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Research Article
Bacterial Causes for Mortality Syndrome in Some Marine Fish Farms with Treatment Trials

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Abstract

Background and Objective: Bacterial fish diseases constitute a major problem in aquaculture, it was found in the environment and under stressors cause severe economic losses to fish. This work aimed to investigate the bacterial causes and suitable treatments of mass mortality in some cultured marine fish farms in Damietta governorate. **Materials and Methods:** The study was performed on 5 farms suffered from mass mortality. Total of 100 diseased fish (10 sea bass and 10 sea bream/farm) and 20 water samples were randomly collected from these farms. Bacteriological examinations were carried out followed by *in vitro* sensitivity tests. Treatment trial was performed using the most effective antibacterial agent on isolated bacteria. **Results:** From fish and water samples *Pseudomonas* spp., *Aeromonas* spp. and *Vibrio* spp. were isolated with the rat of (16, 10%), (22, 10%) and (28, 10%) respectively. These results were confirmed biochemically. Some virulence genes of isolated bacteria were detected using PCR; meanwhile, enrofloxacin reduced significantly the mortality rates in examined farms. **Conclusion:** It could be concluded that, *Pseudomonas* spp., *Aeromonas* spp. and *Vibrio* spp. are the main bacterial species causing mass mortality in marine fish farms. These bacteria were highly sensitive to enrofloxacin *in vitro* and *in vivo*.

Key words: Mortality, Marine Fish, aquaculture, antibacterial agent, enrofloxacin, immunosuppressive agent, antimicrobial resistance

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Fisheries represent an important sector in the Egyptian national income structure. Marine fishes are liable to many environmental stressors as chemicals, natural and biological invaders which induce immune suppression of fish. However, the bacterial invasion is the main immunosuppressive agent[1].

In the long run, water resources will be the most limiting factor for aquaculture development in Egypt. Therefore, marine fisheries are the immediate alternative for water needed in aquaculture[2]. Fish diseases of bacterial origin have become one of the major agents of economic losses since the beginning of marine farming. *Vibrio* spp. are common inhabitants among the aquatic animals. *Vibrio* spp. may be the most devastating bacterial disease in cultured fish[3]. *Vibrio* spp. are widely distributed, Gram-negative bacteria that need sodium and chloride[4-6]. *Pseudomonas* spp. are Gram-negative bacteria that can develop resistance and virulence factors. *P. aeruginosa* produces two types of soluble pigments, a blue pigment, pyocyanin and a fluorescent pigment, pyoverdin[7]. Most *Aeromonas* spp. were catalase and oxidase-positive, motile, able to reduce nitrate to nitrite, sugars fermentative and gas may be produced[8].

Although Aquaculture plays an important role in providing safe, reliable and low priced food, the threat of antimicrobial resistance is increased[9,10] inducing treatment failure. Therefore, identification of antibiotic resistance gene in the virulent bacteria is highly important[11,12]. The outer membrane protein (oprL) of *Pseudomonas* spp. is virulent genes located on the chromosome, that enable it to play a great role in causing diseases[13,14]. Uncontrolled or sub-therapeutic use of antimicrobials may be responsible for the resistance to these chemotherapeutic agents. This explanation is further in harmony with the statement saying that increase use of antimicrobials increases the problem of drug-resistant strain[15,16]. Bla TEM gene is a B-Lactam resistance gene (e.g., ampicillin, amoxicillin), it is in plasmid pUC19[17-20]. It was found in many pathogenic bacteria[12]. Moreover, thermostable direct hemolysin (tdh) and tdh-related hemolysin (trh) genes were found in V. parahaemolyticus inducing outbreaks[21].

Before starting any treatment, feed intake, daily losses and value of the fish should be measured against the cost of the treatment. Oral medications should preferably be given during the early stages of the bacterial diseases since fish in the late stages feed poorly. Antimicrobial therapy is frequently used to control bacterial fish diseases. Antimicrobial susceptibility testing is performed to select the most suitable antibiotics. It should be noted that improper use of antibiotics may led to the presence of resistant bacterial strains[22,23].

The present research aimed to study the role of antibacterial drugs in controlling the bacteria involved in mass mortality in some cultured marine fish farms in Damietta governorate.

MATERIALS and METHODS

Study area: The study was carried out at units of bacteriology and fish diseases Animal Health Research Institute, Kafr El-Sheikh branch, Egypt from December 2018-November 2019.

Fish examination and samples collection

Fish samples: A total of 100 diseased fish were collected from 5 different marine fish farms situated in Damietta, Egypt and suffered from mass mortality (sea bass 100-120 g and sea bream 80-100 g). Fish were carefully examined for symptoms of diseases with a special focus towards the lesions as pale gills, exophthalmia, abdominal distension and skin lesions as blisters, ulcers and hemorrhages. These live fish have transported in battery aerated tanks to the lab for examination.

Water samples: Total 20 water samples from the 5 fish farms (10 samples from the water inlet and 10 samples from farm water) were collected in a sterilized glass bottle. A total 30 mL of water samples were centrifuged at 5000 rpm for 5 min. A total 1 mL of the sediment was incubated into a test tube containing 9 mL of trypticase soya broth at 30°C/24 hrs for bacteriological examination[22].

Clinical and post mortem examination: Naturally infected marine fish were carefully examined in ponds of the farm for swimming, feeding and any abnormal sings on the body[3]. Also, any post mortem lesions were recorded (Fig. 1a-b).

Bacteriological examinations isolation: In complete aseptic conditions, bacteriological isolation was carried out from spleen, liver, kidney, brain and skin lesions of infected fish and inoculated into Tryptic soy broth with NaCl 2% at 30°C for 24-48 hrs then cultured into general bacteriological media (saline Nutrient agar and Tryptic soy agar with NaCl 2%) and incubated[24] at 30°C for 48 hrs. The colonies were streaked on specific medium as Rimler's-Shotts medium (R.S. medium), *Pseudomonas* selective agar base, *Aeromonas* selective agar base with ampicillin supplement and TCBS agar then incubated at 30°C for 24 hrs. Semisolid nutrient agar was used for measuring the motility.

Fig. 1(a-b): Naturally infected sea bass
(a) congestion in the liver and darkening of the skin and (b) Congestion in gallbladder, gills and paleness in liver

Identification

Phenotypic characterization and Biochemical identification: The identification of the isolates was performed according to Bergey[25]. Smears of suspected bacterial colonies were prepared, stained (gram stain) for microscopic examination. For phenotypic characterization of isolated bacteria (Biochemical identification), VITEK2 COMPACT SYSTEM (BIOMERIUX, FRANCE) were used.

Polymerase chain reaction: DNA extraction: DNA extraction from samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH)

Oligonucleotide Primer: Primers from Metabion (Germany) was used. This is shown in Table 1.

PCR amplification: Amplification was carried out through the Applied biosystem 2720 thermal cycler.

Analysis of the PCR products: 1% agarose gel (Applichem, Germany, GmbH) in 1xTBE buffer was used for gel electrophoresis. About 40 µL of the products were loaded in

Table 1: Target genes, primers sequences, applicant sizes and cycling conditions

				Amplification (35 cycles)					
Microbial agent	Target gene	Primers sequences	Amplified segment (bp)	Primary denaturation	Secondary denaturation	Annealing	Extension	Final extension	Reference
Pseudomonas	blaTEM	ATCAGCAATAAACCAGC	516	94°C	94°C	54°C	72°C	72°C	Colom *et al.*[26]
		CCCCGAAGAACGTTTTC		5 min.	30 sec.	40 sec.	45 sec.	10 min.	
Pseudomonas	oprL	ATG GAA ATG CTG AAA TTC GGC	504	94°C	94°C	55°C	72°C	72°C	Xu *et al.*[27]
		CTT CTT CAG CTC GAC GCG ACG		5 min.	30 sec.	40 sec.	45 sec.	10 min.	
Vibrio	trh	GGCTCAAAATGGTTAAG CG	250	94°C	94°C	54°C	72°C	72°C	Mustapha *et al.*[28]
		CATTTCCGCTCTCATATGC		5 min.	30 sec.	30 sec.	30 sec.	7 min.	
Vibrio	tdh	CCATCTGTCCCTTTTCCTGC	373	94°C	94°C	54°C	72°C	72°C	
		CCAAATACATTTTACTTGG		5 min.	30 sec.	30 sec.	40 sec.	7 min.	

the gel holes. Gelpilot100 bp (Qiagen, Germany, GmbH) and GeneRuler 100 bp ladder (Fermentas, Thermo, Germany) were used for measuring the fragments. Photographing the gel was performed utilizing the documentation system (Alpha Innotech, Biometra). The data were analyzed using computer software.

Antibiogram: Antibiogram (sensitivity test) was performed using several antibiotics for the detection of the most effective one for the treatment of diseased fish farms[24,29].

Treatments trials: Enrofloxacin (the most effective *in vitro* antibiotics against isolated bacteria) was used at a level of 50 mg kg^{-1} for 7 days in each bacterial infected farm[30]. One mL blood sample per fish was collected from 20 diseased fish of each farm one day before starting treatment and one day after the end of treatment. Blood was used to obtain serum for some biochemical studies (ALT, AST[31], urea[32] and creatinine[33]).

Statistical analysis: The obtained results were analyzed using SAS.[34].

RESULTS

Bacteriological examination: Table 2 shows bacteria isolated from marine fish farm suffered from mass mortality and situated in Damietta, Egypt. It was found that *Pseudomonas* spp., *Aeromonas* spp. and *Vibrio* spp. were isolated from all farms.

Phenotypic and biochemical characteristics of isolated bacteria

***Pseudomonas* species:** Table 4 shows the Phenotypic and biochemical characteristics of *Pseudomonas* spp. Morphological and biochemical characteristics confirmed that *Pseudomonas* is a rod-shaped motile gram-negative bacterium, oxidative and nitrate reduction positive. The colony color of *Pseudomonas* is yellow (*P. fluorescens* colony is Yellowish green)

***Vibro* species:** Table 5 shows the Phenotypic and biochemical characteristics of *Vibrio* spp. It was observed that *Vibro* is a rod-shaped motile gram-negative bacterium. It is oxidative and catalase-positive. It grows on TCBS (thiosulfate-citrate-bile salts-sucrose agar) producing a yellow colony.

***Aeromonas hydrophila*:** Table 6 shows the Phenotypic and biochemical characteristics of *Aeromonas hydrophila*. It was found that *A. hydrophila* is a rod-shaped motile gram-negative bacterium. It is catalase-positive and hydrolyzes starch and gelatin.

Analysis of the PCR products: Figure 2 showed that isolated *V. parahaemolyticus* and *V. alginolyticus* were positive for the presence of trh and tdh virulence genes respectively,

Table 2: Bacteria isolated from of diseased fish farms

Pathogen	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5
Bacteria					
Aeromonas spp.	+	+	+	+	+
Pseudomonas spp.	+	+	+	+	+
Vibrio spp.	+	+	+	+	+

Bacteriological examinations revealed isolation of *Pseudomonas* spp., *Aeromonas* spp. and *Vibrio* spp. from the examined fish and water samples with rate of (16, 10%), (22, 10%) and (28, 10%), respectively. The isolates were *Pseudomonas* spp. (*P. aeruginosa*, *P. fluorescens*, *P. putida* and *P. alcaligenes*), *Aeromonas* spp. (*A. sobria*, *A. hydrophila* and *A. caviae*) and *Vibrio* spp. (*V. parahaemolyticus*, *V. alginolyticus, V. vulnificus* and *V. harveyi*)

Table 3: Bacteriological examination of water and fish samples

Bacterial strains	Bacterial spp.	No of fish samples	+Ve	No of water samples	+ve	Fish (%)	Water (%)
Pseudomonas spp.	*P. fluorescens*	100	6	20	1	16	10
	P. aeruginosa		6		1		
	P. putida		2		0		
	P. alcaligenes		2		0		
Aeromonas spp.	*A. Hydrophila*	100	10	20	2	22	10
	A. sobria		6		0		
	A. Caviae		6		0		
Vibrio spp.	*V. alginolyticus*	100	8	20	0	28	10
	V. parahaemolyticus		8		1		
	V. vulnificus		7		0		
	V. harveyi		5		1		

meanwhile, isolated *P. aeruginosa* was positive for oprl virulence gene and the examined *P. fluorescens* were positive for bla TEM virulence as shown in Fig. 3. It grows on TCBS (thiosulfate-citrate-bile salts-sucrose agar) producing a yellow colony.

***Aeromonas hydrophila*:** Table 6 shows the Phenotypic and biochemical characteristics of *Aeromonas hydrophila*. It was found that *A. hydrophila* is a rod-shaped motile gram-negative bacterium. It is catalase-positive and hydrolyzes starch and gelatin.

Table 4: Phenotypic and biochemical characteristics of *Pseudomonas* species

	Pseudomonas species		
Test conducted	*P. fluorescens*	*P. aeruginosa*	*P. anguilliseptica*
Color of colony	Yellowish green	Yellow	Yellow
Gram staining	Negative	Negative	Negative
Shape	Round	Round	Round
Fluorescent	+ve	-ve	-ve
Motility	+ve	+ve	+ve
Oxidation/Fermentation	Oxidative	Oxidative	Oxidative
Catalase test	+ve	-ve	+ve
Oxidase	+ve	+ve	+ve
MR-VP	-ve	-ve	+ve
Indole test	-ve	-ve	+ve
Oxidase reaction	+ve	+ve	+ve
Nitrite Reduction	+ve	+ve	+ve
Ornithine decarboxylase	+ve	+ve	-ve
Arginine dihydrolase	+ve	+ve	-ve
β-galactose	+ve	+ve	+ve
Urease production	+ve	+ve	+ve
H2S production	+ve	+ve	+ve
Glucose	+ve	+ve	+ve
Fructose	+ve	+ve	+ve
Dextrose	+ve	+ve	-ve
Galactose	+ve	-ve	+ve
Sucrose	+ve	-ve	+ve
Xylose	+ve	+ve	+ve

-ve : Negative; +ve : Positive, MR-VP: Methyl red -Voges proskauer test

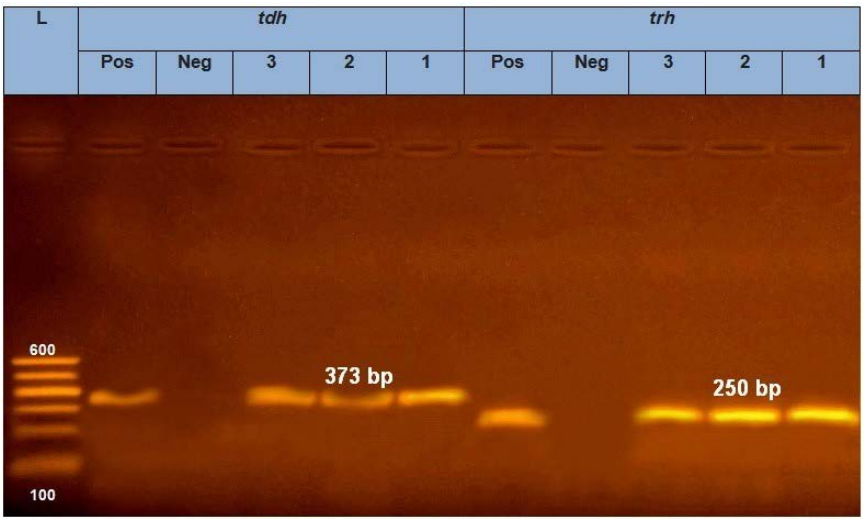

Fig. 2: The examined isolates of *Vibrio parahaemolyticus* and *Vibrio alginolyticu*

Lane L: 100 bp ladder as a molecular size DNA marker, Pos: control positive, Neg: control negative, Lane 1-3: Positive virulence gene (tdh) for *Vibrio parahaemolyticus,* Lane 1-3: positive virulence gene (trh) for *Vibrio Alginolyticus*

Table 5: Phenotypic and biochemical characteristics of *V. harveyi* and *V. alginolyticus*

Biochemical tests	*V. harveyi*	*V. alginolyticus*
Gram stain	-ve	-ve
Growth on TCBS	Yellow colonies	Yellow colonies
Swarming on solid media	-ve	+ve
Motility	+ve	+ve
Oxidase	+ve	+ve
Catalase	+ve	+ve
H2S production	-ve	+ve
Urease	+ve	-ve
Citrate	+ve	-ve
Indole production	+ve	+ve
VP	-ve	+ve
MR	+ve	+ve
Glucose	+ve	+ve
Sucrose	+ve	+ve
Mannitol	+ve	+ve
Lactose	-ve	-ve

-ve : Negative; +ve : Positive, TCBS: Thiosulfate-Citrate-Bile salts-Sucrose agar, MR: Methyl red test, VP: voges proskauer test

Table 6: Phenotypic and biochemical characteristics of *Aeromonas hydrophila*

Test	Result
Shape	Rod
Motility	+ve
Gram staining	-ve
Indole	+ve
MR	+ve
VP	+ve
Citrate utilization	+ve
Catalase	+ve
Urease	-ve
Oxidase	+ve
Carbohydrate utilization	
Lactose	+ve
Glucose	+ve
Trehalose	+ve
Starch hydrolysis	+ve
Gelatin hydrolysis	+ve

-ve : Negative; +ve : Positive, MR: Methyl red test, VP: Voges proskauer test

Table 7: Agar disc diffusion test results showing the sensitivity of isolated bacteria to different antibiotics

Antibiotic	Disc symbol and concentration (µg/disc)	*Aeromonas* spp.	*Pseudomonas* spp.	*Vibrio* spp.
Doxycycline	DO (30)	MS	R	S
Erythromycin	E (15)	R	R	R
Norfloxacin	Nor (10)	S	S	R
Gentamicin	CN(10)	S	R	R
Sulfamethoxazole + trimethoprim	SXT(25)	R	R	R
Enrofloxacin	ENR (5)	S	HS	S
Chloramphenicol	C (30)	S	R	MS
Amoxicillin	AML (10)	MS	R	S
Lincomycin	MY(10)	HR	R	R
Cefotaxime	CTX(30)	R	R	S

S: Sensitive (more than 50 and less than 75% of isolates were susceptible to the antimicrobial agents), MS: Moderately susceptible (50% of the isolates were susceptible to the antimicrobial agents), HS: Highly sensitive (75% or more of isolates were susceptible to the antimicrobial agents), R: Resistant (more than 50 and less than 75% of isolates were resistant to the antimicrobial agents), HR: Highly resistant (more than 75% of isolates were resistant to the antimicrobial agents)

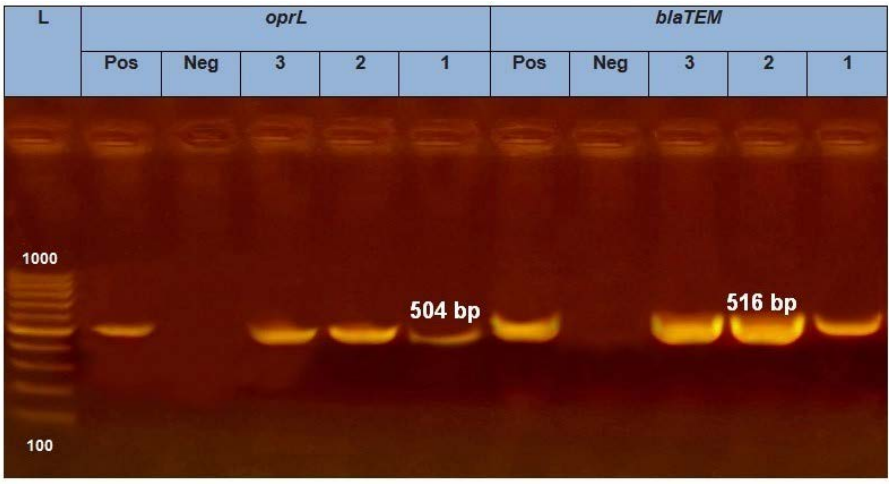

Fig. 3: The examined isolates of *P. aeruginosa* and *P. fluorescens*

Lane L: 100 bp ladder as a molecular size DNA marker, Pos.: control positive, Neg.: Control negative, Lane 1-3: Positive virulence gene (oprl) for *P. aeruginosa,* Lane 1-3: Positive virulence gene (blaTEM) for *P. fluorescens*

Table 8: Effect of enrofloxacin administration (50 mg kg^{-1} feed) for 7 days on mortalities of infected farms

Mortalities	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5
One week before					
Treatment	213	350	370	610	540
One week after treatment	18	30	38	47	52

Table 9: Effect of enrofloxacin on AST, ALT, urea and creatinine levels of diseased fish

	Parameters			
Group	AST (μ mL^{-1})	ALT (μ mL^{-1})	Urea (mg dL^{-1})	Creatinine (mg dL^{-1})
Before enrofloxacin treatment	67.34±5.03^{a}	11.15±1.39^{a}	20.11±1.62^{a}	1.21±0.12^{a}
After enrofloxacin treatment	55.47±5.76^{b}	7.31±0.91^{b}	12.27±2.81^{b}	0.47±0.07^{b}

Aspartate aminotransferase, ALT: Alanine aminotransferase, Means within the same column of different superscript digits are significantly different at ($p<0.05$)

Analysis of the PCR products: Figure 2 showed that isolated *V. parahaemolyticus* and *V. alginolyticus* were positive for the presence of trh and tdh virulence genes respectively, meanwhile, isolated *P. aeruginosa* was positive for oprl virulence gene and the examined *P. fluorescens* were positive for bla TEM virulence as shown in Fig. 3.

Antibiogram: Table 7 explains the effect of different antibiotics on isolated bacteria *in vitro*. The results showed that the most effective antibiotic on isolated bacteria was enrofloxacin, meanwhile, *Pseudomonas* spp. were resistant to many antibacterial agents (Table 7).

Treatments trials: Table 8 shows the effects of dietary enrofloxacin supplementation (50 mg kg^{-1} feed) for 7 days on the mortality rate in examined farms. It was noticed that enrofloxacin reduced significantly the mortality rate. Furthermore, data in Table 9 revealed that enrofloxacin administration induced a significant reduction of serum AST, ALT, urea and creatinine.

DISCUSSION

Fish is an important animal protein source in Egypt. It is important to control fish diseases to avoid high economic losses[35]. The study was performed on 5 farms that suffered from mass mortality. One hundred diseased fish (20 per farm) were randomly collected from these farms. Bacteriological examinations were carried out on the diseased fish. The recorded P.M. lesions were hemorrhages on the liver and darkness of skin as in (Fig. 1a-b). Bacteriological examinations of diseased fish revealed isolation of *Pseudomonas* spp., *Aeromonas* spp. and *Vibrio* spp. from the examined fish and water samples with the rat of (16, 10%), (22, 10%) and (28, 10%) respectively as in Table 3, these results were confirmed with biochemical tests. The isolates were *Pseudomonas* spp. (*P. aeruginosa, P. fluorescens, P. putida* and *P. alcaligenes*), *Aeromonas* spp. (*A. sobria, A. hydrophila* and *A. caviae*) and *Vibrio* spp. (*V. parahaemolyticus, V. alginolyticus V. vulnificus* and *V. harvey*). Bacteriological examinations of ration revealed no isolation of any previous bacteria. These results agree with that recorded by Tison *et al.*[35], Zorrilla *et al.*[36] and Moustafa *et al.*[37] but in low prevalence. Phenotypic and biochemical characteristics of isolated *Pseudomonas, Vibrio* and *Aeromonas* species were nearly similar to that recorded by Moustafa *et al.*[37].

The usage of molecular and conventional methods together for Vibrio species identification is necessary[38]. Further identification for Vibrio species were done for detection of tdh and trh virulence genes, as in Fig. 2, the pathogenicity of Vibrio species was detected by the presence of tdh (B hemolytic nature) and tdh which is cytotoxic to many types of cells[39]. As shown in Fig. 2 the examined isolates of *V. alginolyticus* and *V. parahaemolyticus* were positive for the presence of both trh and tdh virulence genes respectively. Immunological, biological and physicochemical characteristics of trh were found to be similar to those of tdh: 84% sequence identity[40,41]. Both gens are responsible for many outbreaks[21]. The outer membrane protein (oprL) of Pseudomonas spp. plays an important role of antibiotic resistance through efflux systems[42]. As result of mass use of antibiotics agents many pathogenic bacteria acquired virulence genes which made them more pathogenic. As shown in Fig. 3 the examined *P. aeruginosa* were positive for oprl virulence gene and the examined *P. fluorescens* were positive for bla TEM virulence gene. These genes and other virulence genes enable them to play a great role in causing fish diseases[14].

Table 7 showed that, the most effective antibiotic on isolated bacteria was enrofloxacin. *Pseudomonas* spp. were resistant to many antibiotics. Improper use of many antibiotics in aquaculture may lead to the presence of antibiotic-resistant bacteria[10]. For example, amoxicillin is a broad-spectrum beta-lactam antibiotic with a higher absorption rate when given orally[43] but as shown in Fig. 3 blaTEM gene (B-Lactam

resistance gene e.g., ampicillin, amoxicillin) was present in all examined *Pseudomonas* spp., leading to the development of resistance to B-Lactam group[44]. However, data in Table 7 and 8 revealed that the isolated bacterial strains were highly sensitive to enrofloxacin *in vitro* and *in vivo*. Similar results were also obtained by Riviere *et al.*[43] who reported that fluoroquinolones having a piperazine group at position 7 as enrofloxacin is highly active against many pathogenic aerobic Gram-negative bacteria as *Pseudomonas* spp.

The increase in serum ALT, AST, creatinine and urea in infected fish may be attributed to the liver, kidney and gill damage induced by infected bacteria. Serum biochemical analysis of the infected fish after treatment with enrofloxacin revealed a significant improvement of liver and kidney functions. That pointed to the effect of enrofloxacin against infected bacteria. Results are in accordance with the results obtained by Koehler and Ashdown[45], Laganà1 *et al.*[46], Trevesbrown[47] and El-Atta and Tantawy [48] who found that *A. hydrophila*, most *Vibrio* strains, *P. aeruginosa* and *P. fluorescens* were susceptible to ciprofloxacin. Furthermore, the resistance of *Pseudomonas* spp. to amoxicillin was noticed (Table 7) as a result of having blaTEM gene (as shown in Fig. 3). This result was supported by El-Hady and Samy[49] who mentioned that *P. aeruginosa* acquired the resistance to amoxicillin due to heavy contamination of marine water by sewages polluted with antibiotics residues. Finally, improper use of antibiotics may lead to the appearance of resistant strains of bacteria. These bacteria become able to adapt to the antibiotic by mutating and developing resistance gen, so many antibiotics lost their power (amoxicillin in this work). Therefore, using of antibiotics in the veterinary field should be under veterinary supervision with therapeutic doses. Moreover, good environmental conditions should be provided in aquaculture to avoid stresses and immune suppression of fish. Immune compromised fish are more susceptible to the infection.

CONCLUSION

It could be concluded that the high mortalities in some marine fish farms in the Damietta governorate could be due to some pathogenic bacteria mainly *Aeromonas*, *Pseudomonas* and *Vibrio*. Administration of enrofloxacin reduced mortalities in these farms; however, improper mass use of antibacterial agents reduced the chance of using many suitable antibiotics as a result of the development of antibiotic-resistant bacterial strains.

SIGNIFICANCE STATEMENT

This study discovers the possible causes of treatment failure with many antibiotics that can be beneficial for antibiotic selection. This study will help the researchers to uncover the critical area of resistant bacterial-gens were not able to explore. Thus, a new theory on the selection of effective antibiotics based on the absence of antibiotics resistant bacterial-gens may be arrived at.

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