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Original Article

Sequencing and multiple antimicrobial resistance of *Pseudomonas fluorescens* isolated from Nile tilapia fish in Egypt

Sequenciamento e resistência antimicrobiana múltipla de *Pseudomonas fluorescens* isoladas de tilápia-do-nilo no Egito

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Abstract

Pseudomonas fluorescens is one of the main causes of septicemic diseases among freshwater fish, causing severe economic losses and decreasing farm efficiency. Thus, this research was aimed to investigate the occurrence of *P. fluorescens* in Nile Tilapia (*O. niloticus*) fish in Egypt, gene sequencing of 16SrDNA gene, and antimicrobial susceptibility. *P. fluorescens* strains were detected in 32% (128\400) of apparently healthy (9%; 36\400) and diseased (23%; 92\400) Nile tilapia fish. The highest prevalence was observed in gills of fish, 31.3% followed by intestine 26.9%, liver 24.2%, and kidneys 17.6%. The PCR results for *the* 16SrDNA gene of *P. fluorescens* showed 16SrDNA gene in 30% of examined isolates. Moreover, Homogeny and a strong relationship between strains of *P. fluorescens was confirmed using* 16SrDNA sequences. Beside the responsibility of 16SrDNA gene on the virulence of *P. fluorescens*. The results of antimicrobial susceptibility tests revealed that all strains were resistant to piperacillin (100%), followed by ceftazidime (29.7%), and cefepime (25.8%). The strains of *P. fluorescence* were highly sensitive to cefotaxime (74.2%), followed by ceftriaxone and levofloxacin (70.3% each). Interestingly, 29.7% of strains of *P. fluorescens* were multiple antimicrobial-resistant (MAR).

Keywords: 16SrDNA gene, *Pseudomonas fluorescens*, multiple antimicrobial-resistant, nile tilapia fish.

Resumo

Pseudomonas fluorescens é uma das principais causas de doenças septicêmicas em peixes de água doce, causando graves perdas econômicas e diminuindo a eficiência da fazenda. Assim, esta pesquisa teve como objetivo investigar a ocorrência de *P. fluorescens* em peixes de tilápia-do-nilo (*O. niloticus*) no Egito, sequenciamento do gene 16S rDNA e suscetibilidade antimicrobiana. Cepas de *P. fluorescens* foram detectadas em 32% (128\400) de peixes tilápia-do-nilo aparentemente saudáveis (9%; 36\400) e doentes (23%; 92\400). A maior prevalência foi observada nas brânquias dos peixes, 31,3%, seguida pelo intestino 26,9%, fígado 24,2% e rins 17,6%. Os resultados da PCR para o gene 16SrDNA de *P. fluorescens* mostraram o gene 16SrDNA em 30% dos isolados examinados. Além disso, a homogeneidade e uma forte relação entre cepas de *P. fluorescens* foi confirmada usando sequências de 16SrDNA. Além da responsabilidade do gene 16SrDNA na virulência de *P. fluorescens*. Os resultados dos testes de suscetibilidade antimicrobiana revelaram que todas as cepas foram resistentes à piperacilina (100%), seguida pela ceftazidima (29,7%) e cefepima (25,8%). As cepas de *P. fluorescens* foram altamente sensíveis à cefotaxima (74,2%), seguida pela ceftriaxona e levofloxacina (70,3% cada). Curiosamente, 29,7% das cepas de *P. fluorescens* eram multirresistentes a antimicrobianos (MAR).

Palavras-chave: gene 16SrDNA, *Pseudomonas fluorescens*, multirresistente a antimicrobianos, tilápia-do-nilo.

1. Introduction

Pseudomonas fluorescens (P. fluorescens) is a Gram-negative, rod shape organism, motile by polar flagella, and could produce fluorescent pigment (fluorescein) (Darak and Barde 2015). *P. fluorescens* is a common aquaculture opportunistic psychrotrophic microorganism, cause pseudomoniasis in a vertebrate as fresh and salts water fish, and invertebrates as shrimp, wide-spreading in soil, water, plants, and animals grows, and can grow at 25-30 °C (Swain et al., 2007; Wang et al., 2009). Pseudomoniasis; the disease caused by *P. fluorescens*, is usually characterized by ulcerations on the body among fishes, septicemia, petechial hemorrhages, skin darkness, and scales detachment, abdominal ascites and exophthalmia. These pathogenic signs carry the medical

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term of Red Skin Disease which described as related stress disease that causes high economic losses in the fish farm during culturing (Zhang et al., 2009). It occurs throughout the year, especially at low temperatures (Swain et al., 2007). Pseudomonas in fish leading to skin darkness, fin rot and detachment in scales. Infected fish also suffer from ascites, hemorrhages and exophthalmia (Eissa et al., 2010). The changes in histopathology of different infected fish appear in organs as the liver, kidney, gills, and skin (Khalil et al., 2010; Magdy et al., 2014).

Biochemical and physiological identification need more time than polymerase chain reaction (PCR) as molecular techniques aid in rapidly and accurately identifying *Pseudomonas* isolates (Panicker et al., 2004; Raghunath et al., 2007; Rajwar and Sahgal, 2016). Furthermore, the sequencing of 16SrRNA genes could identify an organism by reconstructing its phylogeny, along with the possibility of storing sequences in databases, resulting in the rapid adoption of the 16SrRNA gene by microbiologists. It is also created by multiple heterogeneous copies of the 16SrRNA gene within a genome (Dahllöf et al., 2000; Crosby and Criddle, 2003). Some studies have identified organisms with identical 16SrRNA gene sequences with significant sequence divergence in protein-encoding genes (Pernthaler and Pernthaler, 2005).

Pseudomonas species are naturally resistant to beta-lactam group antibiotics and can quickly develop resistance to antibiotics depending on their various properties. Although there are a limited number of studies about the opportunistic pathogen as *P. fluorescens*, it is also important to investigate the antibiotic resistance of *P. fluorescens* isolates, as they are one of the species frequently isolated from environmental samples and are closely related to public health (Benito et al., 2012; Düyüncü and Ulusoy, 2019). The overuse of antibiotics has led to the emergence of multidrug-resistant bacteria, including foodborne pathogens, making it difficult to treat infections caused by these pathogens (Woappi et al., 2016; Düyüncü and Ulusoy, 2019).

Fish represent very high nutrition value and cheap protein for everyone in Egypt. Thus, this study was carried out to detect the occurrence of *P. fluorescens* in Nile Tilapia *(O. niloticus)* fish in Egypt using conventional and molecular methods and gene sequencing of 16SrDNA gene. In addition, the antimicrobial susceptibility of *P. fluorescens* strains were investigated.

2. Material and Methods

2.1. Samples collection

A total of 400 (*O. niloticus*) fish of various sizes (300 diseased and 100 apparently healthy) was collected from different fish markets and farms in Damietta and Kafr-Elsheikh Governorates, Egypt, from March 2019 to June 2020. Fishes were separately taken in sterile plastic bags, kept in icebox and transferred immediately to the laboratory for further examination. Fish were inspected for clinical and postmortem lesions as observed by Eissa et al. (2010).

2.2. Isolation and identification of P. fluorescens

A total of 4 samples (gills, liver, kidneys, and intestine) from each fish were taken for bacteriological analysis. Each sample was inoculated onto MaCconkey's agar and Bacto Pseudomonas Agar F (Oxoid) and incubated aerobically at 25°C for 24 h. Suspected colonies of *P. fluorescens* were examined morphologically (Gram's staining), motility, and biochemically (oxidase, catalase, triple sugar iron, and citrate utilization tests) as identified by Eissa et al. (2010).

2.3. Genotypic detection of 16SrDNA gene in P. flourescens

A total of 50 representative *P. flourescens* strains were subjected to a polymerase chain reaction for detection of 16SrDNA gene which is responsible for bacterial virulence. The amplification and purification of *P. fluorescens*16SrDNA gene was carried out using the QIAamp DNA mini kit (QIAGEN) Catalogue no.51304. The 16SrDNA from the strains was amplified using the primers F (5´-TGCATTCAAAACTGACTG-3´) and R (5´- AATCACACCGTGGTAACCG -3´) (Machado et al., 2013). PCR was carried out in a total volume of 25 µl containing 12.5 µl of Emerald Amp GT PCR mastermix (Takara) (2x premix), 1 µl of each primer (20 pmol), 6 µl Template DNA and 4.5 µl PCR grade water. The cycling conditions consisted in a first denaturation step at 94 °C for 5 min followed by 35 cycles of amplification (denaturation at 94 °C for 30 sec, annealing at 48 °C for 40 sec and extension at 72 °C for 1 min) followed by a final extension at 72 °C for 10 min. PCR products were electrophoretically separated in 1% agarose gels and visualized in a Gel Documentation (Biometra, Germany).

2.4. Sequencing and analysis of the 16SrDNA in P. fluorescens

Purified PCR product from three representative strains was sequenced in the forward and/ or reverse directions on an Applied Biosystems 3130 automated DNA Sequencer (ABI, 3130, USA) using a ready reaction Bigdye Terminator V3.1 cycle sequencing kit (Perkin-Elmer/Applied Biosystems, Foster City, CA). On GenBank the defrentiation among sequences of our strains and other strains was done by applying PSI-BLAST research (NCBI) and BLAST 2.0. Program of multiple sequence alignment was performed to demonstrate and analyze the sequences, using Pairwise software of Lasergene DNA Star, version 1.83 module of MegAlign, which was designed by Thompson et al. (1994). Maximum likelihood were used to carry out Phylogenetic analyses, neighbor-joining and maximum parsimony in MEGA6 (Tamura et al., 2013).

2.5. Nucleotide accession number

The deposition of nucleotide sequences of *P. fluorescencs* 16SrDNA gene were done in GenBank taking **MT752964**, **MT752968** and **MT753009** as accession numbers.

2.6. Antibiotic sensitivity test

In vitro sensitivity test for *P. fluorescens* isolates was done as recorded by the Clinical and Laboratory Standards Institute using technique of standard diffusion disk.

The sensitivity of *P. fluorescens* isolates against seventeen antibiotic disks (Oxoid, Ltd.) was detected with the consider of their medical need in human and veterinary field; as following: amoxicillin-clavulanic acid (AMC) (30 µg); ceftriaxone (CRO) (30 µg); imipenem (IPM) (10 µg); meropenem (MEM) (10 µg); doxycycline (Do) (30 µg); ceftazidime (CAZ) (30 µg); cefotaxime (CTX) (30 µg); cefipime (FEP) (30 µg); ciprofloxacin (CIP) (5 µg); levofloxacin (LEV) (5 µg); ofloxacin (OFX) (5 µg); pipracillin (PRL) (100); erythromycin (E) (15 µg); cefoperazone (CEP) (75); tobramycin (TOB) (10 µg); pepracillin\tazobactam (TPZ) (110 µg); and gentamicin (GN) (10 µg). The inhibition zone of antibiotic disc with strains was detected by measuring its diameter and noted as sensitive, intermediate sensitive or resistant as recorded by Foysal et al. (2011).

3. Results

3.1. Prevalence of P. fluorescens strains in Nile tilapia fish

P. fluorescens strains were detected in 32% (128\400) of apparently healthy (9%; 36\400) and diseased (23%; 92\400) Nile tilapia fish. Regarding the occurrence of *P. fluorescens* in organs and tissues of examined fish (gills, liver, kidneys and intestine) with a prevalence 22.75% (364\1600), the highest incidence was observed in gills of fish 31.3% (114/364) followed by intestine 26.9% (98/364), liver 24.2% (88\364), and kidneys 17.6%(64\364) as illustrated in Table 1.

Table 1. *P. fluorescens* in different organs of *O. niloticus*.

Examined Organ	Examined fish	No. of isolates (%)
Gills	400	114(31.3%)
Intestine	400	98(26.9%)
Liver	400	88(24.2%)
Kidneys	400	64(17.6%)
Subtotal for organs	1600	364(22.75%)
Total for fish	400	128(32%)

3.2. Genotypic identification and sequence analysis of the 16SrDNA of P. fluorescens

PCR technique for 16SrDNA gene was applied on 50 representatives *P. fluorescens* strains isolated from different organs (liver, gills, kidneys, and intestine) of diseased Nile tilapia fish. The PCR results for 16SrDNA gene of *P. fluorescens* showed 16SrDNA gene in 30% (15\50) of examined isolates. The 16SrDNA gene was amplified, giving product of 850 bp. Additionally, 16SrDNA sequencing analyses and confirms that *P. fluorescens* isolates are closely related, besides the responsibility of 16SrDNA gene on the virulence of *P. fluorescens*. Three isolates (BMS1, BMS2 and BMS3) were subjected to collected 16S rRNA *P. fluorescens* sequences analysis. The close relation and comparison between Studied isolates and other *P. fluorescens* strains were represented phylogenetic tree found in Figure 1.

The sequences of 16SrDNA gene isolates were assembled by statistics program and GenBank deposition numbers. The nucleotide sequences of three sequence-analysed isolates were deposited in GenBank under submitted numbers **MT752964**, **MT752968** and **MT753009** as in Figure 2.

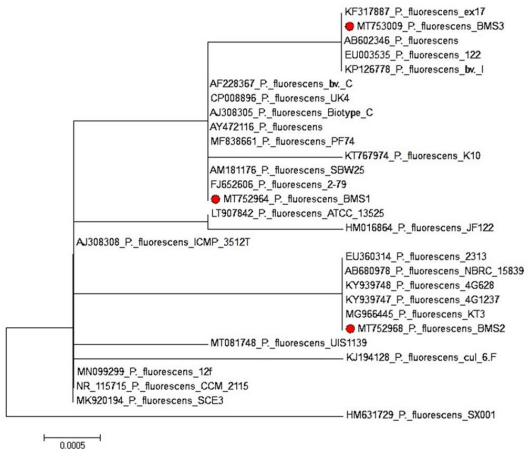

Figure 1. Phylogenetic relatedness of the 16SrDNA gene. Maximum-likelihood unrooted tree generated indicated clustering of the tested strain with different *P. fluorescens* strains.

3.3. Antibiotic sensitivity test results

The antibiotic sensitivity test was carried out on 364 strains of *P. flourescens* obtained from different organs of Nile tilapia fish. The results of *In-vitro* sensitivity test as shown in Table 2 demonstrated that the highest resistance of *P. flourescens* strains was observed to piperacillin (100%), followed by ceftazidime (29.7%), and cefepime (25.8%). Absolute moderate sensitivity was observed to tobramycin, gentamicin (100%), followed by doxycycline (92.3%), amoxicillin\clavulanic acid (89%), peperacillin\tazobactam (85.2%), erythromycin (78%), and cefepime (74.2%). The strains of *P. flourescens* were highly sensitive to cefotaxime (74.2%), followed by ceftriaxone and levofloxacin (70.3% each).

Interestingly, the strains of *P. fluorescens* resistant to more than two antibiotic classes were considered multiple antimicrobial-resistant strains, and characterized by Multiple Antimicrobial Resistant phenomena (MAR). A total of 108 out of 364 (29.7%) strains of *P. fluorescens* isolates were considered multiple antimicrobial-resistant, as in Table 3. All tested strains are resist to pepracillin (penicillin groups), and ceftazidime (Cephalosporins).

4. Discussion

P. fluorescens is one of the most pathogenic microorganism pathogens where it was found to be associated with various freshwater fish diseases throughout the world (Darak and Barde, 2015). In Egyptian farms,

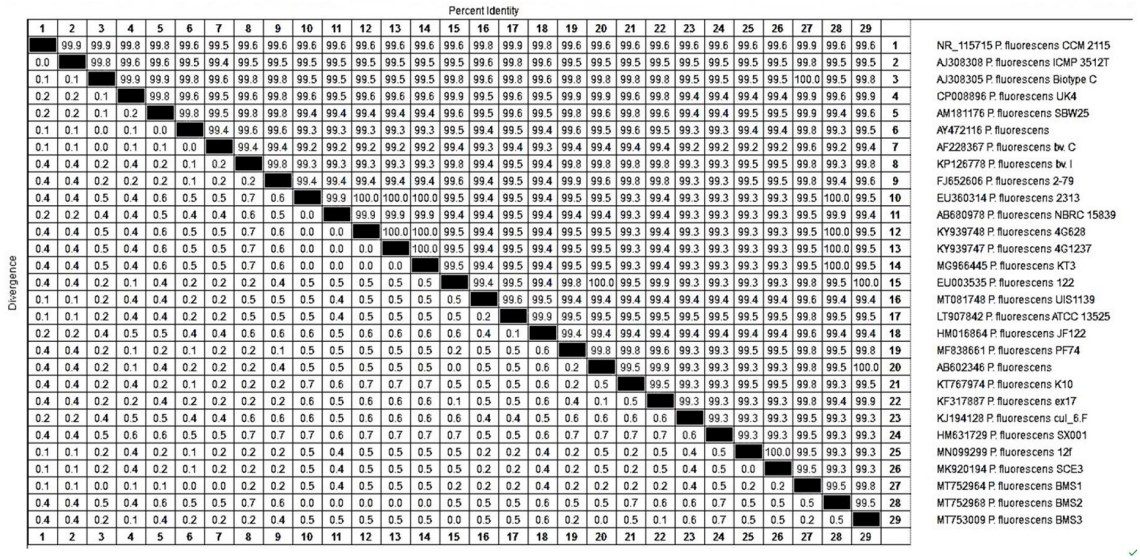

Figure 2. Sequence distance of the 16SrDNA gene of the tested strain (generated by lasergene software) showing identity range of 99.2-100% with the *P. fluorescens* strains included in the analysis.

Table 2. The antibiotic resistance of *P. fluorescens* strains isolated from *O. niloticus* different organs.

Class	Antibiotics	Sensitive	Moderate	Resistant
Penicillins	Peperacillin	0 (Zero%)	0 (Zero%)	364 (100%)
Cephalosporins	Ceftazidime	68 (18.7%)	188 (51.6%)	108 (29.7%)
	Cefepime	0 (Zero%)	270 (74.2%)	94 (25.8%)
	Cefotaxime	270 (74.2%)	68 (18.7%)	26 (7.1%)
	Ceftriaxone	256 (70.3%)	94 (25.8%)	14 (3.9%)
	Cefoperazone	176 (48.4%)	188 (51.6%)	0 (Zero%)
Macrolides	Erythromycin	0 (Zero%)	284 (78.0%)	80 (22.0%)
Penicillins\Compination (tazobactam)	Peperacillin\ Tazobactam	0 (Zero%)	310 (85.2%)	54 (14.8%)
Ambicillins\ Compination(Clavulanic A.)	Amoxicillin\ Clavulanic Acid	0 (Zero%)	324 (89.0%)	40 (11.0%)
Carbapenems	Meropeneme	188 (51.6%)	148 (40.7%)	28 (7.7%)
	Imipenem	148 (40.7%)	202 (55.5%)	14 (3.8%)
Tetracyclines	Doxycycline	14 (3.85%)	336 (92.3%)	14 (3.85%)
Quinolones	Ofloxacin	230 (63.2%)	120 (32.9%)	14 (3.9%)
	Levofloxacin	256 (70.3%)	108 (29.7%)	0 (Zero%)
	Ciprofloxacin	188 (51.6%)	176 (48.4%)	0 (Zero%)
Aminoglycoside	Tobramycin	0 (Zero%)	364 (100%)	0 (Zero %)
	Gentamicin	0 (Zero%)	364 (100%)	0 (Zero %)

P. fluorescens, was identified in different fish species as the main etiological agents of *Pseudomonas* septicemia (Swain et al., 2007; El-Sayyad et al., 2010; Khalil et al., 2010). In this study, *P. fluorescens* overall prevalence was 32% in apparently healthy and diseased fish. *P. fluorescens* incidence in different fish organs was 22.75%. This result was consistent with Eissa et al. (2010), who reported 30.83% Pseudomonas infections, with 23.3% *P. fluorescens* among the examined fish. In contrast, El-Hady and Samy (2011) identified 55.3% of Pseudomonas species with

Table 3. Multiple Antimicrobial Resistance of *P. fluorescense* isolates.

Antibiotics of Multiple Antimicrobial Resistance (MAR)	Classes of Antibiotics	MAR	
		No	%
Cefepime- Ceftazidime-	1- Cephalosporins		
Peperacillin\Tazobactam	2-Penicillins\Compination (tazobactam)		
Imipeneme	3- Carbapenems	14	3.9
Erythromycin	4- Macrolides		
Peperacillin	5- Penicillins		
Cefotaxime-Cefepime- Ceftazidime	1- Cephalosporins		
Doxycycline			
Gentamicin	2- Tetracyclines	11	3.0
Peperacillin	3- Aminoglycoside		
	4- Penicillins		
Ceftazidime	1- Cephalosporins		
Tobramycin	2- Aminoglycoside		
Amoxicillin\ClavulanicAcid	3-Ambicillins\Compination (Clavulanic	12	3.3
Peperacillin	4- Penicillins		
Ceftriaxone-Ceftazidime	1- Cephalosporins		
Erythromycin	2- Macrolides	30	8.2
Peperacillin	3- Penicillins		
Cefepime- Ceftazidime	1- Cephalosporins		
Meropeneme	2- Carbapenems	15	4.1
Peperacillin	3- Penicillins		
Ceftazidime	1- Cephalosporins		
Peperacillin\Tazobactam Peperacillin	2- Penicillins\Compination (tazobactam)	16	4.4
	3- Penicillins		
Cefepime-Ceftazidime- Cefepime Peperacillin\Tazobactam	1- Cephalosporins		
Peperacillin		10	2.8
	2- Penicillins\Compination (tazobactam)		
	3- Penicillins		
Total No. of Isolates of Multiple Antimicrobial Resistance (MAR)		**108**	**29.7%**

55.4% *P. fluorescence* from naturally infected *O. niloticus* in different localities of Egypt. Overall, a wide range of *P.fluorescens* incidence was previously reported from 18.8-60% in diseased Nile tilapia fish in Egypt (Younes et al., 2015; Abd El Tawab et al., 2016).

In this investigation, regarding type of organ samples, the highest incidence was observed in gills (31.3%) followed by the intestine (26.9%), liver (24.2%), and kidneys (17.6%). On the contrary, the previous study demonstrated that the high occurrence of *P. fluorescens was* in kidneys (50.0%), followed by liver (33.3%), gill (16.7%) and (0.0%) from skin (Abd El Tawab et al., 2016).

Morphological and biochemical methods as conventional methods for diagnosing bacterial infections in fish are complex and time-consuming for reaching a definitive diagnosis. Consequently, polymerase chain reaction (PCR) has been used to rapidly diagnose *P. fluorescens as a* member of Pseudomonas group (Scarpellini et al., 2004; Tsai et al., 2012). In the current study, PCR was performed on *P. flourescens* isolates to detect 16SrDNA gene that is specific for *P. fluorescens* and responsible for bacterial virulence. The 16SrDNA gene was detected in 30% of examined strains of *P. fluorescens.* Similarly, previous studies amplify 16SrDNA gene of *P. fluorescens*

strains isolated from diseased fish (Younes et al., 2015; Okasha et al., 2016; El-Gamal et al., 2018).

In this research, 16SrDNA sequencing is important for confirmation the strong connections between *P. fluorescens* strains. Besides the responsibility of 16SrDNA gene on the virulence of *P.fluorescens*. Databases of sequencing explaining the close matching between *P. fluorescens* strains of *P. fluorescens* complex as stated by Anzai et al. (2000) and Yamamoto et al. (2000). The sequence of amplified nucleotides appears 99.2-100% identity with the *P. fluorescens* 16SrDNA sequence described by (Radisic et al., 2020). 16SrRNA sequence results were related with the phenotype identification of the *Pseudomonas* strains. Generally, the 3 isolates (BMS1, BMS2 and BMS3) were recorded due to high agreement and good phenotypic matching with genotypic identification procedures. Genotypically these isolates were identified as *P. fluorescens*.

Accession number in GenBank and statistics of gene sequencing of 16SrDNA gene of *P. fluorescens* isolates are represented in Table 4. In this study, BMS 1(MT752964) has 99.5% identity with SX001 (HM631729) isolated from water and soil in China (Chen et al., 2013), BMS 2 (MT752968) has 99.3% identity with K10 (KT767974) isolated from milk in China (Xin et al., 2017) and BMS 3 (MT753009)

Table 4. Overview of the different *P. fluorescens* 16SrDNA genes detected in nucleotide sequences of the isolates with percent of identity%.

Strains	Other isolates of Identity	Accession number	Country	Year	Isolation source	Identity %
BMS 1	SX001	HM631729	China	2010	water and soil from the sea	99.5%
	cul_6.F	KJ194128	India	2014	Soil	99.5%
MT752964	ex17	KF317887	China	2020	biogas slurry of Tianzhu county of Gansu province	99.8%
	K10	KT767974	China	2015	Milk	99.8%
	PF74	MF838661	Italy	2017	dairy product	99.8%
	JF122	HM016864	China	2010	polluted river bank	99.6%
	KT3	MG966445	Viet Nam	2018	Soil	99.5%
	4G1237	KY939747	Italy	2018	red chicory	99.5%
	4G628	KY939748	Italy	2018	red chicory	99.5%
	Pseudomonas fluorescens bv. C	AF228367	Estonia	2000	river water continuously polluted with phenolic compounds	99.6%
	UK4	CP008896	Denmark	2014	German Collection of Microorganisms and Cell Cultures (DSMZ)	99.9%
BMS 2	SX001	HM631729	China	2010	water and soil from the sea	99.3%
	cul_6.F	KJ194128	India	2014	Soil	99.3%
MT752968	ex17	KF317887	China	2020	biogas slurry of Tianzhu county of Gansu province	99.4%
	K10	KT767974	China	2015	Milk	99.3%
	PF74	MF838661	Italy	2017	dairy product	99.5%
	JF122	HM016864	China	2010	polluted river bank	99.4%
	KT3	MG966445	Viet Nam	2018	Soil	100%

Table 4. Continued...

Strains	Other isolates of Identity	Accession number	Country	Year	Isolation source	Identity %
	4G1237	KY939747	Italy	2018	red chicory	100%
	4G628	KY939748	Italy	2018	red chicory	100%
	Pseudomonas fluorescens bv. C	AF228367	Estonia	2000	river water continuously polluted with phenolic compounds	99.2%
	UK4	CP008896	Denmark	2014	German Collection of Microorganisms and Cell Cultures (DSMZ)	99.6%
BMS 3	SX001	HM631729	China	2010	water and soil from the sea	99.3%
	cul_6.F	KJ194128	India	2014	Soil	99.3%
MT753009	ex17	KF317887	China	2020	biogas slurry of Tianzhu county of Gansu province	99.9%
	K10	KT767974	China	2015	Milk	99.5%
	PF74	MF838661	Italy	2017	dairy product	99.8%
	JF122	HM016864	China	2010	polluted river bank	99.4%
	KT3	MG966445	Viet Nam	2018	Soil	99.5%
	4G1237	KY939747	Italy	2018	red chicory	99.5%
	4G628	KY939748	Italy	2018	red chicory	99.5%
	Pseudomonas fluorescens bv. C	AF228367	Estonia	2000	river water continuously polluted with phenolic compounds	99.4%

has 99.8% identity with PF74 (MF838661) which isolated from dairy product in Italy (Rossi et al., 2018).

The antimicrobial characteristics of pathogenic *P. fluorescens* isolates passed with many resistance steps to different classes of antobiotics. *P. fluorescens*. The results of *in-vitro* sensitivity test for the *P. fluorescens* strains revealed high resistance to piperacillin (100%), followed by ceftazidime (29.7%), and cefepime (25.8%), meanwhile high sensitivity was observed to the most examined antibiotics, particularly cefotaxime ceftriaxone, and levofloxacin. The sensitivity of *P. fluorescens* to different classes of antibiotics as aminoglycosides, quinolones and chloramphenicol were previously detected (El-Hady and El-Khatib, 2009; Darak and Barde, 2015; Younes et al., 2015; Abd El Tawab et al., 2016). The resistance against penicillin group in *P. fluorescens* was common worldwide (Ghosh et al., 2011; Younes et al., 2015; Abd El Tawab et al., 2016); thus, this antibiotic group cannot be used as therapeutic agents for treatment. Also, previous investigations detected the resistance of *P. fluorescens* to amoxicillin, cephalothin, erythromycin, lincomycin, nitrofurantoin and sulphamethoxazole-trimethoprim (Magdy et al., 2014; Roy et al., 2014). As well, their resistance to the cephalosporine group was previously observed (Abd El Tawab et al., 2016). On the contrary, *P. fluorescens* strains isolated from water in Paris were resistant to carbapenems (Girlich et al., 2010). Maja et al. (1996) noted the susceptibility of *P. fluorescens* to gentamicin, kanamycin and neomycin, as well as their resistance to chloramphenicol, erythromycin, penicillin and sulphonamides.

The emergence of multidrug-resistant strains against multiple antibacterial classes threatens humans and animals worldwide. In this study, MAR was detected in

29.7% of *P. flourescens* strains. Godebo et al. (2013) detected 19.3% multidrug resistant strains of *P. aeruginosa* isolated from wounds in Ethiopia. On the other hand, Düyüncü and Ulusoy (2019) found that all examined *P. fluorescens* isolates were multidrug-resistant. Development of mechanisms of antimicrobial resistance, failing control of infection, ineffective treatment, prolonged suffering and may lead to death.

5. Conclusion

In this study, *P. fluorescens* strains was isolated from Nile tilapia (*O. niloticus*) organs (gills, liver, kidney and intestine), Biochemical identification was done and confirmed using PCR and gene sequencing with deposition of studied strains in Genbank. Also Antibiotic sensitivity test was carried out with estimation of multiple antimicrobial resistance. We detected that the prevalence of *P. fluorescens* as a common opportunistic bacterium threatens industrial aquaculture and affects income of fish culturing in Egypt. The emergence of MAR strains of *P. fluorescens* in freshwater fish is alarming. As a rule, precautions of good hygiene must be performed to control infection with *P. fluorescens* in freshwater fish. Also, treatment assays implemented to deal with antibiotic usage should be considered in Egyptian fish farms.

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