), then oxacillin (92.3%), amoxicillin (90.4%), tetracycline (88.5%), cefotaxime (84.6%), and nalidixic acid (78.8%). Out of the 52 isolated *P. aeruginosa* strains, the percentage of MDR strains was 90 % (47/52).

Table 3: In vitro antimicrobial susceptibility of the used *Pseudomonas aeruginosa* isolates

Antimicrobial agents		Disc concentrations	Sensitive		Intermediate		Resistant		AA
			No.	%	No.	%	No.	%	
Ampicillin	AM10	25 µg	0	0.0	3	5.8	49	94.2	R
Oxacillin	OX1	1 µg	1	1.9	3	5.8	48	92.3	R
Amoxicillin	AMX/25	25µg	1	1.9	4	7.7	47	90.4	R
Tetracycline	TE/30	30 µg	2	3.8	4	7.7	46	88.5	R
Cefotaxime	CTX/30	30 µg	1	1.9	7	13.5	44	84.6	R
Nalidixic acid	NA30	30 µg	3	5.8	8	15.4	41	78.8	R
Doxycycline	DO/30	30 µg	7	13.5	36	69.2	9	17.3	I
Trimethoprim/ Sulphamethoxazole	SXT/25	(1.25/23.75) µg	9	17.3	30	57.7	13	25.0	I
Streptomycin	S/10	10 µg	2	3.8	28	53.9	22	42.3	I
Norfloxacin	NOR/10	10 µg	43	82.7	7	13.5	2	3.8	S
Gentamicin	CN/10	10 µg	38	73.1	9	17.3	5	9.6	S
Ciprofloxacin	CIP/5	5 µg	36	69.2	12	23.1	4	7.7	S

No.: Number of isolates; %: Percentage in relation to total number of isolates (n=52)
AA: Antibiogram activity; R: resistant; S: sensitive; I: intermediate

4. PCR identification and detection of some virulence and antimicrobial resistance genes in *Ps. aeruginosa* isolates:

Genotypically, all eight selected *Ps. aeruginosa* isolates have been positive for the *Pseudomonas* gene (16S rDNA), and the specific gene of *Ps. aeruginosa* (16S rDNA), yielding positive bands at 618 bp and 956 bp, respectively (Figs. 1, 2). The virulence gene, exopolysaccharide synthesis locus (*psl*A) gene, was detected in all examined *Ps. aeruginosa* isolates, giving a product of 504 bp. (Fig. 3), and an outer membrane lipoprotein L (*opr*L) in five out of eight examined isolates with positive bands at 656 bp. (Fig. 4). The antimicrobial resistance genes, β- lactam (*bla*TEM); extended spectrum β-lactam (*bla*CTX-M); sulphonamide (*sul*1), streptomycin (*aad*A1) and tetracyclineA *tet*A(A) genes were detected in all eight examined *Ps. aeruginosa* isolates giving products of 516 bp., 593 bp. , 433 bp., 484 bp., 570 bp., respectively (Figs. 5-9), while the quinolones(*qnr*A) gene was detected in six out of eight selected isolates with positive bands at 516 bp. (Fig. 10).

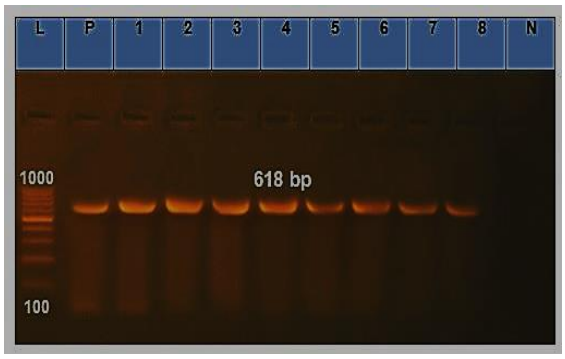

Fig.1: Agarose gel electrophoresis of *Pseudomona*s isolates (16S rDNA) gene.

L: 100-1000 bp. DNA Ladder. N.: Negative control (N. control. (*Staphylococcus. aureus* ATCC®25923 ™), Pos.: Positive control (*Pseudomonas* form Ahri at 618 bp (Lanes 1-4 *Ps. Aeruginosa* isolated from *O. niloticus*
Lanes 5-8 *Ps. Aeruginosa* isolated from *C. gariepinus*), all eight isolates showed positive bands for the 16S rDNA gene at 618bp.

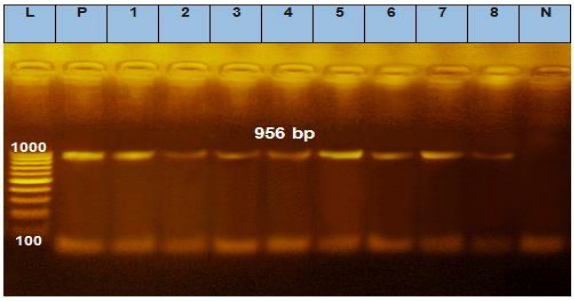

Fig. 2: Agarose gel electrophoresis of specific *Ps. Aeruginosa* isolates (16S rDNA) gene. L: 100-1000 bp. DNA Ladder. N. control (Negative control. (*S. aureus* ATCC®25923 ™), Pos.: Positive control (*Ps. aeruginosa* ATCC®27853™ at 956 bp. Lanes 1-8: tested *Ps. aeruginosa*; Lanes 1-4: *Ps. aeruginosa isolated* from *O. niloticus,* Lanes 5-8: *Ps. aeruginosa* isolated from *C. gariepinus*

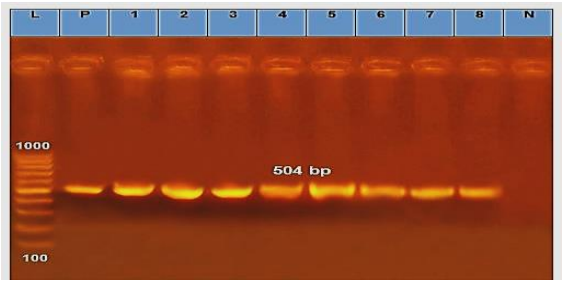

Fig. 3: Agarose gel electrophoresis of exopolysaccharide synthesis locus (*psl*A) gene.

Lane L: 100-1000 bp. DNA Ladder. N, control (Negative control. (*Staphylococcus aureus* ATCC®25923 ™), P.: Positive control (*Ps. aeruginosa* ATCC®27853™ at 504 bp.). Lanes (1 - 8): number of *Ps. aeruginosa* isolates from each sample (1-4 *O. niloticus* and 5-8 *C. gariepinus*), all eight isolates showed positive bands for the specific *psl*A gene at 504bp.

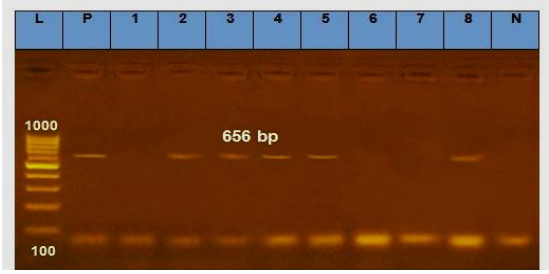

Fig. 4: Agarose gel electrophoresis of outer membrane lipoprotein L (*opr*L) gene.

Lane L: 100-1000 bp. DNA Ladder. N control (Negative control. (*Staphylococcus aureus* ATCC®25923 ™), P.: Positive control (*Ps. aeruginosa* ATCC®27853™ at 656 bp.). Lanes (1, 6 - 7): *Ps. aeruginosa* negative for *opr*L gene, Lanes (2- 5&8): Positive *Ps. aeruginosa* for *opr*L gene at 656 bp.

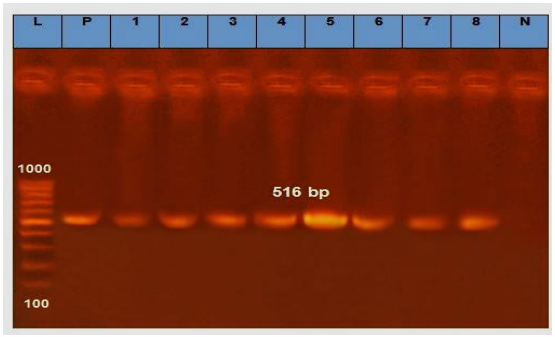

Fig.5: Agarose gel electrophoresis of β- lactam (*bla*$_{TEM}$) gene.
Lane L: 100-1000 bp. DNA Ladder. N.: Negative control (N. control. (*S. aureus* ATCC®25923 ™), P.: Positive control (*Ps. aeruginosa* ATCC®27853™ at 516 bp.). Lanes (1 - 8): positive *Ps. aeruginosa* for *bla*$_{TEM}$ gene at 516 bp.

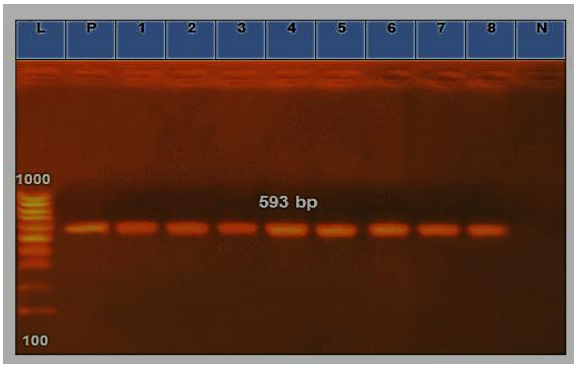

Fig. 6: Agarose gel electrophoresis of extended spectrum β-lactam (bla$_{CTX-M}$) gene.
Lane L: 100-1000 bp. DNA Ladder. N.: Negative control (N. control. (*S. aureus* ATCC®25923 ™), P.: Positive control (*Ps. aeruginosa* ATCC®27853™ at 593 bp.). Lanes (1 - 8): positive *Ps. aeruginosa* for *bla*$_{CTX-M}$ gene at 593 bp.

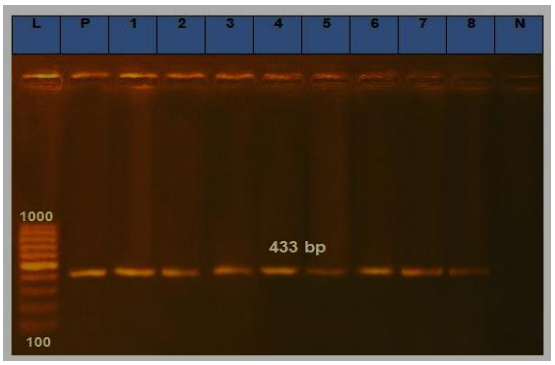

Fig. 7: Agarose gel electrophoresis of sulphonamide (*sul1*) gene.
Lane L: 100-1000 bp. DNA Ladder. N. control (Negative control. (*S. aureus* ATCC®25923 ™), P.: Positive control (*Ps. aeruginosa* ATCC®27853™ at 433 bp.). Lanes (1 - 8): positive *Ps. aeruginosa* for *sul1* gene at 433 bp.

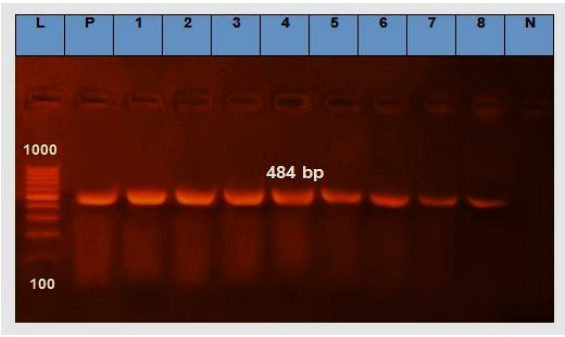

Fig. 8: Agarose gel electrophoresis of the streptomycin (*aad*A1) gene.
Lane L: 100-1000 bp. DNA Ladder. N.: Negative control (N. control. (*S. aureus* ATCC®25923 ™), P.: Positive control (*Ps. aeruginosa* ATCC®27853™ at 484 bp.). Lanes (1 - 8): positive *Ps. aeruginosa* for *aad*A1 gene at 484 bp.

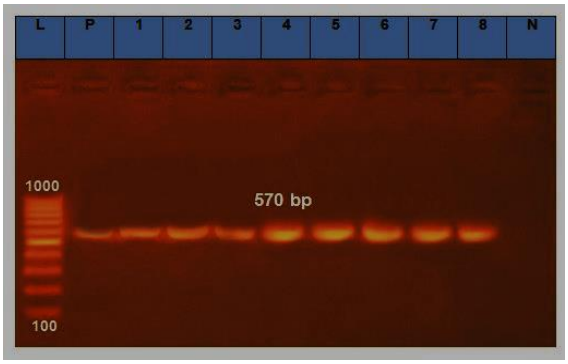

Fig. 9: Agarose gel electrophoresis of tetracycline A *tet*A (A)gene.
Lane L: 100-1000 bp. DNA Ladder. N. control (Negative control. (*S. aureus* ATCC®25923 ™), P.: Positive control (*Ps. aeruginosa* ATCC®27853™ at 570 bp.). Lanes (1 - 8): positive *Ps. aeruginosa* for *tet*A(A) gene at 570 bp

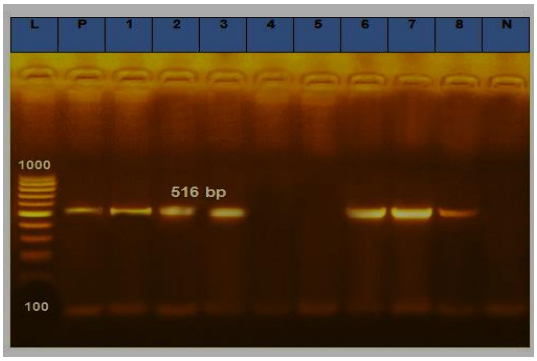

Fig. 10: Agarose Gel electrophoresis of quinolones (*qnr*A) gene.
Lane L: 100-1000 bp. DNA Ladder. N. control (Negative control. (*S. aureus* ATCC®25923 ™), P.: Positive control (*Ps. aeruginosa* ATCC®27853™ at 516 bp.). Lanes (4, 5): *Ps. aeruginosa* negative, Lanes (1 – 3 &6-8): positive *Ps. aeruginosa* for *qnr*A gene at 516 bp.

DISCUSSION

Pseudomonas species, mainly *Ps. aeruginosa*, are widely distributed microorganisms that have been recorded as one of the most robust causes of ulcer-type diseases and septicemia among fishes, resulting in high mortality rates, significant economic losses, and reduced fish farm efficiency (Shahrokhi *et al*., 2022). Additionally, they play a role in causing both gastrointestinal and extra-intestinal infections in humans (Benie *et al*., 2017 and Abdulhaq *et al*., 2020).

The bacteriological findings revealed that 52 *Ps. aeruginosa* isolates were isolated from 250 examined samples (23 from Nile tilapia and 29 from African sharptooth catfish). Nearly comparable findings were recorded by Hanna *et al*. (2014); Abd El- Tawab *et al*. (2016 and 2019a); Algammal *et al*. (2020), and Ali *et al*. (2023). Meanwhile, these findings are contrary to those of Shahrokhi *et al*. (2022), who reported a lower incidence of 5.0%, and with Mohamed e*t al.* (2023), who revealed that the gills, followed by kidneys and livers, had the highest infection rates. These variations in prevalence may be due to differences in host susceptibility, time of sampling collection, geographical area, management and hygienic program, the fish source (from farms or markets), and environmental factors.

The colony morphology, Gram stain, and biochemical character of the isolated *Pseudomonas* species exhibited typical characteristics of *Ps. aeruginosa* and were consistent with previous reports by Austin and Austin (2016); Abd El- Tawab *et al*. (2019a); Algammal *et al*. (2020); Shahrokhi *et al*. (2022); Ali *et al*. (2023); and Mohamed *et al*. (2023).

In our study, *Ps. aeruginosa* isolates were susceptible to norfloxacin, followed by gentamicin and ciprofloxacin, and highly resistant to ampicillin, then oxacillin, amoxicillin, tetracycline, cefotaxime, and nalidixic acid. These findings are consistent with those reported by Abd El Tawab *et al*. (2016, 2019a); Shahrokhi *et al*. (2022); Ali *et al*. (2023), who documented similar sensitivity patterns. MDR is defined as resistance to at least three antimicrobial agents from different classes (Rocha *et al.,* 2019). Therefore, the recorded results have proved that phenotypic MDR is widely spread among *Ps. aeruginosa* isolates (90%) and these results came in harmony with Abd El Tawab *et al*. (2016, 2019a); Shahrokhi *et al*. (2022); Ali *et al*. (2023) and Mohamed *et al*. (2023).

The PCR that uses the 16S rDNA technology offers an effective and reliable tool for the quick and accurate detection of *Pseudomonas* species, providing a speedier alternative to standard, time-consuming biochemical identification techniques (Uma *et al.*, 2007). In the present study, PCR was performed on eight *Ps. aeruginosa* isolates, revealing electrophoresis of the general *Pseudomonas* gene (*16S rDNA*) with a specific band at 618 bp. This result confirmed and aligned with the findings of Spilker *et al*. (2004) and Eid *et al*. (2016). The species-specific *16S rDNA* gene serves as a reliable marker for detecting *Ps. aeruginosa*, providing precise identification even for rare isolates (Spilker *et al*., 2004; Uğur *et al*., 2012)). The PCR results in this study confirmed the presence of this gene in all eight *P. aeruginosa* isolates, with a specific band detected at 956 bp. This finding indicates that all tested isolates were *P. aeruginosa* strains. Similar results were previously reported (Spilker *et al*., 2004; Hanna *et al*., 2014; Abd El-Aziz 2015; Eid *et al*., 2016 and Abd El- Tawab *et al*., 2019b).

Ps. aeruginosa's virulence genes play a significant role in their pathogenicity, because they act in particular ways in the pathogenic mechanism. Each gene may alter host cell signal transduction in ways that accelerate the spread of infection.

The *pslA* gene is a biofilm formation marker that plays an important role in the polysaccharide synthesis locus (psl), which is responsible for secreting extracellular

polysaccharides in *Ps. aeruginosa*. The PCR amplification findings confirmed the presence of the pslA gene in all eight selected Ps. aeruginosa isolates, resulting in a 504 bp fragment. The *pslA* gene was also found in antibiotic-resistant biofilm-forming *Ps. aeruginosa* strains obtained from people, fish, animals, and the environment (Nader *et al*. (2017); Abdulhaq *et al.* (2020); Madaha *et al*. (2020); Ugwuanyi *et al*. (2021); Schimmunech *et al*. (2022) and Ali *et al.* (2023).

Additionally, the outer membrane lipoprotein L (oprL) is a factor, that can cause cell death, and important for how the bacterium interacts with its environment, as well as for Ps. aeruginosa's natural resistance to antibiotics (Markey *et al*., 2013). The PCR tests for the oprL gene in *Ps. aeruginosa* strains showed it was found in five out of the eight tested isolates, producing fragments of 504 bp. Similar results have been previously reported by Abdullahi *et al.* (2013); Khattab *et al*. (2015); Abd El- Tawab *et al*. (2016 and 2019b); Abdulhaq *et al*. (2020); Abd El-Maogoud *et al*. (2021); Ali *et al*. (2023) and Mohamed *et al.* (2023).

The study's results are concerning, since β-lactam, sulphonamide, tetracycline, and streptomycin antimicrobials are often used to treat bacterial infections in fish, animals, and humans.

Our results of PCR described that all eight *Ps. aeruginosa* strains have *bla*TEM, *bla* CTX-M,*sul*1, *aad*A1, and *tet*A(A) antimicrobial resistance genes, but the *qnr*A resistance gene was found in six strains only. These findings are in line with those obtained by Ndi and Barton (2012) ; Bălă¸soiu *et al.* (2014); Fazeli and Momtaz (2014); Al Dawodeyah *et al*. (2018); Algammal *et al.* (2020); Giovagnorio *et al*. (2023) and Mohamed *et al*. (2023), who detected the *bla*TEM and *bla* CTX-M genes, Salimizadeh (2018) ; Alariqi *et al*.(2024) and Ikhrami *et al*. (2024) who found the *sul*1 gene, Ndi and Barton (2012); Salimizadeh (2018) and Mu *et al*. (2023) who exposed *aad*A1 gene, While Algammal *et al*. (2020); Alariqi *et al*. (2024); Ikhrami *et al*. (2024) found the *tet*A (A) gene and Cayci *et al*.(2014); Saki *et al*. (2022); Giovagnorio *et al*. (2023) and Alariqi *et al*. (2024) detected the *qnr*A gene in *Ps. aeruginosa* strains isolated from fish and different sources.

CONCLUSION

The PCR technique offers an important, rapid, and accurate tool for the detection of *Ps. aeruginosa*. Ninety percent of *Ps. aeruginosa* isolates were detected to be multiple drug-resistant (MDR). The prevention of MDR resistance is becoming a must through controlling the antimicrobial overuse to prevent or limit the emergence of new antibiotic-resistant lineages of bacteria. Hence, the *psl*A gene and *opr*L genes are responsible for the virulence and pathogenicity of *Ps. aeruginosa* strains in freshwater fish; their presence in the currently isolated *Ps. aeruginosa* strains may indicate their pathogenicity for freshwater fish (tilapia and catfish). The increasing incidence of community-acquired illnesses is likely to raise the risk of fish infections, which could lead to food poisoning originating from fish. Moreover, the revealed norfloxacin, gentamicin, and ciprofloxacin sensitivity of most isolates indicates the possibility of controlling the *Ps. aeruginosa* strains septicemia in fish.

REFERENCES

Abd El-Aziz and D.M. (2015): Detection of Pseudomonas spp. in Chicken and Fish Sold in Markets of Assiut City, Egypt. J. Food Quality and Hazards Control., 2: 86-89.

AbdEl-Maogoud, H.; Edris, A.M.; Mahmoud, A.H. and Maky, M.A. (2021): Occurrence and characterization of Pseudomonas species isolated from Fish Marketed in Sohag Governorate, Egypt. SVU- Inter. J. Vet. Sci., 4 (2): 76-84.

Abd El-Tawab, A.A.; El Hofy, Fatma, I. El-Sayed and Aya, A. (2019a): Bacteriological studies on Aeromonas and pseudomonas species in Nile tilapia

(Oreochromis niloticus). Benha Veterinary Medical Journal 37: 144-148.

Abd El Tawab, A.A.; Maarouf, A.A.; Ahmed, A. and Nesma, M.G. (2016): Detection of Virulence factors of Pseudomonas species isolated from fresh water fish by PCR. Benha Vet. Med. J. 30(1):199-207.

Abd El Tawab, A.A.; Maarouf, A.A.A.; El Hofy, F.; Amany, O.S. and El-Sayed, A.M. (2019b): Phenotypic and molecular detection of Aeromonas and pseudomonas species isolated from fish with special reference to their virulence factors. Nature and Science, 17(12): 194-205.

Abdulhaq, N.; Nawaz, Z.; Asif, Z.M. and Siddique, A. (2020): Association of biofilm formation with multi drug resistance in clinical isolates of Pseudomonas aeruginosa. EXCLI Journal, 19:201-208.

Abdullahi, R.; Lihan, S.; Carlos, B.S.; Bilung, M.L.; Mikal, M.K. and Collick, F. (2013): Detection of oprLgene and antibiotic resistance of Pseudomonas aeruginosa from aquaculture environment. European J. Experimental Biology, 3(6):148-152.

Alariqi, R.; Almansoob, S.; Senan, A.M.; Raj, A.K.; Rajesh, S.; Shrewastwa, M.K. and Kumal, J.P. (2024): Pseudomonas aeruginosa and related antibiotic resistance genes as indicators for wastewater treatment. Heliyon, 10: 29798-29809.

Al Dawodeyah, Heba, Y.; Obeidat, N.F.; Abu-Qatouseh, Luay, F. and Shehabi, A.A. (2018): Antimicrobial resistance and putative virulence genes of Pseudomonas aeruginosa isolates from patients with respiratory tract infection. GERMS, 8(1): 31-40.

Algammal, A.M.; Mabrok, M.; Sivaramasamy, E.; Youssef, F.M.; Atwa, M.H.; El-Kholy, A.W. and Hozzein, W.N. (2020): Emerging MDR-Pseudomonas aeruginosa in fish commonly harbor oprL and toxA virulence genes and blaTEM, blaCTX-M, and tetA antibiotic resistance genes. Scientific Rep. 10: 1-12.

Ali, H.; Awad, A.; Maarouf, A. and Ahmed, W. (2023): 'Molecular Detection of some Virulence Factors of Pseudomonas aeruginosa Isolated from Freshwater Fishes at Qalubiya Governorate, Egypt', *Benha Veterinary Medical Journal*, 43(2), pp. 80-84.

Archambault, M.; Petrov, P.; Hendriksen, R.S.; Asseva, G.; Bangtrakulnonth, A.; Hasman, H. and Aarestrup, F.M. (2006): Molecular characterization and occurrence of extended-spectrum beta-lactamase resistance genes among Salmonella enterica serovar Corvallis from Thailand, Bulgaria, and Denmark. Microb. Drug Resist.Fall.; 12(3):192-198.

Austin, B. and Austin, D.A. (2016): Bacterial Fish Pathogens, Diseases of Farmed and Wild Fish 6th Ed, Springer International Publishing Switzerland.

Bălă,soiu, M.; Bălă,soiu, A.T.; Mănescu, R.; Avramescu, C. and Ionete, O. (2014): Pseudomonas aeruginosa Resistance Phenotypes and Phenotypic Highlighting Methods. Curr. Health Sci., J. 40: 85-94.

Benie, C.K.D.; Dadié, A.; Guessennd, N.; Kouadio, N.A.; Kouame, N.D.; N'golo, D.C.; Aka, S.; Dako, E.; Dje, K.M. and Dosso, M. (2017): Characterization of virulence potential of Pseudomonas aeruginosa isolated from bovine meat, fresh fish, and smoked fish.European J. Microbiology and Immunology, 7 (1): 55–64.

Cayci, Y.T.; Coban, A. and Gunaydin, M. (2014): Investigation of Plasmid-Mediated Quinolone Resistance in Pseudomonas aeruginosa Clinical Isolates. Indian J. Med. Microbiol., 32, 285–289.

CLSI" Clinical Lab Standards Institute". (2018): Performance Standards for Antimicrobial Disk Susceptibility Tests. 13th ed. CLSI standard M02. Wayne, PA.

Colom, K.; Pèrez, J.; Alonso, R.; Fernández-Aranguiz, A.; Lariño, E. and Cisterna, R. (2003): Simple and reliable multiplex PCR assay for detection of *bla*$_{TEM}$, *bla*$_{SHV}$ and *bla*$_{OXA-1}$ genes in Enterobacteriaceae. FEMS Microbiology Letters, 223: 147-151.

Eid, H.M.; El-Tabiy, A.A. and Fathy, S.M. (2016): Prevalence and Molecular Characterization of Pseudomonas

Species Isolated From Fish Markets in Port-Said. SCVMJ, XXI (1) :1-12.

Esposito, S. and De Simone G. (2017): Update on the main MDR pathogens: prevalence and treatment options. Infez. Med. 25, 301-310.

Fazeli, N. and Momtaz, H. (2014): Virulence gene profiles of multidrug-resistant Pseudomonas aeruginosa isolated from Iranian hospital infections. Iran Red Crescent Med J. , 16(10): e15722.

Ghadaksaz, A.; Fooladi, A.A.I.; Hosseini, H.M. and Amin, M. (2015): The prevalence of some Pseudomonas virulence genes related to biofilm formation and alginate production among clinical isolates.J. Applied Biomedicine, 13: 61–68.

Ghafoor, A.; Hay, I.D. and Rehm, B.H.A. (2011): Role of exopolysaccharides in *Pseudomonas aeruginosa* biofilm formation and architecture. Appl. Environ. Microbiol., 77(15):5238–5246.

Giovagnorio, F.; De Vito, A.; Madeddu, G.; Parisi, S.G. and Geremia, N. (2023): Resistance in *Pseudomonas aeruginosa*: A Narrative Review of Antibiogram Interpretation and Emerging Treatments. Antibiotics, 12, 1621-1646.

Hanna, M.I.; El-Hady, M.A.; Hanaa, A.A.; Elmeadawy, S.A. and Kenwy, A.M. (2014): Contribution on *Pseudomonas aeruginosa* infection in African Catfish (*Clarias gariepinus*) Research J. Pharmaceutical, Biological and Chemical Sciences., 5 (5) :575-588.

Ibekwe, A.M.; Murinda, S.E. and Graves, A.K. (2011): Genetic Diversity and Antimicrobial Resistance of *Escherichia coli* from Human and Animal Sources Uncovers Multiple Resistances from Human Sources. PLoS ONE, 6(6): 20819.

Ikhrami, M.A.; Sari, D.W.K. and Putra, M.P. (2024): Emergence of antibiotic resistance genes *sul*1, *tet*A, *bla*GES, and *mex*F in sapon irrigation canal and aquaculture pond in Kulon Progo Regency, Indonesia. J. Ecological Engineering, 25(2), 85–92.

Khattab, M.A.; Nour, M.S. and El-Sheshtawy, N.M. (2015): Genetic identification of *Pseudomonas aeruginosa* virulence genes among different isolates. J. Microbiol. Biochem. Technol., 7(5): 274-277.

Madaha, E.L.; Gonsu, H.K.; Bughe, R.N.; Fonkoua M.C.; Ateba, C.N. and Mbacham, W.F. (2020): Occurrence of *bla*TEM and *bla* CTX-M genes and biofilm forming ability among clinical isolates of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* in Yaoundé Cameroon. Microorganism, 8:708-715.

Markey, B.K.; Leonard, F.C.; Archambault, M.; Cullinane, A. and Maguire, D. (2013): Clinical Veterinary Microbiology, 2nd Ed. MOSBY. Elsevier Ltd. Edinburgh London New York Oxford Philadelphia St Louis Sydney Toronto.

Mavrodi, D.V.; Bonsall, R.F.; Delaney, S. M.; Soule, M.J.; Phillips, G. and Thomashow, L.S. (2001): Functional analysis of genes for biosynthesis of pyocyanine and phenazine-1-carboxamide from Pseudomonas aeruginosa PAO1. J. Bacteriol., 183(21): 6454-6465.

Mohamed, D.S.; Ragab, A.M.; Ibrahim, M.S. and Talat, D. (2023): Prevalence and Antibiogram of Pseudomonas aeruginosa Among Nile Tilapia and Smoked Herring, with an Emphasis on their Antibiotic Resistance Genes (blaTEM, blaSHV, blaOXA-1 and ampC) and Virulence Determinant (oprL and toxA). *Journal of Advanced Veterinary Research*, *13*(6), 1166-1172.

Mu, X.; Li, X.; Yin, Z.; Jing, Y.; Chen, F.; Gao, H.; Zhang, Z.; Tian, Y.; Guo, H.; Lu, X.; He, J.; Zheng, Y.; Zhou, D.; Wang, P. and Dai, E. (2023): Abundant diversity of accessory genetic elements and associated antimicrobial resistance genes in *Pseudomonas aeruginosa* isolates from a single Chinese hospital. Annals of Clinical Microbiology and Antimicrobials, 22:51-67.

Nader, M.I.; Kareem, A.A.; Rasheed, M.N. and Issa, M.A.S. (2017): Biofilm formation and detection of *psl*Á gene in multidrug resistant *Pseudomonas aeruginosa* isolated from Thi-Qar, Iraq.Iraqi J. Biotechnology, 16(4): 89-103.

Ndi, O.L. and Barton, M.D. (2012): Resistance Determinants of Pseudomonas Species

from Aquaculture in Australia. J. Aquac Res. Development, 3: 1-11.

Overhage, J.; Schemionek, M.; Webb, J.S. and Rehm, B.H. (2005): Expression of the psl Operon in *Pseudomonas aeruginosa* PAO1 Biofilms: *psl*A performs an essential function in biofilm formation. Appl Environ Microbiol., 71(8):4407–4413.

Randall, L.P.; Cooles, S.W.; Osborn, M.K.; Piddock, L.J.V. and Woodward, M.J. (2004): Antibiotic resistance genes, integrons and multiple antibiotic resistance in thirty-five serotypes of *Salmonella enterica* isolated from humans and animals in the UK. J. Antimicrobial Chemotherapy, 53: 208–216.

Robicsek, A.; Strahilevitz, J.; Jacoby, G.A.; Macielag, M.; Abbanat, D.; Park, C.H.; Bush, K. and Hooper, D.C. (2006): Fluoroquinolonemodifying enzyme: a new adaptation of a common aminoglycoside acetyltransferase. Nat. Med., 12: 83-88.

Roberts, R.J. (2012): Fish Pathology.4th. Ed, Wiley-Blackwell, Iowa, 581.

Rocha, A.J.; Barsottini, M.R.; Rocha, R.R.; Laurindo, M.V.; Laurindo De Moraes, F.L. and Rocha, S.L. (2019): Pseudomonas aeruginosa: Virulence factors and antibiotic resistance genes. Brazilian Archives of Biology and Technology.62: 1-15.

Roulová, N.; Mot'ková, P.; Brožková, I. and Pejchalová, M. (2022): Antibiotic resistance of *Pseudomonas aeruginosa* isolated from hospital wastewater in the Czech Republic. J. WateR Health, 20:692-701.

Saki, M.; Farajzadeh Sheikh, A.; Seyed-Mohammadi, S.; Asareh Zadegan Dezfuli, A.; Shahin, M.; Tabasi, M.; Veisi, H.; Keshavarzi, R. and Khani, P. (2022): Occurrence of plasmid-mediated quinolone resistance genes in *Pseudomonas aeruginosa* strains isolated from clinical specimens in Southwest Iran: A multicentral study. Sci. Rep., 12. 2296.

Salimizadeh, Z. and Karouei, S.M.H. Hosseini, F. (2018): Dissemination of Class 1 Integron among different multidrug resistant *Pseudomonas aeruginosa* strains. Medical Laboratory J., 12(4): 36-42.

Sambrook, J.; Fritsch, E. and Montias, T. (1989): Molecular Biology. In: Molecular cloning. Laboratory manual, 2nd Ed. Cold Spring Harbor Laboratory press, USA.

Schimmunech, M.S.; Lima, E.A.; Silveira, A.V.B.; De Oliveira, A.F., Moreira, C.N.; De Souza, C.M.; De Paula, E.M.N. and Stella, A.E. (2022): Pseudomonas aeruginosa isolated from the environment of a veterinary academic hospital in Brazil - resistance profile. Acta Scientiae Veterinariae, 50: 1854-1861.

Shahrokhi, G.R.; Rahimi, E. and Shakerian, A. (2022): The prevalence rate, pattern of antibiotic resistance, and frequency of virulence factors of *Pseudomonas aeruginosa* strains isolated from fish in Iran.J. Food Quality Volume 2022, Article ID 8990912:1- 8.

Spilker, T.; Coenye, T.; Vandamme, P. and Lipuma, J. (2004): PCR- Based assay for differentiation of *Pseudomonas aeruginosa* from other Pseudomonas species recovered from cystic fibrosis patients. J. Clin. Microbiol., 42(5): 2074-2079.

Uğur, A.; Ceylan, O. and Ashm, B. (2012): Characterization of Pseudomonas spp. from seawater of the southwest coast of Turkey. J. Biol. Environ. Sci., 6(16): 15-23.

Ugwuanyi, F.C.; Ajayi, A.; Ojo, D.A.; Adeleye, A.I. and Smith, S.I. (2021): Evaluation of efflux pump activity and biofilm formation in multidrug resistant clinical isolates of Pseudomonas aeruginosa isolated from a Federal Medical Center in Nigeria. Ann. Clin. Microbiol. Antimicrob. 20: 11-19.

Uma, A.; Reddy, Y.K.; Meena, S.; Saravanabava, K. and Muralimanohar, B. (2007): Application of 16S rDNA amplification and sequencing for detection of fish and shrimp bacterial pathogens. Ind. J. Comp. Microbiol. Immunol. Inf. Dis.28:7-9.

Xu, J.; Moore, J.E.; Murphy, P.G.; Millar, B.C. and Elborn, J.S. (2004): Early detection

of *Pseudomonas aeruginosa* - comparison of conventional versus molecular (PCR) detection directly from adult patients with cystic fibrosis (CF). Annals of Clinical Microbiology and Antimicrobials, 3:21.

Yang, L.; Hu, Y.; Liu, Y.; Zhang, J.; Ulstrup, J. and Molin, S. (2011): Distinctroles of extracellular polymeric substances in *Pseudomonas aeruginosa* biofilm development. Environ Microbiol. 13(7):1705– 1717.

دراسات بكتيرية وجزيئية على بكتيريا السودوموناس ايروجينوزا المعزولة من سمك القرموط الافريقي وسمك البلطي النيلي

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الزائفة الزنجارية (Pseudomonas aeruginosa) بكتيريا سالبة الجرام شائعة في البيئات المائية، ومعروفة بقدرتها على إحداث الأمراض الانتهازية في الأسماك. تُشكل تهديدًا خطيرًا لتربية الأحياء المائية نظرًا لقدرتها على التسبب في عدوى شديدة، وارتفاع معدلات النفوق في تجمعات الأسماك، وخسائر مالية فادحة. لذلك، تسلط هذه الدراسة الضوء أيضًا على المخاوف الصحية العامة المتعلقة بالسودوموناس ايروجينوزا بالنسبة للمستهلكين. أجريت الدراسة الحالية على ٢٥٠ عينة من الأعضاء التي تظهر عليها اعراض مرضية في العضلات والكبد والكلى والأمعاء والطحال لأسماك البلطي النيلي المريضة (*O. niloticus*) والقرموط الأفريقي (*C. gariepinus*) بأحجام مختلفة. تم جمع الأسماك من أسواق الأسماك المختلفة بمحافظة القليوبية بمصر خلال الفترة من سبتمبر ٢٠٢٣ إلى فبراير ٢٠٢٤ لعزل وتوصيف المظهر والوراثة لبكتيريا (*Ps. aeruginosa*) *Pseudomonas aeruginosa* . أظهرت النتائج أن ٥٢ عزلة aeruginosa تم استخلاصها من أعضاء أسماك المياه العذبة وتم عزلها في الغالب من ٢٥ عينة عضلية (٤٨,١٪) تليها الأمعاء ١٣ (٢٥,٠٪) ثم الكبد ٨ (١٥,٤٪)؛ الكلى ٥ (٩,٦٪)؛ وعينة واحدة من الطحال من سمك القرموط الافريقي (١,٩٪). أظهر اختبار الانتشار القرصي وجود مقاومة للأدوية المتعددة (MDR) في جميع العزلات ضد فئات المضادات الميكروبية المستخدمة. كانت *Ps. aeruginosa* حساسة للغاية للنورفلوكساسين يليه الجنتاميسين والسيبروفلوكساسين. في المقابل، كان لديهم مقاومة عالية للأمبيسيلين ثم الأوكساسيلين, أموكسيسيلين, التتراسيكلين, سيفوتاكسيم وحمض الناليديكسيك. تم اكتشاف جينات ١٦ s-Pseudomonas(جنس) وجينات ١٦ s-Pseudomonas aeruginosa(خاصة بالنوع) بواسطة تقنية تفاعل البلمرة المتسلسل في جميع العزلات المختارة. تم العثور على جينات (pslA و oprL) في ثمانية وخمسة نباتات تمت دراستها ps. aeruginosa.على التوالي. تم الكشف عن جينات ١٦ s-Pseudomonas(جنس) وجينات ١٦ s-Pseudomonas aeruginosa (خاصة بالنوع) بواسطة تفاعل البلمرة المتسلسل في جميع العزلات. لقد تم التوصل إلى أن الجين الرئيسي المسؤول عن ضراوة Ps. Aeruginosa المعزولة من أسماك المياه العذبة هو pslA.