ISSN: 2356-7767

Characterization of *Pseudomonas Putida* isolated from mullet (*Mugil Cephalus*) in Port said, Egypt.

***Ahmed, Sittien; **Jehan, I. Abdellatif; **Soad, S. A. Salama; *Rasha, Elkenany and *Amal Awad**

*Department of Bacteriology, Mycology, and Immunology, Faculty of Veterinary Medicine, Mansoura University, Egypt.

**Fish Diseases Department, Animal Health Research Institute, 12618, Dokki, ARC, Egypt.

Research

Corresponding author:
Jehan I. Abdellatif
E.mail: Jjibrahim2001@gmail.com

Received in 26 /11/2024
Accepted in 30 /12/2024

Abstract

This study aimed to examine the occurrence and antibiotic sensitivity of *Pseudomonas putida* in mullet fish (*Mugil Cephalus*) in Baher Al-Baqar, Egypt. Out of 150 moribund fish, *P. putida* was found in 49% (73/150) of the samples. The bacteria were identified in various organs of the fish, with the highest prevalence in the kidneys (32.9%, 24/73), followed by the liver (28.8%, 21/73), spleen (15%, 11/73), intestine (12.3%, 9/73), and gills (11%, 8/73). The genetic characterization of the *Pseudomonas putida* isolates revealed the presence of the exoS virulence gene and the resistance genes blaTEM and ampC. However, the toxA and blaSHV genes were absent. Additionally, the 16S rRNA PCR product from one representative strain was sequenced and deposited in the GenBank database under the accession number PP829285.1. This genetic profiling provides valuable insight into the virulence factors and antibiotic resistance mechanisms of *P. putida* in the context of fish health. The phylogenetic tree of one representative strain showed high nucleotide identity with other *P. putida* strains in the GenBank database. The results of antimicrobial susceptibility of isolates revealed that the highest resistance was to trimethoprim sulphamethoxazol (67.1%), followed by oxytetracycline (56.2%), amikacin (52%), lincomycin (49.3%), nitrofurantoin (49.3%), oxalinic acid (48 %), gentamicin (46.6%), amoxicillin (45.2%), tetracycline (43.8%), and erythromycin (41.1%). The study provides valuable insights into the dynamics of managing and controlling *Pseudomonas putida* infections in Mugil cephalus (mullet fish). By examining prevalence, antibiotic resistance patterns, and potential treatment options, this research enhances our understanding of how to effectively combat *P. putida* infections in aquaculture, ultimately supporting healthier fish populations and more sustainable aquaculture practices. It emphasizes the importance of continuous monitoring of bacterial prevalence and resistance patterns, as well as the development of innovative treatment methods. These efforts are crucial for ensuring the sustainability of aquaculture practices in Egypt and other regions, highlighting the need for effective management strategies to address fish health and prevent the spread of antimicrobial-resistant pathogens.

Keywords: *Pseudomonas putida, Mugil Cephalus, 16S rRNA gene, antimicrobial susceptibility,*

Introduction

Pseudomonas spp. are the most common pathogens among human , food ,water seafood and fish **Milligan *et al.* (2023).** Pseudomonas species are among the most virulent pathogens affecting fish, leading to conditions such as ulcerative syndrome and pseudomonas septicemia. These infections can cause severe damage to fish health, resulting in significant economic losses in aquaculture **El-Nagar (2010), Algammal *et al.* (2020).** Pseudomonas spp. (including *P. aeruginosa*, *P. putida*, and *P. fluorescens*) are zoonotic bacterial pathogens that usually cause disease and significant mortality among both cultured and freshwater fish. *Pseudomonas putida* is a non-fermenting Gram-negative, aerobic, rod-shaped, and motile bacteria and non-spore forming, straight or slightly curved rods bacilli, usually motile for the presence of one or more flagella, *Pseudomonas putida* is unable to grow at a pH lower than 4.5 **De Jonghe e*t al.* (2011)**. Their optimal growth temperature is equal to 25°C, but they can live in presence of lower temperatures **Decimo *et al.* (2014),** increasing their survival capability. It is a fast-growing bacterium found in soil and water habitats where oxygen is present **Krieg and Holt (1984).**

Aquaculture is considered as one of the most important food-producing sectors supplying the needs for animal protein and seafood sources globally **Troell *et al.* (2017)**. In Egypt in particularly mullet fish attain a great economic importance in the aquaculture market due to its high protein nutritional value. Among bacterial diseases, *P. putida* is an opportunistic human pathogen responsible for bacteremia and sepsis in neonatal, neutropenic and cancer patients, as well as for urinary tract infections **Altinok *et al.* (2006).** Naturally disease occurs through waterborne infection, bacteria attach itself to the gills (the main portal of entry) and spread to the internal organs through the bloodstream **Salama and Gharib (2009).**

The disease is characterized by petechial hemorrhage, darkness of the skin, detached scales, abdominal ascites, and exophthalmia in fish **Altinok *et al.* (2006); Austin& Austin (2007).**

Antibiotics are widely used in aquaculture to prevent and manage diseases. However, prolonged, or excessive use of antibiotics not only leads to residue accumulation but also contributes to the emergence of **antibiotic-resistant bacteria (ARB)** and the spread of **antibiotic resistance genes (ARGs)**. This poses a significant threat to both aquatic and human health, highlighting the need for responsible antibiotic use and alternative disease management strategies in aquaculture. The Pseudomonas species possesses a variety of virulent factors that may contribute to its pathogenicity. ***Pseudomonas putida*** has significantly important virulence factors such as exotoxin S and exotoxin A genes, which affect the severity of pathogenicity and mortality rate if both are present in aqua culture as mentioned by **Khattab *et al.* (2015).** In addition, the presence of resistance genes such as *bla*TEM, *bla*SHV and *amp*C often demonstrate resistance to antibiotics belonging to other classes which makes strategies of treatment more complex. Antibiotics, **antibiotic-resistant bacteria (ARB)**, and **antibiotic resistance genes (ARGs)** are extensively prevalent in aquaculture ecosystems. Their widespread presence poses significant risks to aquatic health, environmental sustainability, and public health, emphasizing the urgent need for improved management practices and alternative solutions to mitigate the development and spread of resistance. **Yuan *et al.* (2023**). Therefore, the present study was aimed to investigate the prevalence of *P. putida* from mullet fish (*Mugil Cephalus*) in Bahr Al-Baqar, Egypt, antimicrobial susceptibility testing, and detection of their virulence and antimicrobial resistance genes.

Materials and Methods

1. Fish sampling:

A total of 150 moribund Mullet (*Mugil Cephalus*), with an average weight of 90–120 g, were randomly collected from Bahr Al Baqar, Egypt, during different seasons from March 2022 to February 2023. Samples were transferred and analyzed at the Lab. of Fish Diseases Research Dept. at Animal Health Research Institute, Al-Dokki, Egypt. The fish samples were clinically examined for abnormal external and internal disease findings.

2. Bacteriological and Biochemical identification Findings:

Fish samples were subjected to bacteriological

examination under sterile conditions, and bacteria were isolated from the various organs of each fish (liver, kidney, spleen, gills, and any external lesions if present) using a sterile loop. Samples were streaked onto pseudomonas agar base medium (9222) (Lab M®, UK) supplemented with glycerol and gentamycin and incubated at 28°C for 18 h. The suspected colonies were further examined based on cultural characteristics, Gram stain microscopy, motility testing, and biochemical tests (oxidase, catalase, methyl red, Voges Proskauer, **Buller (2014); Duman *et al.* (2021).**

3. Molecular identification of *Pseudomonas Putida and* detections of *virulence genes*:
The Pseudomonas isolated are submitted for identification by 16S rDNA. The isolates were inoculated into brain heart infusion broth (BHIB) and incubated for 24 h. According to the manufacturer's instructions, DNA was extracted using the Patho Gene-spin™ DNA Extraction Kit.

3.1. Genotypic characterization and identification 16srRNA:
PCR was conducted using an oligonucleotide primer for the **16srRNA** gene **(F: AGAGTTTGATCCTGGCTCAG and R: AAG GAG GTG ATC CAG CCG CA)**. The PCR protocol was as follows an initial denaturation at 95°C for 5 min, followed by 35 cycles at 94°C for 2 min; 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s; 72°C for 5 min. the primer used in the amplification studies was obtained from **Benga *et al.* (2014)**. The *Pseudomonas putida* isolate was examined for the presence of antibiotic-resistance genes (*bla*TEM, *bla*SHV, and *parC*) and virulence genes (*exo*S, and *tox*A) Table (**1**).

3.2. Sequencing and phylogenetic analysis:
PCR products of *P. putida* were purified using a QIAquick PCR Product extraction kit (Qiagen, Valencia**).** Bigdye Terminator V3.1 cycle sequencing kit (Perkinelmer) was used for the sequence reaction and then it was purified using a Centrisep spin column. DNA sequences were obtained by Applied Biosystems3130 genetic analyzer (HITACHI, Japan), a BLAST® analysis (Basic Local Alignment Search Tool) **Altschul *et al.* (1990)** was initially performed to establish sequence identity to GenBank accessions. The MegAlign module of BioEdit created the phylogenetic tree, and Phylogenetic analyses used Neighbour joining in MEGA X **Kumar *et al.* (2018).**

4.1. Antimicrobial susceptibility of *Pseudomonas Putida*:
The disk diffusion method was used to assess the antimicrobial susceptibility of *P. Putida* strains according to **Quinn *et al.* (2002)** on Mueller-Hinton agar. 14 different antibiotic discs (Oxoid, Basingstoke, UK） were selected to cover the different antibiotic groups used in the aquaculture as follow: gentamycin (CN) (10 µg), lincomycin (MY) (10 µg), colistin sulphate (CT) (10 µg), erythromycin (E) (15 µg), nitrofurantoin (F) (300 µg), amoxycillin (AML) (10 µg), nalidixic acid (NA) (30 µg), oxalinic acid OA (2 µg), oflicin (OFX)(10 µg), tetracycline (TE) (30 µg), trimethoprim-sulphamethoxazol (SXT) (25 µg),flumequine (UB) (30 µg), amikacin (AK) (30 µg), oxytetracycline (T) (30 µg). Inhibition zone diameters were interpreted as sensitive (S), intermediate (I), and resistant (R), according to **CLSI (2024).**

Table (1). Oligonucleotide primers and cycling conditions of the primers of Antibiotic resistance genes.

	Oligonucleotide sequence (5′ → 3′)	Condition	Product size	References
Antibiotic resistance genes				
***bla*TEM**	F: TTGCTCACCCAGAAACGCTGGTG	94˚C/ 5 min, 94˚C/ 30, 61˚C/40 sec, 72˚C/ 45 finally 72˚C/ 10 min	708 bp	**Mataseje *et al*. (2012)**
	R: TACGATACGGGAGGGCTTACC			
***bla*SHV**	F: CGCCGGGTTATTCTTATTTGTCGC		1016 bp	
	R: TCTTTCCGATGCCGCCGCCAGTCA			
***amp*C**	F: GATCGTTCTGCCGCTGTG	94˚C/ 5 min, 94˚C/ 30, 56˚C/40 sec, 72˚C/ 45 finally 72˚C/ 10 min	271bp	**Oliver *et al*. (2002)**
	R: GGGCAGCAAATGTGGAGCAA			
Virulence genes				
exoS	F: CTT GAA GGG ACT CGA CAA GG	94˚C/ 5 min, 94˚C/ 30, 55˚C/40 sec, 72˚C/ 45 finally 72˚C/ 10 min	504 bp	**Khattab *et al.* (2015)**
	R: TTC AGG TCC GCG TAG TGA AT			
toxA	F: GGT AAC CAG CTC AGC CAC AT	94˚C/ 5 min, 94˚C/ 30, 58˚C/40 sec, 72˚C/ 45 finally 72˚C/ 10 min	352 bp	
	R: TGA TGT CCA GGT CAT GCT TC			

5.1. Statistical analysis

Statistical analysis of the results by calculation of the ratio was made using the SPSS (Statistical Set for Social Science) Statistics 17.0 software program.

6.1. Biosafety measures

This study applied biosafety measures in accordance with pathogen safety data sheets: Infectious substances- *Vibrio parahaemolyticus*, Pathogen Regulation Directorate, Public Health Agency of Canada (2010).

Results

1. Clinical and postmortem observations:

The moribund fish exhibited ocular hemorrhages with abdominal dropsy, hemorrhagic areas on the skin surface and at fin bases, skin lesions that ranged from detached scales to skin ulcers of variable depth and tail and fin rot (**Figure 1: A, B, C, D).** Internally, fish revealed accumulation of bloody ascitic fluids in the abdominal cavity, congestion of the gills and spleen, enlargement, paleness, and appearance of petechial hemorrhages on the liver, distended gallbladder, enlargement and congestion of spleen, as well as posterior kidney **(Figure 2: A& B).**

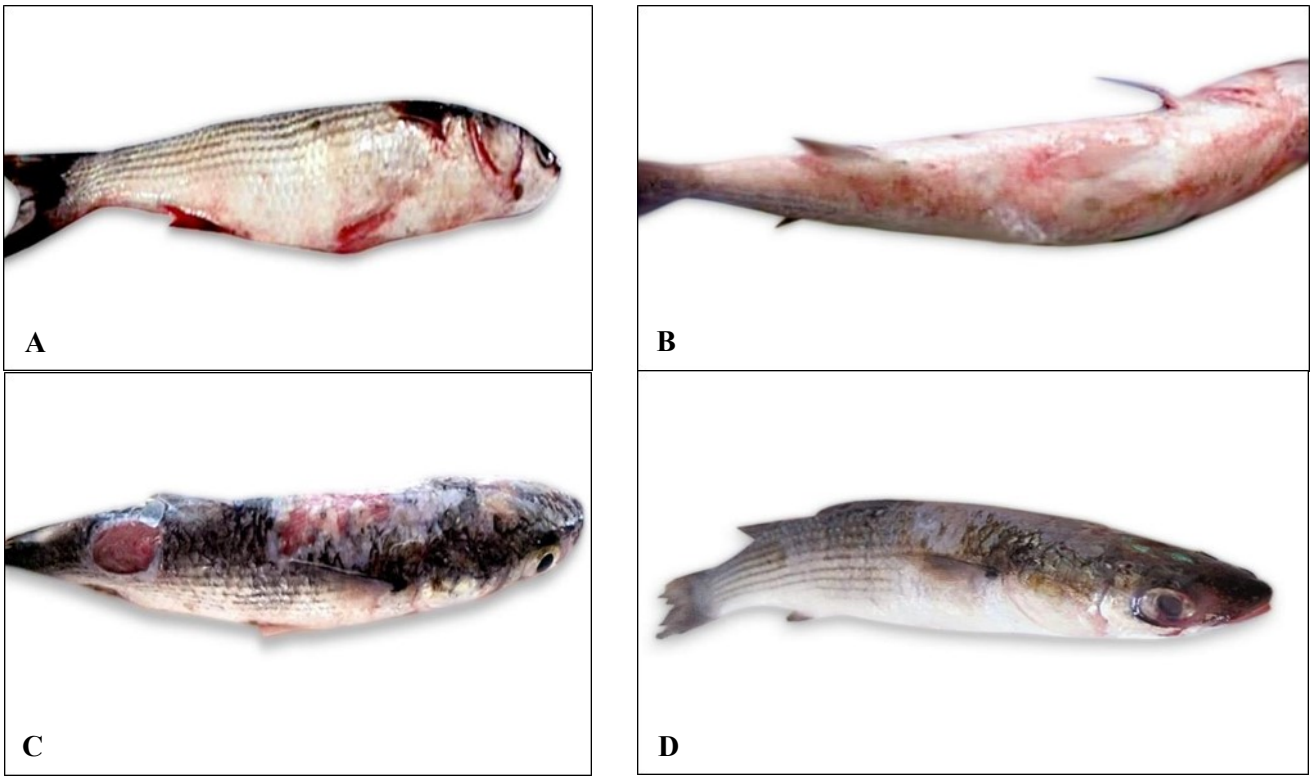


Figure (1). Clinical signs findings of moribund mullet showing: **(A)** Extensive cutaneous hemorrhages at caudal peduncle and at fin bases with scales loss and Abdominal distension, **(B)** Scale loss and hemorrhagic areas on the skin surface **(C)** Deep cutaneous lesions and hemorrhage at muscles **(D)** Scale loss with tail and fin rote and ocular hemorrhages.

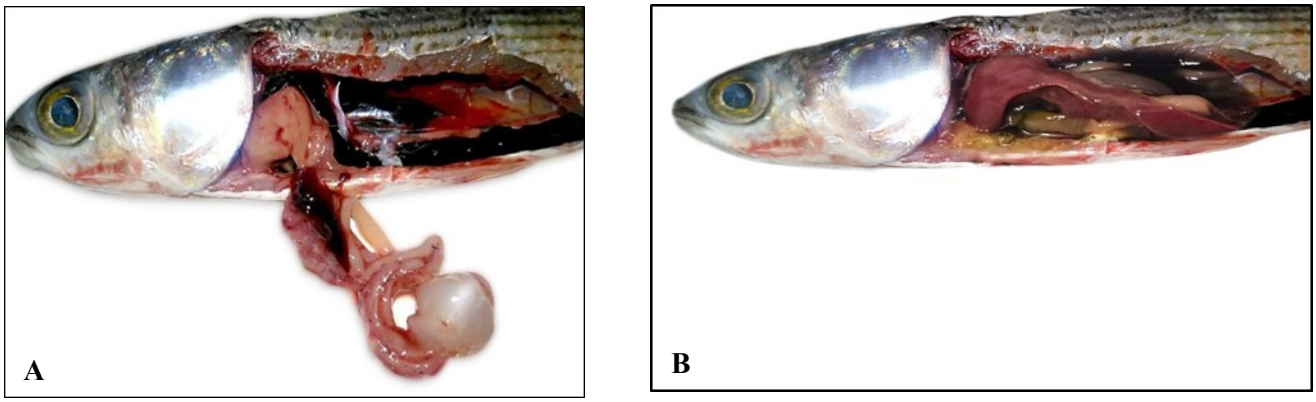


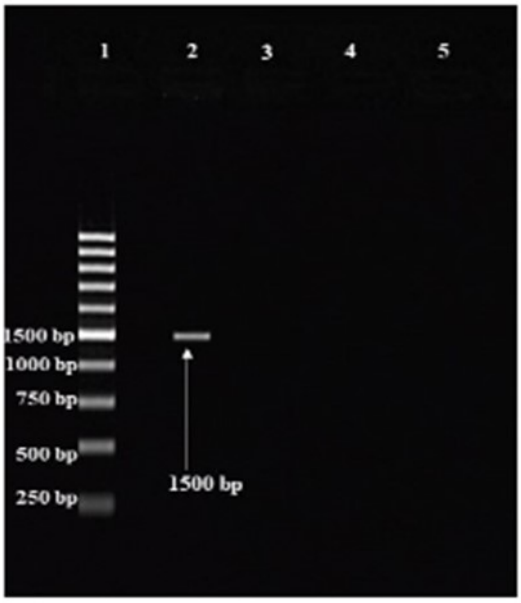
Figure (2). Necropsy findings of moribund mullet showing: **(A)** accumulation of bloody ascitic fluids in the abdominal cavity, congestion of spleen, enlargement, paleness, and appearance of petechial hemorrhage on the liver, distended gallbladder. **(B)** Enlargement and congestion of the spleen, as well as the posterior kidney, with hemorrhagic patches on the surface of the liver.

2. Prevalence of *Pseudomonas putida* in diseased fish

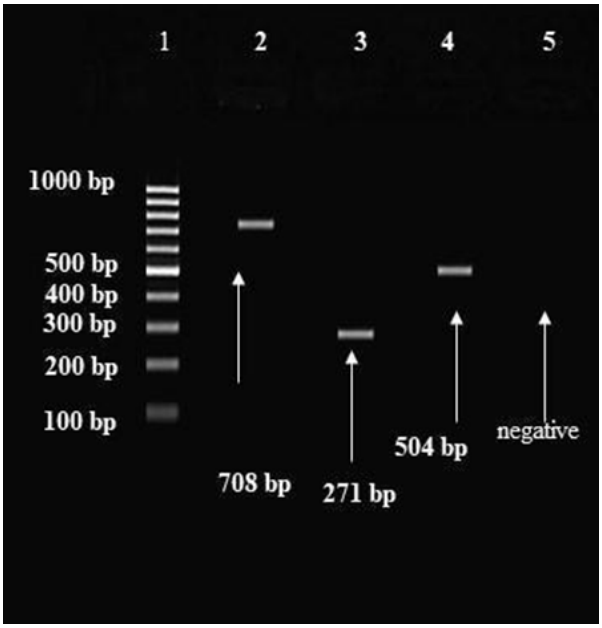
Pure *P. putida* isolates were diagnosed by culture character , biochemical assy, and confirmed by 16S rRNA gene . giving a specific band at 356 bp. The prevalence of *P. putida* in diseased *Mugil Cephalus* fish screened from Bahr El Baqar, was 49% (73/150). Out of the 73 samples, *P. Putida* were observed in multiple organ samples including 32.9% (24/73) of the kidneys,28.8% (21/73) liver, 15% (11/73) spleen, 12.3% (9/73) intestine, and 11% (8/73) gills.

3. Sequence analysis of *Pseudomonas putida* 16S rRNA gene

PCR products of 16S rRNA for one representative strain of our collection were purified and sequenced and sent for the GenBank database under the accession number PP829285.1. The resulting sequence isolate showed high nucleotide identity with other *P. putida* strains. The phylogenetic tree for the obtained sequence were constructed and showed high nucleotide identity with other isolates such as PP455756.1 isolated from Freshwater Fish specimen, ON491125.1 and ON491000.1 isolated from Catla as illustrated in **Figure (4) and (5)**. The strain (PP829285.1) was harboring *bla*TEM, ampC antibiotic resistance genes, and *exoS* virulence gene at the same time but it did not contain the ***bla*SHV** and ***tox*A** gene.



A



B

Figure (3.A). Agarose gel electrophoresis showing amplification of *P. putida* 16srRNA, **Lane (1)**:1000 bp DNA Ladder, **Lane (2)**: positive isolates for 16s gene.
Figure (3.b). Agarose gel electrophoresis of *Pseudomonas putida* antibiotic resistance and virulence genes.
Lane (1):100 bp DNA Ladder
Lane (2): positive for *bla*TEM gene at (708 bp) and negative for *bla*SHV at (1016 bp).
Lane (3): positive for *ampC*gene at (271bp)
Lane (4): positive for *exo*S gene at (504 bp)
Lane (5): negative for *tox*A gene at (352 bp)

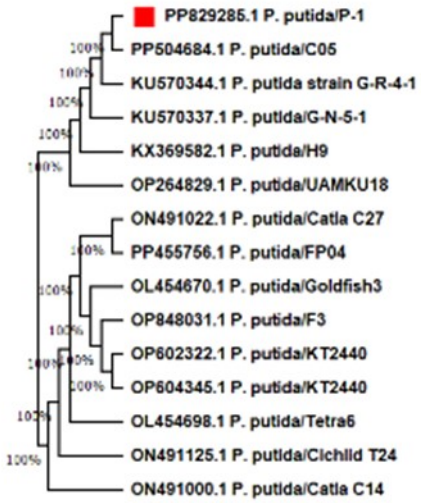
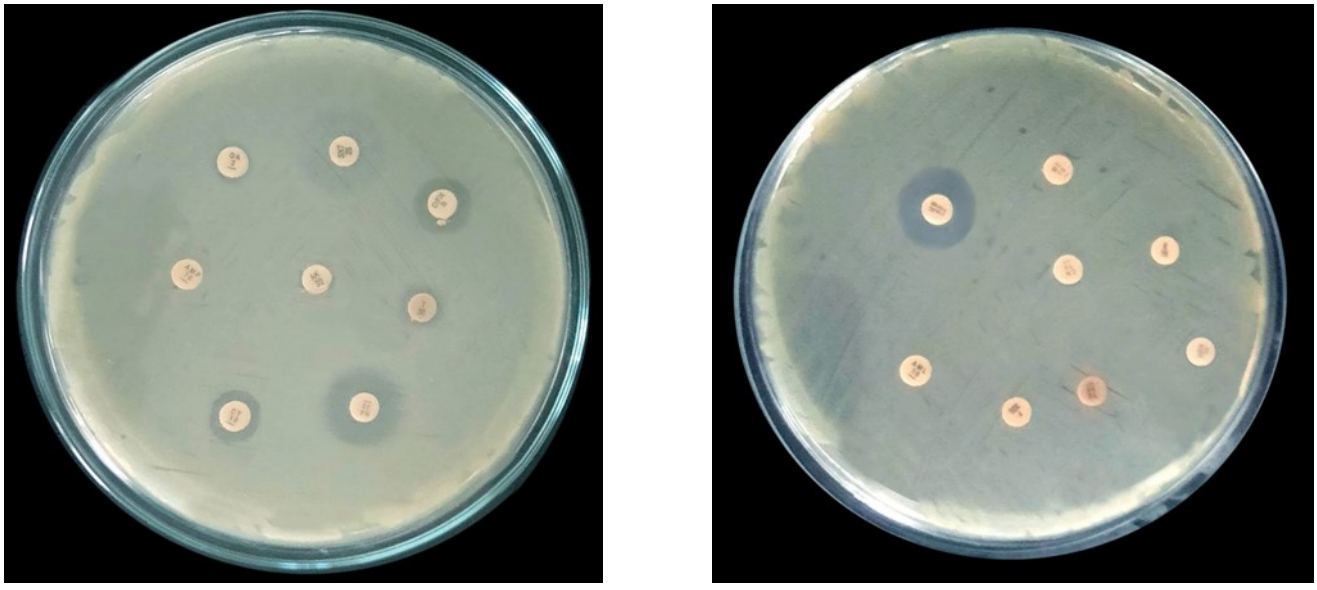


Figure (4). phylogenetic tree of *P. putida* strain by 16srRNA gene.
This isolate PP829285.1 showed high nucleotide identity with other isolates PP455756.1 isolated from Freshwater Fish specimens as well as ON491125.1, and ON 491000.1 isolated from Catla.

4. Antibiogram of *Pseudomonas putida* strains

Antimicrobial susceptibility testing was performed on all recovered isolates of *P. putida*. The results indicated marked resistance to several antibiotics, with the tested isolates showing the following resistance rate : trimethoprim sulphamethoxazol (67.1%), oxytetracycline (56.2%), amikacin (52%), lincomycin (49.3%), nitrofurantoin (49.3%), oxalinic acid (48 %), gentamicin (46.6%), amoxicillin (45.2%), tetracycline (43.8%) and erythromycin (41.1).In contrast, the isolates exhibited sensitivity to colistin sulfate (67.1%), oflicin (56.2%), flumequine (53.4%) and nalidixic acid (45.2%) as presented in **Table (2)** and **Figure (5)**.



A **B**

Figure (5). Antimicrobial resistance results of *P. putida* isolate by the disk diffusion method**.**

Table (2). Antimicrobial resistance results of *P. putida* isolates (n=73)

Antibiotic agent	Antimicrobial Class	Disc code	Total (n=73)		
			Sensitive	Intermediate	Resistant
Trimethoprim Sulfa-methoxazole	Folate Pathway Inhibitors	SXT	4 (5.5%)	20 (27.4%)	49 (67.1%)
Oxytetracycline	Tetracyclines	T	13 (17.8%)	19 (26%)	41 (56.2%)
Amikacin	Aminoglycosides	AK	14 (19.2%)	21 (28.8%)	38 (52%)
Lincomycin	Lincosamides	MY	16 (22%)	21 (28.7%)	36 (49.3%)
Nitrofurantoin	Nitrofurans	F	11 (15.1%)	26 (35.6%)	36 (49.3%)
Oxalinic acid	Quinolones	OA	14 (19.1%)	24 (32.9%)	35 (48%)
Gentamycin	Aminoglycosides	CN	21 (28.7%)	18 (24.7%)	34 (46.6%)
Amoxicillin	Penicillin	Aml	19 (26%)	21 (28.8%)	33 (45.2%)
Tetracycline	Tetracyclines	TE	18 (24.7%)	23 (31.5%)	32 (43.8%)
Erythromycin	Macrolides	E	21 (28.8%)	22 (30.1%)	30 (41.1%)
Nalidixic acid	Quinolones	NA	33 (45.2%)	21 (28.8.4%)	19 (26%)
Oflicin	Quinolones	OFX	41 (56.2%)	19 (26%)	13 (17.8%)
Flumequine	Fluoroquinolone	UB	39 (53.4%)	21 (28.8%)	13 (17.8%)
Colistin sulfate	Lipopeptides	CT	49 (67.1%)	18 (24.7%)	6 (8.2%)

Discussion

Various bacterial pathogens could infect a wide range of fish species, leading to significant economic losses in aquaculture. These infections can cause high mortality rates, reduced fish production, and substantial financial damage, highlighting the importance of effective disease management and prevention strategies. The presented study was carried to assess and characterize the *P. putida* infection in *Mugil Cephalus* from Bahr El Baqar, Egypt. The samples presented from kidney, spleen, intestine, gills and liver were microbiologically tested for the presence of *P. putida*. The prevalence of *P. putida* in diseased *Mugil Cephalus* fish screened from Bahr El Baqar, was 49% (73/150).The prevalence of *P. putida* was higher than that reported by **El-Hady and Samy (2011)** who recorded that prevalence of Pseudomonas spp. in Mugil cephalus was (36%), and lower than that-reported by **Eid *et al*. (2016) and Salem *et al*. (2018)** who recorded that prevalence of Pseudomonas spp. in Mugil cephalus was (66%) and (70 %),respectively. Out of the 73 *P. putida* isolates were observed in 32.9% (24/73) of the kidneys, 28.8% (21/73) liver, 15% (11/73) spleen, 12.3% (9/73) intestine and 11% (8/73) gills. These results were also confirmed by **Eid *et al.* (2016)** who reported the presence of *P.putida* was 9.09% in the kidneys, 18.18% in liver, 45.46% in intestine in *Mugil cephalus* samples The moribund *Mugil Cephalus* samples exhibited external signs consistent with hemorrhagic septicemia such as extensive surface hemorrhages all over the body surface especially at the ventral part of abdomen, erosions, loss of scales, erosion of fins and tail and some samples even showed exophthalmia and eye cataract and skin discoloration. The postmortem examination displayed signs of deterioration, there was enlargement of gall bladder, pale liver and pigmentation on surface of the liver as reported by **Altinok *et al.* (2006); Austin & Austin (2007); Abdel Rahman *et al.* (2024**).

The *exoS* virulence gene was detected in examined isolates. The presence of the *exo*S gene indicates that the isolates have the potential to exert significant pathogenic effects in infected fish. Similar findings were made by **Abdullahi *et al*. (2013) and Abd El Tawab *et al*. (2016).** A recent study reported that *P. putida* caused higher mortalities in Nile tilapia as it carried virulence-linked genes (ToxA, Nan1, and ExoS) **Alzahrani *et al.* (2023) and Abdel Rahman *et al.* (2024).**

The molecular biology analysis of the 16S rRNA gene sequence of *P. Putida* isolate, submitted to GenBank under accession number PP829285.1. The resulted sequence isolate showed high nucleotide identity with other *P. putida* isolates such as PP455756.1 isolated from Freshwater Fish specimens as well as ON491125.1, and ON 491000.1 isolated from Catla. This high level of interest in *P. Putida* has led to intense genome-scale metabolic modeling efforts of strain KT2440; the best-characterized strain and the first to be completely sequenced **Nelson *et al*. (2002).**

Antibiotics are commonly used to prevent and control diseases in aquaculture. However, the long-term overuse of antibiotics not only leaves residues but results in the development of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) **Yuan *et al.* (2023**). The antimicrobial susceptibility testing of *P. putida* isolates revealed significant variation in their responses to different antibiotics. *P. putida* isolates demonstrated resistance to multiple commonly used antibiotics, including trimethoprim sulfamethoxazole, oxytetracycline, amikacin, lincomycin, nitrofurantoin, oxalinic acid, gentamycin, amoxicillin, tetracycline, and erythromycin. Similarly, **Eid *et al.* (2016)** and **El-Hady and Samy (2011)** found that P. *putida* was resistant to penicillin, amoxicillin, and ampicillin with the presence of the blaTEM and ***amp*C** antibiotic resistance genes in the isolates. Moreover, the strains possessed the resistance genes blaTEM and ***amp*C**, indicating resistance to beta-lactam and cephalothin, cefazolin, cefoxitin, penicillin, and β-lactamase inhibitor-lactam combinations. Antibiotics, respectively as discussed by **Jacoby (2009).**

This pattern of resistance highlights the difficulties in controlling bacterial infections in aquaculture, particularly considering the extensive usage of these antibiotics, which may fuel the danger of microbial resistance in aquaculture. Multiple studies have demonstrated the transfer of antibiotic resistance genes (ARGs) from *Pseudomonas putida* to *Pse-*

udomonas aeruginosa, highlighting the role of *P. putida* as a reservoir and exchange platform for ARGs. This genetic exchange underscores the potential for *P. putida* to contribute to the spread of antibiotic resistance among bacterial populations, posing significant challenges for infection control and public health. **Juan *et al.* (2010)**. However, the isolates exhibited notable sensitivity to colistin sulfate, oflicin, flumequine, and nalidixic acid, these antibiotics could be effective in treating *P. Putida* infection in *Mugil Cephalus.* The presence of multidrug resistance highlights the need for alternative approaches, such as the use of probiotics, Therapeutic bacteriophages, often referred to as phages, represent a promising alternative to antibiotics for managing bacterial infections in a variety of organisms, including cultured fish. Their natural ability to target and kill specific bacteria, combined with their immunogenic properties, can stimulate diverse immune responses in various immune cells, enhancing bacterial clearance. However, to develop standardized and effective phage-based treatments for aquaculture and to minimize potential side effects in farmed fish, further research is needed to deepen our understanding of phage biology, their interactions with host immune systems, and the genetic mechanisms underlying their function. This will ensure their safe and effective application in practical settings. **Ramos *et al.* (2021).**

The increasing global demand for food has driven the intensification of aquaculture production, creating a need for economically viable and environmentally sustainable practices. Improved health management strategies are essential to meet these goals. While antibiotics and chemotherapies have been widely used in aquaculture to control infectious diseases and enhance growth, their overuse has led to concerns such as antibiotic resistance, environmental contamination, and residue accumulation in seafood. As a result, there is a growing emphasis on developing alternative approaches, such as vaccination, probiotics, prebiotics, phage therapy, and improved biosecurity measures, to ensure sustainable and responsible aquaculture practices. **Carrizo, Juan Cruz *et al.* (2021).** However, since antibiotics lead to bacterial resistance generation, for further research, it should focus on exploring the mechanisms of resistance in *P. Putida* and identifying new therapeutic agents or strategies to combat infections in aquaculture, hence preserving the industry. Additionally, studies on the environmental factors contributing to the spread of resistant strains and the impact of aquaculture practices on pathogen dynamics would provide a comprehensive understanding of the challenges in managing *P. Putida* infections in different aquatic species.

Conclusion

The study showed the prevalence of multidrug resistance and virulent *P. putida* strains in *Mugil Cephalus* in Egypt. Also, identifying effective antibiotics, such as colistin sulfate, oflicin, flumequine, and nalidixic acid, that provide developmental treatment protocols. These findings have significant implications for aquaculture in the region, highlighting the need for effective monitoring and management strategies to control *P. Putida* infections in aquaculture. However, the presence of multidrug resistance underscores the need for alternative approaches, such as implementing biosecurity and decisive veterinary hygienic regulation to control such infections. The careful utilization of antibiotics in the fisheries industry has become mandatory to prevent antibiotic residues and mitigate significant losses arising from multidrug-resistant *P. Putida* strains.

References

Abd El Tawab, A.A.; Maarouf, A.A. and Ahmed, N.M. (2016). Detection of Virulence factors of Pseudomonas species isolated from freshwater fish by PCR. Benha Veterinary Medical Journal, 30 (1), 199-207.

Abdullahi, R.; Lihan, S.; Carlos, B.S.; Bilung, M.L.; Michal, M.K. and Collick, F. (2013). Detection of oprL gene and antibiotic resistance of *Ps. aeruginosa* from aquaculture environment. European J. Experimental Biol. 3(6), 148-152.

Abdel Rahman, A.F.; Heba, H. Mahboub; Gehad, E. Elshopakey; Mahmoud, I.M. Darwish; Heba, Said Abdel-Rahman Gharib; Mohamed, Shaalan; Esraa, M. Fahmy; Heba, M. Abdel-Ghany; Sameh,

H. Ismail and Hassnaa, Mahmoud Elsheshtawy (2024). *Pseudomonas putida* infection induces immune-antioxidant, hepatorenal, ethological, and histopathological/ immunohistochemical disruptions in *Oreochromis niloticus:* the palliative role of titanium dioxide nanogel. BMC Veterinary Research (2024) 20:127 https://doi.org/10.1186/s12917-024-03972-6

Algammal, A.M.; Mabrok, M.; Sivaramasamy, E.; Youssef, F.M.; Atwa, M.H.; Elkholy, A.W.; Hetta, H.F. and Hozzein, W.N. (2020). Emerging MDR-*Pseudomonas aeruginosa* in fish commonly harbors oprL and toxA virulence genes and blaTEM, blaCTX-M, and tetA antibiotic-resistance genes. *Sci* Rep. 2020;**10**(1):15961.

Altinok, I.l.; Kayis, S. and Capkin, E. (2006). Pseudomonas Putida infection in rainbow trout. Aquaculture. 261. 850-855. 10.1016/j.aquaculture.2006.09.009.

Altschul, S.F.; Gish, W.; Miller, W.; Myers, E.W. and Lipman, D.J. (1990). Basic local alignment search tool. J. Mol. Biol., 215: 403410.https://www.sciencedirect.com/science/article/abs/pii/S0022283605803602

Alzahrani, O.M.; Elumalai, P.; Nada, H.S.; Ahmed, S.A.A.; Zaglool, A.W.; Shawky, S.M.; Alkafafy, M. and Mahboub, H.H. (2023). *Pseudomonas putida*: Sensitivity to various antibiotics, genetic diversity, virulence, and role of formic acid to modulate the immune-antioxidant status of the challenged Nile tilapia compared to carvacrol oil. In: Fishes. 2023; 8:6.

Austin, B. and D.A. Austin (2007). Bacterial Fish Pathogens. Diseases of Farmed and Wild Fish Springer-Praxis Publishing, Ltd., United Kingdom.

Benga, L.; Benten, W.P.M.; Engelhardt, E.; Köhrer, K.; Gougoula, C. and Sager, M. (2014). 16S ribosomal DNA sequence-based identification of bacteria in laboratory rodents: a practical approach in laboratory animal bacteriology diagnostics. Laboratory animals, *48*(4), pp.305-312.

Buller, N.B. (2014). Bacteria from fish and other aquatic animals: A practical identification manual. CABI Publishing, Oxford shire, UK.

Carrizo, J.C.; Griboff, J.; Bonansea, R.I.; Nimptsch, J.; Valdés, M.E.; Wunderlin, D.A. and Amé, M.V. (2021). Different Antibiotic Profiles in Wild and Farmed Chilean Salmonids. Which Is the Main Source for Antibiotic in Fish? Sci. Total. Environ. 2021; 800:149516. doi: 10.1016/j. scitotenv. 2021. 149516.

CLSI (2024). Clinical and laboratory standards institute. Performance standards for antimicrobial susceptibility testing; 34th Edition. CLSI supplement M100 https://cir.nii.ac.jp/crid/1572261550694185984

Duman, M.; Mulet, M.; Altun, S.; Saticioglu, I. B., Ozdemir, B., Ajmi, N... and García-Valdés, E. (2021). Thediversity of Pseudomonas species isolated from fish farms in Turkey. Aquaculture, 535, 736369

Decimo, M.; Morandi, S.; Silvetti, T. and Brasca, M. (2014). Characterization of Gram negative psychrotrophic bacteria isolated from Italian bulk tank milk. *J Food Sci* 79:2081–90.

De Jonghe, V.; Coorevits, A.; Van Hoorde, K.; Messens, W.; Van Landschoot, A.; De Vos, P. and Heyndrickx, M. (2011). Influence of storage conditions on the growth of Pseudomonas species in refrigerated raw milk. Appl Environ Microbiol 77:460–70.

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.

El-Hady, M. and Samy, A. (2011). Molecular Typing of Pseudomonas Species Isolated from Some Cultured Fishes in Egypt. Global Veterinaria. 7.

El-Nagar, R.M.A. (2010). Bacteriological studies on pseudomonas microorganisms in cultured M.V.Sc. thesis, Fac. Vet. Med., Zag. University.

Jacoby, G.A. (2009). AmpC beta-lactamases. Clin Microbiol Rev. 2009 Jan;22(1):161-82, Table of Contents. doi: 10.1128/CMR.00036-08. PMID: 19136439; PMCID: PMC2620637.

Juan, C.; Zamorano, L.; Mena, A.; Albertí, S.; Pérez, J.L. and Oliver, A. (2010). Metallo-β-lactamase-producing *Pseudomo-*

nas putida as a reservoir of multidrug resistance elements that can be transferred to successful *Pseudomonas aeruginosa* clones, *Journal of Antimicrobial Chemotherapy*, Volume 65, Issue 3, March 2010, Pages 474–478, https://doi.org/10.1093/jac/dkp491.

Khattab, Mona and Elsheshtway, Nadia. (2015). Genetic identification of Pseudomonas aeruginosa virulence genes among different isolates. Journal of Microbial & Biochmeical Technology.07.10.4172/1948-5948.1000224

Krieg, N.R. and Holt, J.G. (1984). Bergey's Manual of systematic Bacteriology." vol.1 Williams and Wilkins Co., Balimore, pp.161 -172.

Kumar, S.; Glen, Stecher; Michael, Li; Christina, Knyaz and Koichiro, Tamura (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular* Biology and Evolution 35:1547-1549

Milligan, E.G.; Calarco, J.; Davis, B.C.; Keenum, I.M.; Liguori, K.; Pruden, A. and Harwood, V.J. (2023). A systematic review of culture-based methods for monitoring antibiotic-resistant Acinetobacter,Aeromonas, and Pseudomonas as environmentally relevant pathogens in wastewater and surfacewater. Current Environmental Health Reports, 10(2), 154-171.

Mataseje, L.F.; Bryce, E.; Roscoe, D.; Boyd, D.A.; Embree, J.; Gravel, D.; Katz, K.; Kibsey, P.; Kuhn, M.; Mounchili, A. and Simor, A. (2012). Carbapenem-resistant Gram-negative bacilli in Canada 2009-10: results from the Canadian Nosocomial Infection Surveillance Program (CNISP). J Antimicrob Chemother; 67:1359-67.

Nelson, K.E.; Weinel, C.; Paulsen, I.T.; Dodson, R.J.; Hilbert, H. and Martins dos Santos, V.A.P., (2002). Complete genome sequence and comparative analysis of the metabolically versatile *Pseudomonas putida* KT2440. Environ Microbiol 4: 799–808.

Oliver, A.; Weige, L.M.; Rasheed, J.K.; Mcgowan JR., J.E.; Raney, P. and Tenover, F.C. (2002). Mechanisms of Decreased Susceptibility to Cefpodoxime in *Escherichia coli.* Antimicrobial Agents and Chemotherapy, 46(12): 3829-836. 0066-4804/02/ $04.000.

Quinn, P.J.; Markey, B.K.; Carter, M.E.; Donnelly, W.J.C. and Leonard, F.C. (2002). Bacterial colonization, tissue invasion and clinical disease. Chapter, 7 In: Veterinary Microbiology and Microbial Disease. Blackwell Science Ltd, Oxford

Ramos-Vivas, J.; Superio, J.; Galindo-Villegas, J. and Acosta, F. (2021). Phage Therapy as a Focused Management Strategy in Aquaculture. Int J Mol Sci. 2021 Sep 28;22(19):10436. doi: 10.3390/ijms 221910436. PMID: 34638776; PMCID: PMC8508683.

Salama, S.A. and A. Gharib, A. (2009). Parasitic protozoa accompanied with *Pseudomonas putida* infection in cultured *Oreochromis niloticus*- Egypt J Exp Biol.

Salem, A.M.; Osman, I.M. and Shehata, S.M. (2018). Assessment of psychrotrophic bacteria in frozen fish with special reference to Pseudomonas species. Benha Veterinary Medical Journal, *34*(2), 140-148.

Troell, M.; Kautsky, N.; Beveridge, M.; Henriksson, P.; Primavera, J.; Rönnbäck, P.; Folke, C. and Jonell, M. (2017). Aquaculture, In Reference Module in Life Sciences, Elsevier, 2017, ISBN: 978-0-12-809633-8, http://dx.doi.org/ 10.1016/ B978-0-12-809633-8.02007-0

Yuan, X.; Lv, Z.; Zhang, Z.; Han, Y; Liu, Z. and Zhang, H. (2023). A Review of Antibiotics, Antibiotic Resistant Bacteria, and Resistance Genes in Aquaculture: Occurrence, Contamination, and Transmission. *Toxics*, *11* (5), 420. https://doi.org/10.3390/ toxics11050420.