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Prevalence and antibiogram of *Pseudomonas aeruginosa* Among Nile Tilapia and Smoked Herring, with an Emphasis on their Antibiotic Resistance Genes (bla_{TEM}, bla_{SHV}, bla_{OXA-1} and *ampC*) and Virulence Determinant (*opr*L and *tox*A)

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Abstract

Bacterial diseases are one of the most challenging issues facing aquaculture sector. *Pseudomonas aeruginosa* (*P. aeruginosa*) has been regarded as one of the most significant threats to the fishing industry, which also affects public health. We aimed to elucidate the occurrence and antibiogram profile of *P. aeruginosa* recovered from Nile tilapia (*Oreochromis niloticus*) and smoked herring (*Clupea harengus*) with emphasis on their antibiotic resistance genes (bla_{TEM}, bla_{SHV}, bla_{OXA-1} and *ampC*) and virulence determinant genes (*opr*L and *tox*A). A total of 150 fish samples (110 diseased Nile tilapia, and 40 smoked herring) were collected randomly from retails of Gharbia Governorate, Egypt. The retrieved isolates were phenotypically characterized using standard methods of culturing and biochemical tests. Then, verified using molecular assay, 16S rRNA gene was detected in 100% of the tested isolates. The overall incidence of *P. aeruginosa* was 33.3%, out of which 45% from smoked herring and 29% from Nile Tilapia. The occurrence of *P. aeruginosa* in various infected organs of *O. niloticus* showed that the gills were the most obviously infected organ followed by kidney, liver, and spleen, respectively. A significant difference ($P< 0.05$) was noticed in the distribution of *P. aeruginosa* among *O. niloticus* internal organs. The phenotypic susceptibility to nine commonly used antimicrobial agents was detected using disc diffusion assay. The tested strains were extremely susceptible to ciprofloxacin, amikacin, and imipenem, whereas exhibited remarkable resistance to oxacillin, cefpodoxime, amoxicillin with clavulanic acid, ceftriaxone, and nalidixic acid. Interestingly, 100% of *P. aeruginosa* isolates were multiple antimicrobial resistant (MAR). Three resistance phenotypes profiles were identified with MAR index ranged from 0.4-0.5. Screening for antibiotic resistance genes revealed a diversity of β-lactamases in *P. aeruginosa* isolates, with bla_{TEM} being the most dominant gene (100%), followed by bla_{SHV}, bla_{OXA-1} and *ampC* with a total prevalence of 66.6% to all of them. The identified antimicrobial resistance phenotypes and genotypes were found to be significantly correlated. Subsequently, the distribution of virulence determinants in these strains was identified. These isolates had 100% prevalence of *opr*L and *tox*A virulence genes. In conclusion, the emergence of MDR *P. aeruginosa* in fish particularly ESBL and *AmpC* beta-lactamases producers could pose a potential health hazard to consumers. Thus, antimicrobial susceptibility must be continuously monitored to assess potential risks to human health. Ciprofloxacin, amikacin, and imipenem were the most efficient antibiotics for treatment of the identified *P. aeruginosa*, ESBL and *AmpC* beta-lactamases producers.

KEYWORDS
P. aeruginosa, MDR, β-lactamases, *AmpC*, Virulence determinant

INTRODUCTION

Aquaculture is a global crucial source of sustainably produced animal protein for the growing population. However, the main challenge is high mortality and disease spread, especially bacterial infections that substantially decrease the overall production of farmed fish (Jayaprakashvel and Subramani, 2019). Moreover, the emergence of antibiotic-resistant bacteria (ARBs) is currently threatening the aquatic sector and poses hazards of transferring on their resistance to human and animal pathogens (Abdullahi *et al*., 2013; Sherif *et al*., 2021).

One of the most prevalent bacteria that affect fish is *Pseudomonas aeruginosa* (Ndi and Barton, 2012; Shahrokhi *et al*., 2022). It is a Gram-negative pathogen of the Pseudomonadaceae family and is widespread in nature with a substantial susceptibility to several classes of antibiotics (Abdullahi *et al*., 2013; Tang *et al*., 2017; Spagnolo *et al*., 2021). The emergence of MDR *P. aeruginosa* strains is growing worldwide, resulting in fewer effective treatments (Breidenstein *et al*., 2011). It is well known that *P. aeruginosa* uses a large variety of intrinsic and acquired resistance mechanisms to combat most antibiotics (Pang *et al*., 2019; Roulová *et al*., 2022).

Beta (β)-lactam antibiotics are employed to treat a wide range of bacterial illnesses (Bozcal and Dagdeviren, 2017). However, the hydrolysis of antibiotics by different β-lactamases is one of the main significant mechanisms of resistance to β-lactam antibiotics (Salimi and Eftekhar, 2013). The most frequently reported β-lactamases are penicillinases of the molecular classes A serine (CTX-M families, TEM and SHV), and D (OXA-type) (Weldhagen *et al*., 2003). Moreover, class C of cephalosporinase (*AmpC*-β-lactamases) contributes to β-lactam resistance in *P. aeruginosa* (Mirsalehian *et al*., 2014; Torrens *et al*., 2019), providing a resistance to cephalosporins, penicillins, and β-lactamase inhibitors like

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amoxycillin-clavulanic acid (Chika *et al*., 2017).

One of the most popular and easily accessible fish for Egyptians is the Nile tilapia (*Oreochromis niloticus*) (Ibrahim, 2020) also, smoked herring (Renga) is a widely used traditional fish product in Egypt (Osheba, 2013; Abdel-Naeem *et al*., 2021). Thus, the present study aimed to clarify the prevalence and antibiogram profile of *P. aeruginosa* in Nile tilapia and smoked herring. As well as, to verify the presence of antimicrobial resistance genes (bla_{TEM}, bla_{SHV}, bla_{OXA-1} and *ampC*) and virulence genes (*opr*L and *tox*A).

MATERIALS AND METHODS

Ethical approval

All protocols and fish handling were approved by Ethics Committee of the Animal Health and Welfare of Damanhour University, Egypt.

Fish sampling

A total of 150 fish samples (110 Nile tilapia and 40 smoked herring) were randomly collected from Gharbia governorate, Egypt. Then, the collected samples were placed in sterile plastic bags and immediately transported in an icebox under aseptic conditions to the microbiology Laboratory at the Faculty of Veterinary Medicine, Damanhour University.

Clinical and postmortem examinations

Fish were subjected to clinical postmortem inspections in accordance with Austin *et al*. (2007) method.

Isolation and identification of Pseudomonas aeruginosa

Under aseptic conditions, 440 swabs were obtained from the gills, kidney, liver and spleen of each *Oreochromis niloticus* fish as well as 40 swabs from smoked herring abdomen. All collected swabs were inoculated separately into nutrient broth (Oxoid, UK) and incubated at 37°C for 24 h for primary enrichment. Then, a loopful of broth was streaked onto cetrimide agar (Himedia, India) and incubated at 37°C for 24 h under aerobic condition. Suspected colonies were purified and kept onto semisolid nutrient agar for further identification. The presumptive identification of isolates was based on their cultural characteristics, Gram staining and biochemical reactions such as catalase, oxidase, indol, methyl red, Vogues Proskauer, citrate utilization, H_2S production, urease, gelatin liquefaction and motility according to Austin and Austin (2007) and Markey *et al*. (2013).

Molecular identification of P. aeruginosa

P. aeruginosa genomic DNA was extracted using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) according to the manufacturer's instructions.

PCR assay was performed for verification of bacteria using the *P. aeruginosa* 16S rRNA gene (Spilker *et al*., 2004). Primers sequences for 16S rRNA gene of *P. aeruginosa* and their cycling conditions are shown in Table 1.

Antimicrobial susceptibility testing

P. aeruginosa isolates were tested for antimicrobial susceptibility using the modified Kirby-Bauer disk-diffusion technique on Muller Hinton agar (Oxoid, UK), and interpreted according to Clinical and Laboratory Standard Institute guidelines (CLSI, 2017). The following antimicrobial discs (Oxoid) were selected nalidixic acid (NA, 10 µg), ciprofloxacin (CIP, 5 µg) oxacillin (OX, 10 µg), cefpodoxime (CPD, 10 µg), ceftriaxone (CRO, 30 µg), ceftazidime (CAZ, 30 µg), amoxicillin-clavulanic (AMC, 30 µg), amikacin (AK, 30 µg) and imipenem (IMP, 10 µg).

Table 1. Oligonucleotide primer sequences and their cycling conditions used in this study.

Target gene	Oligonucleotide sequence 5′–3′	Size (bp)	PCR conditions				
			Primary denaturation	Secondary denaturation	Annealing	Extension	Final extension
P. aeruginosa 16S rDNA	GGGGGATCTTCGGACCTCA TCCTTAGAGTGCCCACCCG	956 bp	94˚C 5 min.	94˚C 30 sec.	52˚C 40 sec.	72˚C 50 sec.	72˚C 10 min.
*tox*A	GACAACGCCCTCAGCATCACCAGC CGCTGGCCCATTCGCTCCAGCGCT	396 bp			55˚C 40 sec.	72˚C 40 sec.	
*opr*L	ATG GAA ATG CTG AAA TTC GGC CTT CTT CAG CTC GAC GCG ACG	504 bp			55˚C 40 sec.	72˚C 45 sec.	
bla_{TEM}	ATCAGCAATAAACCAGC CCCCGAAGAACGTTTTC	516 bp			54˚C 40 sec.	72˚C 45 sec.	
bla_{SHV}	AGGATTGACTGCCTTTTTG ATTTGCTGATTTCGCTCG	392 bp			54˚C 40 sec.	72˚C 40 sec.	
bla_{OXA-1}	ATATCTCTACTGTTGCATCTCC AAACCCTTCAAACCATCC	619 bp			54˚C 40 sec.	72˚C 45 sec.	
ampC	TTCTATCAAMACTGGCARCC CCYTTTTATGTACCCAYGA	550 bp			50˚C 40 sec.	72˚C 45 sec.	

Table 2. Overall prevalence of *P. aeruginosa* among the examined Nile tilapia and smoked herring.

Fish species	No. of Examined fish	Positive for *P. aeruginosa*		Chi-square value	*P* value
		No	%		
Nile tilapia	110	32	29.09	3.34	0.07
Smoked herring	40	18	45		
Total	150	50	33.3		

Determination of MAR index

In brief, the MAR index was calculated by using the formula MAR = a/b, where "a" refers to the number of antibiotics to which isolates demonstrated resistance and "b" accounts for all of the used antibiotics. A value higher than 0.2 suggests that the isolates were obtained from high-risk sources (Osundiya *et al*., 2013).

Detection of antibiotic resistant genes and virulence of P. aeruginosa isolates

Four sets of primers that target the genes (bla_{TEM}, bla_{SHV}, bla_{OXA-1} and *ampC*) were used to assess the resistance of some representative *P. aeruginosa* strains to the commercially available antibiotics (Colom *et al*., 2003; Srinivasan *et al*., 2005). Additionally, two sets of primers targeting the (*opr*L and *tox*A) genes were chosen to evaluate the virulence of these selected strains according to Matar *et al*. (2002) and Xu *et al*. (2004). The used oligonucleotide primers (Metabion, Germany) and PCR cycling conditions are listed in Table 1. The PCR products were electrophoresed on a 1.5% agarose gel (Applichem, Germany, GmbH)) with Ethedium bromide staining. Generuler 100 bp ladder (Fermentas, Thermo Scientific, Germany) was used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra).

Statistical Analysis

Using SAS software (version 9.4, SAS Institute, Cary, NC, USA), the Chi-square test was used to assess the data frequencies; the level of significance was p-value< 0.05.

RESULTS

Clinical and postmortem findings

Naturally infected Nile tilapia displayed hemorrhage on different body surfaces, notably at the ventral part of the abdomen, ulcerative skin, tail rot, detached scales, erosion, and redness at the base of the fins. Internal examination also showed abdominal distention, liver paleness and congested kidney and spleen. On the other hand, infected herring fish exhibited obvious discolorations, dull surface areas and unpleasant odour.

Morphological and biochemical characterization of Pseudomonas aeruginosa

All the recovered *P. aeruginosa* isolates displayed large greenish colonies on cetrimide agar medium as result of pyocyanin exo-pigments. Microscopic examination of these colonies showed typical Gram negative, medium sized, straight, motile, and none sporulated bacilli. The biochemical characterization of these isolates revealed positive for oxidase, catalase, reduction of nitrate to nitrite, citrate utilization and gelatin hydrolysis whereas, react negatively to indole, methyl red, VP, urea hydrolysis and H_2S production.

Molecular identification of Pseudomonas aeruginosa

All identified *P. aeruginosa* isolates gave positive amplicons at 956 bp for the 16S rRNA gene. (Fig. 1).

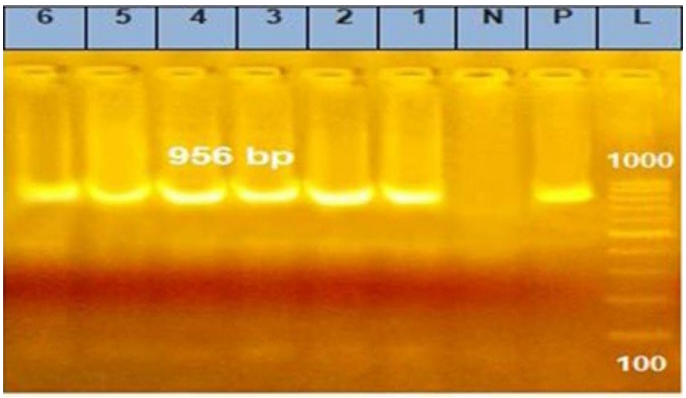

Fig. 1. Gel electrophoresis of specific gene of *P. aeruginosa* (16S rRNA); Lane L: 100-1000 bp DNA Ladder; P: Positive control (at 956 bp.); N: Negative control; Lane (1-6): Represent positive *P. aeruginosa* isolates for 16S rRNA gene.

Occurrence of P. aeruginosa among the examined Nile tilapia and smoked herring

A total of 150 fish were investigated for presence of *P. aeruginosa*, out of which 50 (33.3%) were positive for the infection. The highest incidence was observed in herring fish (18/40, 45%) followed by Nile tilapia (32/110, 29%) (Table 2). There were no statistically significant differences between the type of fish and the incidence of *P. aeruginosa* (P > 0.05). The occurrence of *P. aeruginosa* in different infected organs of *O. niloticus* showed that the gills were the most obviously infected organ (41.6%) followed by the kidney (35%), liver (16.9%) and spleen (6.5%) (Table 3). A significant difference (P< 0.05) was noticed in the distribution of *P. aeruginosa* among the *O. niloticus* internal organs.

The antimicrobial susceptibility testing of P. aeruginosa isolates

A total of 50 *P. aeruginosa* isolates were tested for antimicrobial susceptibility, (one isolate/each infected fish). As shown in Table 4, the tested strains were extremely susceptible to ciprofloxacin (100 %), amikacin (100 %), imipenem (98 %) and Ceftazidime (36 %).. On the contrary, the isolates displayed remarkable resistance to oxacillin (100 %), cefpodoxime (100 %), and amoxicillin with clavulanic acid (100 %), ceftriaxone (86 %) and nalidixic acid (50 %). Interestingly, all isolates exhibited a remarkable resistance to four or five antimicrobial agents, indicating multidrug resistance. Furthermore, *P. aeruginosa* isolates showed three resistance patterns with MAR index ranged from 0.4-0.5 (Table 5).

Determination of virulence and antimicrobial resistance genes in the recovered P. aeruginosa

Table 6 and Figs. 2-7 show the distribution of antimicrobi-

Table 3. Incidence of *P. aeruginosa* in different organs of Nile tilapia

Type of fish	No of isolates	Organs								Chi-square value	*P* value
		Gills		Kidney		Liver		Spleen			
		No	%	No	%	No	%	No	%		
O. niloticus	77	32	41.6	27	35	13	16.9	5	6.5	32.19	*0.001

al resistance genes and virulence gene among the examined *P. aeruginosa* isolates. The detection of genes encoding antimicrobial resistance revealed that the bla_{TEM} gene was found in all the selected *P. aeruginosa* isolates (100%), whereas the detection rates for the bla_{SHV}, bla_{OXA-1}, and *ampC* were 66.6%. Additionally, *opr*L and *tox*A, two genes were selected as detection targets for virulence from these resistant isolates, and all were tested positive for *opr*L and *tox*A.

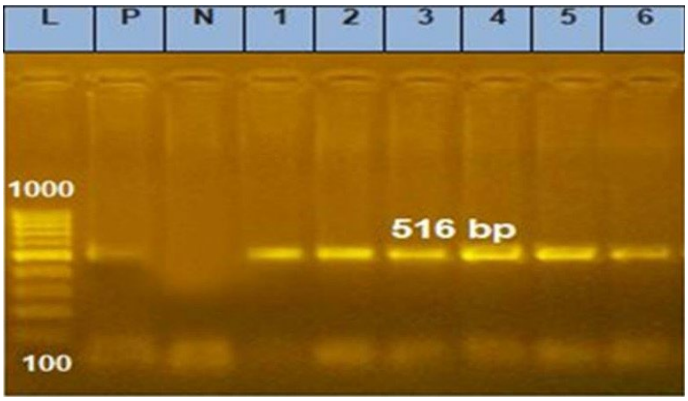

Fig. 2. Agarose gel electrophoresis of bla_{TEM} gene of *P. aeruginosa*. Lane L: 100-1000 bp DNA Ladder; P.: Positive control (at 516 bp); N: negative control; Lanes 1–6: the specific DNA product (516 bp) amplified from representative isolates of *P. aeruginosa*.

Fig. 3. Agarose gel electrophoresis of bla_{SHV} gene of *P. aeruginosa*. Lane L: 100-1000 bp DNA Ladder; P.: Positive control (at 392 bp); N: negative control; Lanes 1–6: Representative isolates of *P. aeruginosa*.

Egypt, Nile tilapia and smoked herring fish are popular seafood, implying that there is a public health issue regarding the safety of these foods. In the present study, the occurrence of *Pseudomonas aeruginosa* among fish samples randomly collected from Gharbia governorate, Egypt was investigated through clinical examination, bacteriological isolation, phenotypic characterization, and molecular identification. Additionally, the antibiogram profile, antimicrobial resistance genes and virulence genes of the recovered isolates were identified.

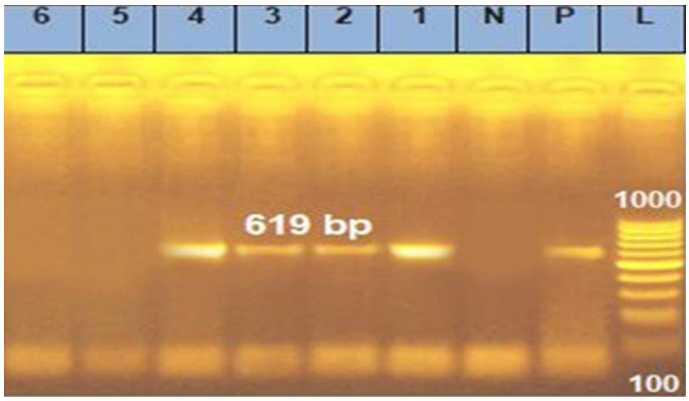

Fig. 4. Gel electrophoresis for *P. aeruginosa bla*OXA1 gene; Lane L: 100-1000 bp DNA Ladder; P.: Positive control (619 bp); N: negative control; Lanes 1–6: Representative isolates of *P. aeruginosa*.

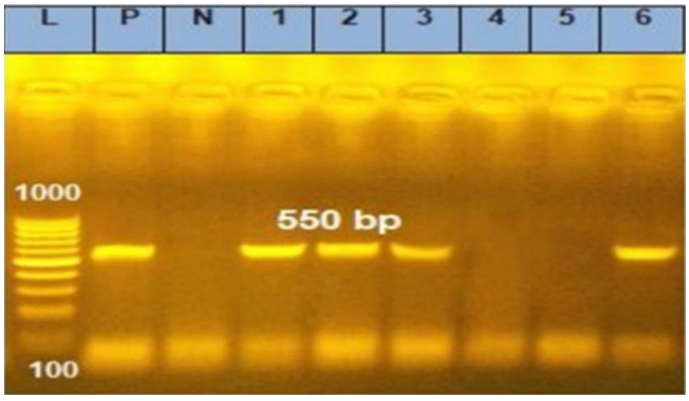

Fig. 5. Gel electrophoresis of *ampC* gene of *P. aeruginosa*. Lane L: 100-1000 bp DNA Ladder; P.: Positive control (550 bp); N: negative control; Lanes 1–6: Representative isolates of *P. aeruginosa*.

DISCUSSION

Pseudomonas species are one of the most prevalent bacterial fish pathogens that cause excessive fish mortalities and substantial economic losses (Eissa *et al*., 2010; López *et al*., 2012). In

A total of 150 fresh fish samples (110 Nile tilapia and 40 smoked herring) were screened for presence of *P. aeruginosa*. *O. niloticus* showed hemorrhage on various body surfaces, particularly at the ventral portion of the abdomen, ulcerative skin, tail rot, detached scales, erosion, and redness at the base of the fins. Meanwhile, internal examination revealed abdominal disten-

Table 4. Antimicrobial susceptibility of *P. aeruginosa* isolates from Nile tilapia and smoked herring (n. = 50).

Antimicrobial Family	Antimicrobial agents	Sensitive		Intermediate		Resistant	
		Number	%	Number	%	Number	%
Quinolone	Nalidixic acid	0	0	25	50	25	50
	Ciprofloxacin	50	100	0	0	0	0
Penicillin	Oxacillin	0	0	0	0	50	100
Cephalosporin (3rd generation)	Cefpodoxime	0	0	0	0	50	100
	Ceftriaxone	0	0	7	14	43	86
	Ceftazidime	18	36	32	64	0	0
β-Lactam-β-lactamase-inhibitor combinations	Amoxicillin/clavulanic acid	0	0	0	0	50	100
Aminoglycoside	Amikacin	50	100	0	0	0	0
Carbapenem	Imipenem	49	98	1	2	0	0

tion, pale enlarged liver and congested kidney and spleen. These clinical and postmortem findings were typical of *Pseudomonas* septicemia and similar to those reported by El-Nagar (2010); Amrevuawho *et al*. (2014); Magdy *et al*. (2014); Abd El Tawab *et al*. (2016); Elham *et al*. (2017); Algammal *et al*. (2020) and Yaseen *et al*. (2020). Diverse virulence factors that *P. aeruginosa* encodes may havevtriggered these findings (Todar, 2008; Tuon *et al*., 2022).

The retrieved isolates were phenotypically characterized using standard methods of culturing and biochemical tests. Presumptive *P. aeruginosa* colonies on cetrimide agar media appeared rounded large and irregular with a fruity odor and blue green pigmentation. These colonies were Gram negative, medium sized rods, and positive for oxidase, catalase, nitrate reduction, citrate utilization and gelatin liquefaction. Conversely, they react negatively to indol, MR, VP, H_2S production. These phenotypic characteristics are typical of *P. aeruginosa* and are nearly identical to those reported by many previous authors such as Abd El Tawab *et al*. (2016); Algammal *et al*. (2020) and Spagnolo *et al*. (2021). Additionally, further verification was performed using the 16S ribosomal RNA gene, revealing that 100% of the tested isolated were positive for *Pseudomonas*.

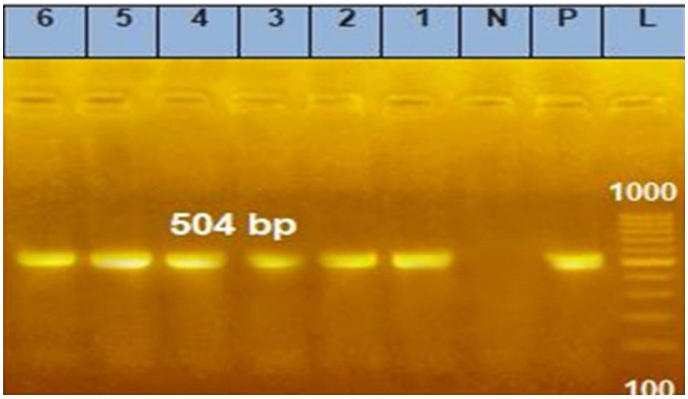

Fig. 6. Gel electrophoresis for *P. aeruginosa* outer membrane lip*opr*otein L (*opr*L); Lane L: 100-1000 bp DNA Ladder. Neg.: Negative control. Pos.: Positive control (504 bp) Lane 1- 6: Representative *P. aeruginosa* isolates.

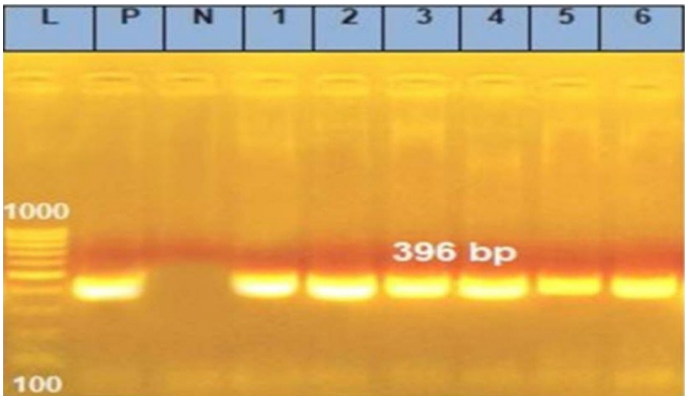

Fig. 7. Agarose gel electrophoresis of *tox*A gene of *P. aeruginosa*. Lane L: 100-1000 bp DNA Ladder; P.: Positive control (at 396 bp); N: negative control; Lanes 1–6: Representative isolates of *P. aeruginosa*.

Table 5. Antimicrobial resistance patterns of *P. aeruginosa* isolates (n.=50).

No of isolates	Resistance pattern	MAR index
25	OX, AMC, CPD, CRO,	0.4
18	OX, AMC, CPD, CRO, NA	0.5
7	OX, AMC, CPD, NA	0.4

OX: oxacillin; AMC: amoxicillin-clavulanic acid; CPD: cefpodoxime; CRO: ceftriaxone; NA: nalidixic acid.

Data shown in Table 2 revealed that the overall incidence of *P. aeruginosa* in the examined fish samples was 33.3%. Concerning, Nile tilapia, *P. aeruginosa* was isolated by percentage of 29.09 %. Our results are nearly similar to those obtained by Magdy *et al*. (2014); Abd El Tawab *et al*. (2016); Benie *et al*. (2017); Dadié and Dosso (2019); Algammal *et al*. (2020); Ali *et al*. (2023) and Farag *et al*. (2023). Meanwhile, our findings disagree with those of Shahrokhi *et al*. (2022) who recorded lower incidence from fresh fish (5.0%).

Additionally, the statistical analysis revealed a significant difference in the occurrence of *P. aeruginosa* in different internal organs ($P < 0.05$). The gill was the most frequently infected internal organ, followed by the kidney, liver, and spleen respectively. this is on the contrary to the findings obtained by Eissa *et al*. (2010); Abd El Tawab *et al*. (2016) and Algammal *et al*. (2020), who revealed that the liver had the highest infection rates.

Smoking is a common method of fish preservation (Belichovska *et al*., 2019). However, the presence of spoilage and pathogenic bacteria in smoked fish and food borne illnesses still pose hazards in developing countries (Abigaba *et al*., 2021). We found high prevalence of *P. aeruginosa* in smoked fish (45%). These findings are nearly similar to those published by Benie *et al*. (2017) and Dadié and Dosso (2019) who reported that *P. aeruginosa* was present in smoked fish at a rate of 20.0 % and 15.7 % respectively. Poor fish handling, environmental contamination and improper smoking techniques used by fishmongers can all be *bla*med for the higher amounts of *P. aeruginosa* found in smoked fish and making it hazardous for consumption. Meanwhile, Shahrokhi *et al*. (2022) detected a lower frequency of the *P. aeruginosa* in smoked fish (2.8 %) that may have been caused by the sanitary conditions that were followed both before and during the smoking process.

A total of 50 verified *P. aeruginosa* isolates were tested for susceptibility to a total of 9 distinct antibiotics from 6 different antibiotic classes. All isolates exhibited a 100 % susceptibility rate to ciprofloxacin and amikacin, whereas 98 % to imipenem. These results are consistent with Carol *et al*. (2013); Nasreen *et al*. (2015); Abd El Tawab *et al*. (2016); Eid *et al*. (2016) and Ali *et al*. (2023). Also, Fazeli *et al*. (2012) and Mahmoud *et al*. (2013) who found that amikacin and imipenem were the most efficient medications against *P. aeruginosa*. Carbapenems are extremely potent antimicrobials for treating diseases resulted by the most resistant bacteria (Aurilio *et al*., 2022). Thus, we recommend that ciprofloxacin, amikacin, and imipenem be used to treat infections caused by *P. aeruginosa*. On the contrary, Benie *et al*. (2017) determined that *P. aeruginosa* isolates were primarily resistant to imipenem and ciprofloxacin.

Notably, all isolates revealed multi-drug resistance (MDR) to

Table 6. The distribution of the antimicrobial resistance coding genes and virulence gene among the *P. aeruginosa* isolates.

No of examined isolates	Antimicrobial resistance gene								Virulence gene			
	bla$_{TEM}$		*bla*$_{SHV}$		*bla*$_{OXA-1}$		*ampC*		*opr*L		*tox*A	
	No	%	No	%	No	%	No	%	No	%	No	%
6	6	100	4	66.6	4	66.6	4	66.6	6	100	6	100

four or five selected antimicrobial agents. Multidrug resistance was described as resistance to at least three antimicrobial agents from different classes (Horcajada *et al*., 2019). The MAR index ranged from 0.4 to 0.5 and showed three antibiotic resistance profiles (Table 5). Similarly, MDR was found in *P. aeruginosa* strains identified in Egypt according to Abd El Tawab *et al*. (2016); Algammal *et al*. (2020) and Ali *et al*. (2023). We found that the highest rates of resistance were for oxacillin (100 %), cefpodoxime (100 %) and amoxicillin with clavulanic acid (100 %). The isolates also showed resistance to ceftriaxone (86 %) and nalidixic acid (50%).

Consequently, to understand the underlying genetic causes of AMR, we intended to determine correlation between resistance phenotype and genotype through detecting the existence of antibiotic resistance genes (bla_{TEM}, bla_{SHV}, bla_{OXA-1} and *ampC*) in representative *P. aeruginosa* isolates. All the selected *P. aeruginosa* isolates contained the bla_{TEM} gene (100%), almost a comparable study was carried out in Egypt by Algammal *et al*. (2020) revealed bla_{TEM} with a total prevalence of 83.3% %. While the detection rates for the bla_{SHV}, bla_{OXA-1}, and *ampC* were 66.6% (Table 6). Extended-spectrum--lactamases (ESBLs) and *AmpC*s are among the lactamases that are responsible for the emergence of resistance to third generation cephalosporins, penicillins, and β-lactamase inhibitor-β-lactam combination (Pfeifer *et al*., 2010; El Shamy *et al*., 2021). co-resistance to numerous additional antibiotic classes is prevalent in organisms that produce ESBLs. Thus, the presence of these antibiotic resistance genes could potentially be responsible for the observed phenotypic resistance to penicillin, cephalosporin and β-Lactam-β-lactamase-inhibitor combinations and could trigger the emergence of MDR strains.

Concerning, the distribution of the virulence genes, all the examined strains harbored *opr*L and *tox*A genes. Similarly, a study conducted in Egypt reported 100% of *P. aeruginosa* isolates were positive for the *opr*L and *tox*A gene (Algammal *et al*., 2020).

CONCLUSION

Our finding revealed that the overall frequency *P. aeruginosa* was 33.3%, smoked herring accounted for 45% and Nile tilpia for 29%. Ciprofloxacin, amikacin, and imipenem showed potential antimicrobial effects against emerged *P. aeruginosa* in fish. The highest resistance rates were detected against oxacillin, cefpodoxime, amoxicillin with clavulanic acid, ceftriaxone and nalidixic acid, respectively. Whereas, several lactamases, primarily bla_{TEM}, were present in *P. aeruginosa* isolates. The association of virulence factors (*opr*L and *tox*A) with ESBLs and *AmpC* beta-lactamases could increase the pathogenicity potential and severity of infection. Also, there is a significant potential for transfer of antibiotic resistance genes to the microbial community and are potential human hazard. We suggest crucial, urgent action to strictly regulate the excessive and unnecessary use of antibiotics. Additionally, the emphasis on non-antibiotic alternative control methods for bacterial infections in farmed fish should be encouraged.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Abd El Tawab, A.A., Maarouf, A.A., Ahmed, N.M., 2016. Detection of Virulence factors of *Pseudomonas* species isolated from fresh water fish by PCR. Benha Vet. Med. J. 30, 199-207.

Abdel-Naeem, H.H., Sallam, K.I., Malak, N.M., 2021. Improvement of the microbial quality, antioxidant activity, phenolic and flavonoid contents, and shelf life of smoked herring (*Clupea harengus*) during frozen storage by using chitosan edible coating. Food Control 130, 108317.

Abdullahi, R., Lihan, S., Carlos, B.S., Bilung, M. L., Mikal, M.K., Collick, F., 2013. Detection of *opr*L gene and antibiotic resistance of *Pseudomonas aeruginosa* from aquaculture environment. European Journal of Experimental Biology 3, 148-152.

Abigaba, R., Nakavuma, J.L., Kankya, C., Kabasa, J.D., 2021. Microbiological Quality of Traditionally Smoked Fish from Lake Victoria Crescent, Uganda. Food Science and Quality Management 104, 47-53.

Algammal, A.M., Mabrok, M., Sivaramasamy, E., Youssef, F.M., Atwa, M.H., El-Kholy, A.W., Hozzein, W.N., 2020. Emerging MDR-*Pseudomonas aeruginosa* in fish commonly harbor *opr*L and *tox*A virulence genes and bla_{TEM}, bla_{CTX-M}, and *tet*A antibiotic-resistance genes. Scientific Rep. 10, 1-12.

Ali, H., Awad, A., Maarouf, A., Ahmed, W., 2023. Molecular Detection of some Virulence Factors of *Pseudomonas aeruginosa* Isolated from Freshwater Fishes at Qalubiya Governorate, Egypt. Benha Vet. Med. J. 43, 80-84.

Amrevuawho, O.M., Akinyemi, A.A., Ezeri, G.N.O., BANKOLE, M., Takeet, O.V.A., 2014. Pathological study of Clarias gariepinus (Burchell, 1822) sub-adult artificially infected with *Pseudomonas aeruginosa*. Braz. J. Aquat. Sci. Technol. 18, 65-70.

Aurilio, C., Sansone, P., Barbarisi, M., Pota, V., Giaccari, L. G., Coppolino, F., Pace, M.C., 2022. Mechanisms of action of carbapenem resistance. Antibiotics 11, 421.

Austin, B., Austin, D.A., 2007. Characteristics of the pathogens: Gram-negative bacteria. Bacterial Fish Pathogens. Springer Praxis Books. Springer, Dordrecht. pp. 81-150. https://doi.org/10.1007/978-1-4020-6069-4_4

Austin, B., Austin, D.A., Munn, C.B., 2007. Bacterial fish pathogens: disease of farmed and wild fish. Chichester: Springer. 26, 552.

Belichovska, D., Belichovska, K., Pejkovski, Z., 2019. Smoke and smoked fish production. Sci. J. Meat Technol. 60, 37-43.

Benie, C. K. D., Nathalie, G., Adjéhi, D., Solange, A., Ferniquekonan, K., Desire, K., 2017. Prevalence and antibiotic resistance of *Pseudomonas aeruginosa* isolated from bovine meat, fresh fish and smoked fish. Arch. Clin. Microbiol. 8, 1-9.

Bozcal, E., Dagdeviren, M., 2017. Toxicity of β-lactam antibiotics: pathophysiology, molecular biology and possible recovery strategies. Poisoning: From Specific Toxic Agents to Novel Rapid and Simplified Techniques for Analysis; InTechOpen: Rijeka, Croatia, pp. 87-105.

Breidenstein, E.B., de la Fuente-Núñez, C., Hancock, R.E., 2011. *Pseudomonas aeruginosa*: all roads lead to resistance. Trends Microbiol. 19, 419-426.

Carol, G.R., Jeyasanta, K.I., Mani, A.E., Patterson, J., 2013. Prevalence of *Pseudomonas* sp. in fin fishes and their antibiotic susceptibility. J. Pure Appl. Microbiol. 7, 677-681.

Chika, E., Carissa, D., Chijioke, E., Ifeanyichukwu, I., 2017. Prevalence of *AmpC* β-lactamase-producing *Pseudomonas aeruginosa* isolates from feacal matter of cow. J. Microbiol. Exp. 4, 00124.

CLSI (Clinical and Laboratory Standards Institute), 2017. Performance standards for antimicrobial susceptibility testing. CLSI supplement M100, pp. 106-112.

Colom, K., Pérez, J., Alonso, R., Fernández-Aranguiz, A., Lariño, E., Cisterna, R., 2003. Simple and reliable multiplex PCR assay for detection of bla_{TEM}, bla_{SHV} and *bla*OXA–1 genes in Enterobacteriaceae. FEMS Microbiol. Lett. 223, 147-151.

Dadié, T., Dosso, K.M.D.M., 2019. High Prevalence of Multidrug Resistant *Pseudomonas aeruginosa* Strains Recovered from Bovine Meat, Fresh and Smoked Fish in Côte d'Ivoire. Microbiology and Nature 1, 44-54.

Eid, H., El Tabiy, A., Fathy, S., 2016. Prevalence and molecular characterization of *Pseudomonas* species isolated from fish markets in port-said. Suez Canal Veterinary Medical Journal. SCVMJ. 21, 1-12.

Eissa, N. M. E., El-Ghiet, E. A., Shaheen, A., Abbass, A., 2010. Characterization of *Pseudomonas* species isolated from tilapia "*Oreochromis niloticus*" in Qaroun and Wadi-El-Rayan lakes, Egypt. Global Vet. 5, 116-121.

El Shamy, A.A., Zakaria, Z., Tolba, M.M., Salah Eldin, N., Rabea, A.T., Tawfick, M.M., Nasser, H.A., 2021. *AmpC* β-Lactamase Variable Expression in Common Multidrug-Resistant Nosocomial Bacterial Pathogens from a Tertiary Hospital in Cairo, Egypt. Inter. J. Microbiol. 2021, 6633888.

Elham, I., Ismail, M., El Lamei, M., Heba A., 2017. Studies on *Pseudomonas* septicemia in some tilapia in Ismailia. Suez Canal Veterinary Medical Journal. SCVMJ. 22, 107-117.

El-Nagar, R.M.A., 2010. Bacteriological studies on *Pseudomonas* microorganisms in cultured. MSc. thesis Fac. Vet. Med. Zag. University, Egypt.

Farag, H.A., Abd El Tawab, A. Maarouf, A., Ahmed, W., 2023. Molecular Detection of some Virulence Factors of *Pseudomonas aeruginosa* Isolated from Freshwater Fishes at Qalubiya Governorate, Egypt. Benha Vet. Med. J. 43, 80-84.

Fazeli, H., Sadighian, H., Nasr-Esfahani, B., Pourmand, M.R., 2012. Identifi-

cation of class-1 integron and various β-lactamase classes among clinical isolates of *Pseudomonas aeruginosa* at children's medical center hospital. J. Med. Bacteriol. 1, 25-36.

Horcajada, J.P., Montero, M., Oliver, A., Sorlí, L., Luque, S., Gómez-Zorrilla, S., Grau, S., 2019. Epidemiology and treatment of multidrug-resistant and extensively drug-resistant *Pseudomonas aeruginosa* infections. Clin. Microbiol. Rev. 32, e00031-19.

Ibrahim, T., 2020. Diseases of Nile tilapia with special emphasis on water pollution. J. Environ. Sci. Technol. 13, 29-56.

Jayaprakashvel, M., Subramani, R., 2019. Implications of quorum sensing and quorum quenching in aquaculture health management. Implication of quorum sensing and biofilm formation in medicine, agriculture and food Industry, Springer, Singapore. pp. 299-312. https://doi.org/10.1007/978-981-32-9409-7_18

López, J.R., Navas, J.I., Thanantong, N., de la Herran, R., Sparagano, O.A.E., 2012. Simultaneous identification of five marine fish pathogens belonging to the genera Tenacibaculum, Vibrio, Photobacterium and *Pseudomonas* by reverse line blot hybridization. Aquaculture 324, 33-38.

Magdy, I.H., El-Hady, M.A., Ahmed, H.A., Elmeadawy, S.A., Kenwy, A.M., 2014. A contribution on *Pseudomonas aeruginosa* infection in African catfish (*Clarias gariepinus*). Res. J. Pharm. Biol. Chem. Sci. 5, 575-588.

Mahmoud, A.B., Zahran, W.A., Hindawi, G.R., Labib, A.Z., Galal, R., 2013. Prevalence of multidrug-resistant *Pseudomonas aeruginosa* in patients with nosocomial infections at a university hospital in Egypt, with special reference to typing methods. J. Virol. Microbiol. 2013, Article ID 290047 DOI: 10.5171/2013.290047

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

Matar, G.M., Ramlawi, F., Hijazi, N., Khneisser, I., Abdelnoor, A.M. 2002. Transcription levels of *Pseudomonas aeruginosa* exotoxin A gene and severity of symptoms in patients with otitis externa. Curr. Microbiol. 45, 350-354.

Mirsalehian, A., Kalantar-Neyestanaki, D., Nourijelyani, K., Asadollahi, K., Taherikalani, M., Emaneini, M., Jabalameli, F., 2014. Detection of *AmpC*-β-lactamases producing isolates among carbapenem resistant *P. aeruginosa* isolated from burn patient. Iran. J. Microbiol. 6, 306.

Nasreen, M., Sarker, A., Malek, M.A., Ansaruzzaman, M.D., Rahman, M., 2015. Prevalence and resistance pattern of *Pseudomonas aeruginosa* isolated from surface water. Adv. Microbiol. 5, 74.

Ndi, O.L., Barton, M.D., 2012. Resistance determinants of *Pseudomonas* species from aquaculture in Australia. J. Aqua. Res. Develop. 3, 1.

Osheba, A.S., 2013. Technological attempts for production of low sodium smoked herring fish (Renga). Adv. J. Food Sci. Technol. 5, 695-706.

Osundiya, O.O., Oladele, R.O., Oduyebo, O.O., 2013. Multiple antibiotic resistance (MAR) indices of *Pseudomonas* and *Klebsiella* species isolates in Lagos University Teaching Hospital. Afri. J. Clin. Exp. Microbiol. 14, 164-168.

Pang, Z., Raudonis, R., Glick, B.R., Lin, T.J., Cheng, Z., 2019. Antibiotic resistance in *Pseudomonas aeruginosa*: mechanisms and alternative therapeutic strategies. Biotechnol. Adv. 37, 177-192.

Pfeifer, Y., Cullik, A., Witte, W., 2010. Resistance to cephalosporins and carbapenems in Gram-negative bacterial pathogens. Inter. J. Med. Microbiol. 300, 371-379.

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

Salimi, F., Eftekhar, F., 2013. Coexistence of *AmpC* and extended-spectrum β-lactamases in metallo-β-lactamase producing *Pseudomonas aeruginosa* burn isolates in Tehran. Jundishapur J. Microbiol. 6, 8.

Shahrokhi, G.R., Rahimi, E., 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 2022, 1-8.

Sherif, A.H., Gouda, M., Darwish, S., Abdelmohsin, A., 2021. Prevalence of antibiotic-resistant bacteria in freshwater fish farms. Aqua. Res. 52, 2036-2047.

Spagnolo, A.M., Sartini, M., Cristina, M.L., 2021. *Pseudomonas aeruginosa* in the healthcare facility setting. Rev. Med. Microbiol. 32, 169-175.

Spilker, T., Coenye, T., Vandamme, P., LiPuma, J.J., 2004. PCR-based assay for differentiation of *Pseudomonas aeruginosa* from other *Pseudomonas* species recovered from cystic fibrosis patients. J. Clin. Microbiol. 42, 2074-2079.

Srinivasan, V., Nam, H.M., Nguyen, L.T., Tamilselvam, B., Murinda, S.E., Oliver, S.P., 2005. Prevalence of antimicrobial resistance genes in Listeria monocytogenes isolated from dairy farms. Foodbourne Pathog. Dis. 2, 201-211.

Tang, Y., Ali, Z., Zou, J., Jin, G., Zhu, J., Yang, J., Dai, J., 2017. Detection methods for *Pseudomonas aeruginosa*: history and future perspective. Rsc. Adv. 7, 51789-51800.

Tuon, F.F., Dantas, L.R., Suss, P.H., Tasca Ribeiro, V.S., 2022. Pathogenesis of the *Pseudomonas aeruginosa* Biofilm: A Review. Pathogens (Basel, Switzerland) 11, 300.

Todar, K., 2008. *Pseudomonas aeruginosa*. Online textbook of Bacteriology. https://textbookofbacteriology.net/*Pseudomonas*.html

Torrens, G., Hernández, S.B., Ayala, J. A., Moya, B., Juan, C., Cava, F., Oliver, A., 2019. Regulation of *AmpC*-driven β-lactam resistance in *Pseudomonas aeruginosa*: different pathways, different signaling. Msystems 4, e00524-19.

Weldhagen, G.F., Poirel, L., Nordmann, P., 2003. Ambler class A extended-spectrum β-lactamases in *Pseudomonas aeruginosa*: novel developments and clinical impact. Antimicrob. Agent Chem. 47, 2385-2392.

Xu, J., Moore, J.E., Murphy, P.G., Millar, B.C., Elborn, J.S., 2004. Early detection of *Pseudomonas aeruginosa*–comparison of conventional versus molecular (PCR) detection directly from adult patients with cystic fibrosis (CF). Ann. Clin. Microbiol. Antimicrob. 3, 1-5.

Yaseen, M., Abdelaziz, M., Abdel-moneam, D., Abd-Elhay, E., Wassif, I., Fawzy, M., Moustafa, M., 2020. Phenotypic and Genotypic characterization of the pathogenic *Pseudomonas aeruginosa* isolated from cultured Pangasianodon hypophthalmus in Egypt. Egyptian J. Aqua. Biol. Fish 24, 453-467.