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Molecular Study on some Virulence and Fluoroquinolone Resistance Genes of *Pseudomonas aeruginosa* Isolated from Naturally Infected Cultured Sea Bream Fish (*Sparus aurata*) in Egypt

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Abstract

This study carried out to investigate the presence of some virulence and fluoroquinolone resistant genes by PCR and comparing with antibiotic diffusion disc for isolated *P. aeruginosa*. A total number of 100 alive cultured Sea bream, showing clinical signs were collected from a private fish farm at Damietta, Egypt then subjected to clinical, postmortem (PM), bacteriological examination, biochemical and serological identification of the isolated bacteria, *P. aeruginosa* were more prevalent and subjected to VITEK2 and PCR for detection of outer membrane lipoprotein gene (*oprL*) at "504 bp" and exotoxin A gene (*toxA*) at "270 bp" which indicated the virulent isolates of *P. aeruginosa* and DNA *gyrA*se (*gyr*A) at "287 bp" and topoisomerase IV (*parC*) at "267 bp" for determining fluoroquinolone resistance genes compared with antibiotic sensitivity test by disc diffusion using 3 of fluoroquinolone members. The more prevalent isolates were *P. aeruginosa* (43.02%), of them 12 isolated showed by PCR the presence of *oprL*, *toxA*, *gyrA* and *parC* genes and confirmed with antibiogram resistance to tested 3 fluoroquinolone members. The present study explored that pathogenic and fluoroquinolone resistant isolates of *P. aeruginosa* were more prevalent that necessitate more rapid hygienic programs and narrow uses of antibiotics for Sea bream aquaculture fisheries in Egypt.

Keywords: *P. aeruginosa;* PCR; Virulence; Fluoroquinolone; Sea bream

Introduction

Sea bream (*Sparus aurata*) is straightaway one of the devastating consumable species around the globe. The world catch establishment of this species in 2004 and was around 10% of the total aquaculture yield [1]. Fish is a principal inception of food for people and contributes around 60% of the worldwide's supply of protein. 60% of the producing countries decide 30% of their yearly protein from fish [2]. Sea bream (*Sparus aurata*) fisheries were known starting late in Egypt and necessity for element progression especially in feeding and microbiological aspects [3].

Opportunistic diseases brought about by *P. aeruginosa* are a genuine medicinal issue, and quinolone resistant *P. aeruginosa* could be recovered from clinical cases. As of not long ago, the *gyr*A mutation in quinolone resistance has been examined in a few species of bacteria [4-6]. Albeit numerous studies have concentrated on *E. coli* [7] and *S. aureus* [8], concentrates on with *P. aeruginosa* are less cutting-edge. As of late, [9] cloned the wild kind of the *gyr*A gene in *P. aeruginosa* and watched the Thr-83-Ile, Asp-87-Asn, and Asp-87-Tyr mutations in *gyr*A from clinical isolates.

Fluoroquinolones are basic category of antimicrobial operators used as a part of the treatment of *Pseudomonas* infections. Fluoroquinolones are members from the quinolone family which act as bactericidal workers by limiting bacterial DNA gyrase and topoisomerase IV, therefore quelling DNA transcription and replication. DNA gyrase is ordinarily the target in Gram -ve microorganisms; *Pseudomonas* speedily makes resistance to these professionals, along these lines diminishing their effectiveness. The rule segments of resistance are mutations in the genes that encode DNA gyrase (*gyr*A) and topoisomerase IV (*par*C). Fluoroquinolone resistance can provoke treatment dissatisfaction in *P. aeruginosa* infection [10].

The nucleic corrosive modifications that happened in those codons changed the amino acid profile from Thr to Ile and Asp to Asn (or Asp to Tyr), individually, predictable with past records [10-12].

Regular transformations in fluoroquinolone resistant strains happened in codons 83 and 87 of *gyr*A gene [13]. No mutations were found in *par*C. A novel transformation identified with fluoroquinolone resistance in codon 126 of the *mex*R gene, altering amino acid Val to Glu, was distinguished [13].

Infections are fortified by climatic changes that consider antagonistically the marine environment, which is a conventional media for different microbes and initiated by human interference that lead not simply to fabricate the damaging tendency of these pathogens also to the rise and advancements of new microorganisms not present some time as of late [14].

Septicemic bacterial infection with *Pseudomonads*, *Vibrios*, *Photobacteria*, *Aeromonads*, *Streptococci* and *Staphylococci* have been found in some fingerlings, juveniles, grown-ups and brood supplies of some marine fish species [15]. This may to some degree recognized to the general existence of such pathogens in seawater, residue and furthermore digestive tract of marine fishes [16]. Hence, the increase of rotted normal environments, similar to change in temperature or hardship of supplement would ordinarily drive them to effervescently beating fish [17].

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The predominant parts of bacterial infection in fish are achieved by Gram -ve organisms including: *Pseudomonas*, *Vibrio*, *Aeromonas*, *Citrobacter*, *Edwardsiella* and *Streptococcus* as Gram +ve family. These species may be the vital causes of some fish disorders. Most of bacterial fish pathogens are specific inhabitants of the marine environment or fresh water [18].

New and developing ecological pathogens represent a portion of the best dangers to current aquaculture, a basic wellspring of nourishment protein worldwild. Likewise with other serious cultivating works on, expanding our comprehension of the science of diseases is vital to enhance creature welfare and farming [19].

Material and Methods

Fish samples

In this investigation, a total number of 100 alive cultured Sea bream fish (*Sparus aurata*) with different body weight ranged from (50 g to 150 g) showing abnormal signs were collected from a private fish farm at Mahallat El-Diba region at Damietta governorate, Egypt. The sources of water in that region are numerous; drainage of canal originated from El-Manzalah Lake and El-headway downstream from Mediterranean Sea. Fish were clinically examined [20] and transferred in ice box soon to the laboratory located in the same farm where sampling occurs,. The freshly dead collected Sea bream fish were subjected to full postmortem (PM) examination [21].

Bacterial isolation and identification

Samples collected after fish surfaces swapped with 70% ethyl alcohol for surface sterilization and then inoculum were taken from liver, kidney, spleen and heart under complete aseptic condition and cultured on Trypticase Soya Broth+3% NaCl (Difco®) and incubated at 25°C for 24-48 hrs then subcultured on different types of media for primary isolation and identification; Trypticase Soya agar+3% NaCl (TSA); *Pseudomonas* base agar (Himdia®) with Cetrimide-Fucidin-Cephalosporin (CFC) supplement (Oxoid®), Thiosulphate citrate bile salt sucrose agar (TCBS) (Oxoid®), Blood agar media using 5% sheep RBCs for hemolytic activity, MacConkey bile salt lactose agar (Oxoid®), Mannitol salt agar medium (Oxoid®), Semi–solid 0.5% agar medium for preservation of bacterial isolates, Muller-Hinton agar (oxoid®) for antibiogram activity. The inoculated plates were incubated at 25°C for 24-48 hrs for bacteriological examination [22], biochemical identification by conventional test [23]. Followed by serological identification of the isolated bacteria; the anti-sera for slide agglutination test used for serotyping of the bacterial isolates were obtained from (Deben Diagnostics Ltd, United Kingdom) and performed at Lab. of Fish Diseases at El Wafaa hatchery center – Ismailia governorate. Further confirmatory identification of the more prevalent isolated *P. aeruginos*a by automatically colorimetric biochemical confirmation VITEK2 COMPACT SYSTEM (BIOMERIUX, FRANCE) according to manufacturer's instructions by matching the results of the bacterial isolates with those involved in its data base platform. After these, PCR assay performed as followed

Molecular characterization of isolated *P. aeruginosa*

- Extraction of DNA of isolated *P. aeruginos*a using the Qiagen Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions and was used as the DNA template for PCR.
- The oligonucleotide primers were synthesized and supplied by Metabion (Germany) which indicated the virulent isolates of *P. aeruginosa,* Table 1 and Table 2 were used.
- Multiplex PCR was used for detection of both outer membrane lipoprotein gene (*opr*L) at "504 bp" [24] and exotoxin A gene (*tox*A) at "270 bp" [25].
- Conventional PCR used to explore the presence of DNA *gyr*Ase (*gyr*A) at "287 bp" and topoisomerase IV (*par*C) at "267 bp" [13] for detection of fluoroquinolone resistance genes of *P. aeruginosa* isolated from naturally infected cultured Sea bream fish.

Gene		Primer Sequence(5' - 3')	Size (bp)	Reference
*tox*A	F	CTGCGCGGGTCTATGTGCC	270	Stover et al. [25]
	R	GATGCTGGACGGGTCGAG		
*opr*L	F	ATG GAA ATG CTG AAA TTC GGC	504	Xu et al. [24]
	R	CCT CTT CAG CTC GAC GCG ACC		
F: Forward; R: Reverse; bp: Base Pair				

Table 1: Oligonucleotide primer sequences for detecting *tox*A and *opr*L genes of p.aeruginosa isolated from Sea bream Fish.

Gene		Primer Sequence(5' - 3')	Size (bp)	Reference
*gyr*A	F	GTGTGCTTTATGCCATGAG	287 bp	Gorgani et al. [13]
	R	GGTTTCCTTTTCCAGGTC		
*par*C	F	CATCGTCTACGCCATGAG	267 bp	
	R	AGCAGCACCTCGGAATAG		
F: Forward; R: Reverse; bp: Base Pair				

Table 2: Oligonucleotide primer sequences for detection of Fluoroquinolone resistance genes *gyr*A and *par*C of p.aeruginosa isolated from Sea bream Fish.

The reaction was conducted in a total volume of 50 µl in 0.5 ml microfuge tube as 25 µl Green Master Mix, 10 µl DNA template, 5 µl upstream primer, 5 µl downstream primer and 5 µl nuclease free water and the reaction mixture was over laid with 50 µl nuclease free mineral oil to prevent evaporation during thermocycling, DNA marker (100 bp DNA ladder) (Gene Ruler ™) was used as a DNA molecular weight marker for PCR product in agarose gel electrophoresis, followed by the use of ethidium bromide for staining nucleic acid [26].

Antibiogram activity of *P. aeruginosa*

Finally, PCR assay on fluoroquinolone resistance genes was compared with the antibiogram activity using antibiotic diffusion disc (Oxoid®) against 3 of fluoroquinolone members (Ciprofloxacin, Flumequine and Enrofloxacin), Gentamycin, Trimethoprim/ Sulphamethoxazole, Oxytetracycline, Amoxycillin and Erythromycin according to the method described [27].

Results and Discussion

Clinical signs and PM lesions of examined sea bream fish

Some of clinically examined Sea bream showed hemorrhages at pelvic and anal fins, moreover showed petechial hemorrhages at the operculum and around the eye (Figure 1), while others showed erythema in the mouth and severe exophthalmia, severe hemorrhagic patches at the body and severe hemorrhages at base of pectoral fins, severe hemorrhagic swollen protruded anal dopening (Figure 2), While by PM, some of examined Sea bream showed hemorrhages at gills, intestine and muscles (Figure 3), hemorrhages at heart (Figure 4), congestion and hemorrhages in the internal organs and distended gall bladder (Figure 5) others showed serous ascetic fluid tinged with blood dropping on behind paper upon opening the fish, while the others showed congestion of different degrees on the liver of examined fish.

Results of bacteriological examination of sea bream fish

Prevalence of Gram –ve and Gram +ve bacterial serotypes among naturally infected sea bream fish: Bacteriological examination of a

Figure 1: Petechial haemorrhages as the sperculum and around the eye, corneal capacity and purulent material in the eye.

Figure 2: Severe haemorrhagic swollen protruded anal opening.

Figure 3: Haemorrhages in the gills, intestine and muscles.

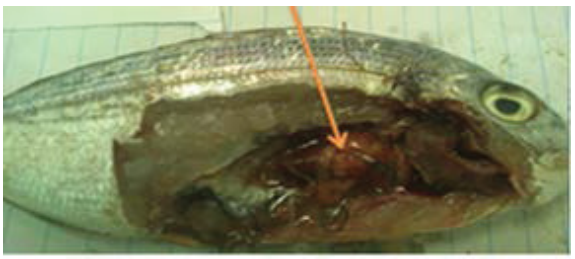

Figure 4: Haemorrhages in the heart.

total number of 100 naturally infected cultured Sea bream fish samples explored the isolation of 77 (89.53%) Gram –ve and 9 (10.47%) Gram +ve, totally; 86 (100.00%) bacterial isolates (Table 3). This confirmed with Saad who recorded in naturally infected fish Gram -ve bacteria were (78.00%) while Gram +ve bacteria were (22.00%). It has also been agreed that Gram -ve rods are the more causative agent in producing diseases in fish and to lesser extent Gram +ve cocci [28].

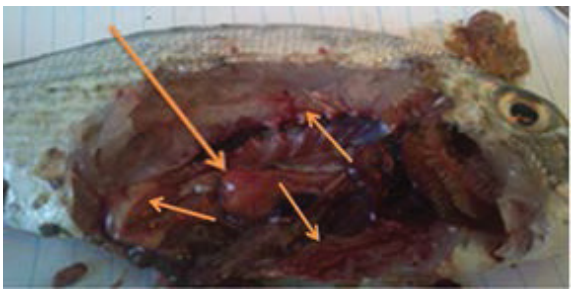

Figure 5: Congestion and Haemorrhages in the intestinal organs and distended gall bladder.

Examined Fish Species	Bacterial Isolates					
	Gram -ve		Gram +ve		Total no. of Isolates	
	No.	%	No.	%	No.	%
Sea bream	77	89.53	9	10.47	86	100.00
Gram -ve: Gram Negative; Gram +ve : Gram Positive						

Table 3: Prevalence of Gram +ve and Gram –ve bacterial serotypes among naturally infected Sea bream fish.

Colonial, morphological, conventional biochemical and VITEK2 **identification of yielded bacterial isolate:** Basic morphological and conventional biochemical characterization identified the yielded isolates into *P. aeruginosa*, *V. alginolyticus*, *V. anguillarum serotype* 01, *Strept. iniae* and *Ph. damsela subsp. piscicida* these agreed with Suanyuk, Eissa, Das [15,17,29]. All yielded isolates *P. aeruginosa* were identified with 100% probability by colorimetric VITEK2 COMPACT SYSTEM which used as a recent aid for more confirmation for identification of the more isolates of P. aeruginosa. Chatzigeorgiou et al. [30] have been concluded that VITEK2 GN card should be considered for identifying gram -ve non fermentative bacilli while Paim [31] found that VITEK2 COMPACT SYSTEM software version 5.03 is considered for identifying Gram +ve cocci. COMPACT SYSTEM software version 5.03 properly identified isolated Gram +ve cocci.

Prevalence of bacterial serotypes among naturally infected sea bream fish

According to Table 4, the more prevalent bacterial serotype in naturally infected cultured Sea bream fish was *P. aeruginosa* (43.02%), followed by *V. alginolyticus* (20.93%), *V. anguillarum serotype 01* (16.28%), *Strept. iniae* (10.47%), and *Ph. damsela subsp. piscicida* (9.30%). These disagreed with Zorrilla [32] who found that *Ph. damselae subsp. piscicida* was more prevalent in Sea bream infection, but he recorded that *Pseudomonas* spp. and *Vibrio* spp. known as pathogenic bacteria for Sea bream fish and also agreed with Hossain [33] who isolated *Strept. iniae* from marine fish. Also agreed with Yiagnisis and Athanassopoulou [34] who isolated *Ph. damsela subsp. Piscicida* from diseased cultured Sea bream, El-Moghazy and Elham [1,35] also isolated *Pseudomonas* spp. from Sea bream fish. Also these results supported by Snoussi [16] reported that *Vibrio* found in tropical and temperate waters worldwide seaside water of the Mediterranean Sea. In addition, Moustafa [36] who said that Gram -ve bacteria isolates including *V. alginolyticus*, *Pasteurella piscicida (P. damsela* subspp *piscicida)* were the more isolated pathogens from marine fish species, while *S. iniae* was the most Gram +ve bacteria.

The prevalence of bacterial isolates in the internal organs of the naturally infected sea bream fish

The incidence of bacterial isolates retrieved from different internal organs of naturally infected cultured Sea bream fish was illustrated in Table 5, in which; the more bacterial isolation recorded in liver (41.86%) followed by spleen (25.58%), heart (16.28%) and kidney (16.28%), these results agreed with Eissa [37] who reported that liver was the highest organ followed by kidney and spleen of marine fishes.

Total prevalence of bacterial serotypes retrieved from the different internal organs of naturally infected sea bream fish

The total prevalence of different bacterial serotypes retrieved from different internal organs of naturally infected cultured Sea bream fish was illustrated in Table 6 that showed liver infection was high incidence by *P. aeruginosa* (52.78%) followed by *V. alginolyticus* (19.4%), *V. anguillarum serotype 01* (16.67%), *Strept. iniae* (8.33%) and *Ph. damsela subsp. Piscicida* (2.78%) while in heart, isolation of *P. aeruginosa* was also more prevalent (50.00%) but *Ph. damsela subsp. piscicida, Strept. iniae* and *V. alginolyticus* all isolated with the same percentage (14.29%) and the lowest was *V. anguillarum serotype 01*(7.14%). About kidney, *V. alginolyticus* was the more prevalently isolated (42.86%) followed by *Ph. damsela subsp. piscicida* (28.57%), *Strept. iniae* (14.28%) and both *P. aeruginosa* and *V. anguillarum serotype 01* were isolated by the same percentage (7.14%). In spleen, *P. aeruginosa* was more prevalent (45.45%) followed by *V. anguillarum serotype 01*(27.28%), *V. alginolyticus* (13.64%), *Strept. iniae* (9.10%) and *Ph. damsela subsp. piscicida* (4.54%).

Results of PCR of the more prevalent isolates of *P. aeruginosa* from examined sea bream fish

Results of PCR for detection of virulence genes of *P. aeruginosa*

No. of Examined Sea fish species	Bacterial isolates	No	%
100 sea bream	*P. aeruginosa*	37	43.02
	V. alginolyticus	18	20.93
	V. anguillarum serotype 01	14	16.28
	Strept. iniae	9	10.47
	Ph. damsela subsp piscicida	8	9.30
	Total no. of Isolates	86	100.00
P: Pseudomonas; v: virio; strept: streptococcus; ph: photobacterium			

Table 4: Prevalence of different bacterial serotypes of infection in Sea Bream Fish.

Examined Sea fish species	Examined organs	Bacteriologically positive	
		No	%
Sea Bream	Liver	36	41.86
	Heart	14	16.28
	Kidney	14	16.28
	Spleen	22	25.58
	Total	86	100

Table 5: The Prevalence of bacterial isolates in different organs of naturally infected Sea Bream Fish.

Organs/Bacterial isolates	Liver		Heart		Kidney		Spleen	
	No	%	No	%	No	%	No	%
P. aeruginosa	19	52.78	7	50.00	1	7.14	10	45.45
V. alginolyticus	7	19.4	2	14.29	6	42.86	3	13.64
V. anguillarum serotype 01	6	16.67	1	7.14	1	7.14	6	27.28
Strept. iniae	3	8.33	2	14.29	2	14.28	2	9.10
Ph. damsela subsp piscicida	1	2.78	2	14.29	4	28.57	1	4.54
Total no. of Isolates	36	100	14	100	14	100	22	100
P: Pseudomonas; v: virio; strept: streptococcus; ph: photobacterium								

Table 6: The total Prevalence of bacterial serotypes isolated from different internal organs of naturally infected cultured Sea Bream Fish.

isolates from sea bream fish: In Figure 6, the primer sequence showed maximum identity with the sequence of virulence genes of all tested 12 isolates of *P. aeruginosa* with 100% homology to outer membrane lipoprotein gene *oprL* at "504 bp" and exotoxin A gene *toxA* at "270 bp" which indicated that these are virulent isolates of *P. aeruginosa*. These results agreed with those reported by De Vos, Pirnay, Xu, Deschaght and Billard-Pomares [24,38-41] who confirmed that PCR assay using the *opr*L gene was highly specific and more suitable than culture for detecting *P. aeruginosa*.

PCR for detection of fluoroquinolone resistance genes of *P. aeruginosa* isolates from sea bream fish

The primer sequence showed maximum identity with the sequence of fluoroquinolone resistance genes of all tested 12 isolates *P. aeruginosa* with 100% homology to DNA *gyrA*se (*gyrA*) at "287 bp" (Figure 7), and topoisomerase IV (*parC*) at "267 bp" (Figure 8), PCR amplified the correctly sized products which indicated that these are fluoroquinolone resistant isolates of *P. aeruginosa*.

Results of antibiogram activity of the more prevalent isolates of *P. aeruginosa* from examined sea bream fish

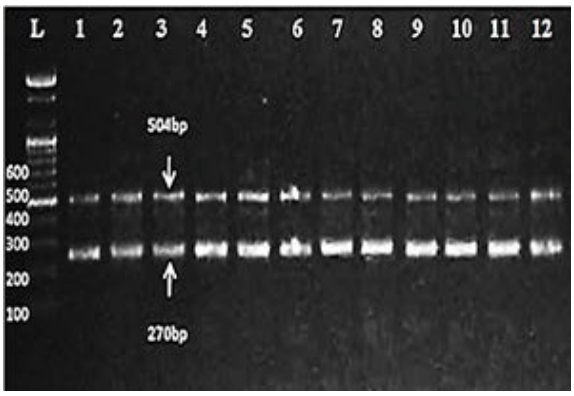

Figure 6: Agarose gel electrophoresis contacting the PCR products along with 100 bp DNA Ladder (lane L) While lane from 1 to 12 are the tested isolates of *P. aeruginosa* isolated from Sea Bream Fish in which all showed positive bands for both outer membrane lipoprotein gene(*opr*L) at 504 bp and exotoxin A gene (*tox*A) at 270 bp.

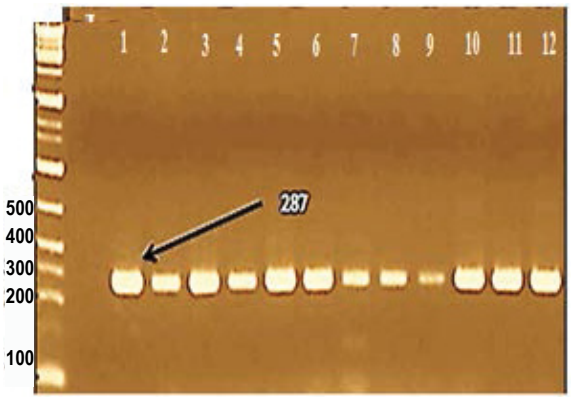

Figure 7: Agarose gel electrophoresis contacting the PCR products along with 100 bp DNA Ladder (lane L) While lane from 1 to 12 are the tested isolates of *P. aeruginosa* which all showed fluoroquinolone resistance gene; DNA *gyrA*se (*gyrA gene*) at 287 bp.

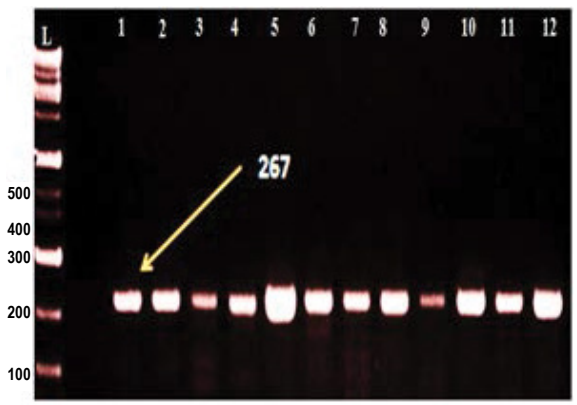

Figure 8: Agarose gel electrophoresis contacting the PCR products along with 100 bp DNA Ladder (lane L) While lane from 1 to 12 are the tested isolates of *P. aeruginosa* which all showed fluoroquinolone resistance gene; topoisomerase IV (*parC gene*) at 267 bp.

Type of used antimicrobial disc	Disc code	Disc concentration(µg)	Sensitivity of isolated *P. aeruginosa*
Ciprofloxacin	CIP5	5	-
Flumequine	UB30	30	-
Enrofloxacin	ENR5	5	-
Gentamycin	GN10	10	+++
Trimethoprim/ Sulphamethoxazole	SXT25	1.25+23.75	-
oxytetracycline	OT30	30	-
Amoxycillin	AML10	10	+
Erythromycin	E15	15	-
Resistant: - ; Intermediate: +,+; Sensitive: +++,++++			

Table 7: Antibiotic sensitivity test of isolated *P. aeruginosa* from Sea Bream Fish by using different antimicrobial discs

As shown in Table 7; *P. aeruginosa* isolates showed resistance to 3 of fluoroquinolone members: Ciprofloxacin, Flumequine and Enrofloxacin and these confirmed the result of positive detection of fluoroquinolone resistance genes by PCR, as well as *P. aeruginosa* isolates were resistant to Trimethoprim/Sulphamethoxazole, Oxytetracycline and Erythromycin while sensitive to Gentamycin and intermediately resistant to Amoxycillin. These agreed with Foti [42] who reported that *P. aeruginosa* resistant to oxytetracycline and amoxicillin and discussed these finding to contamination of marine water by polluted sewages residues of antibiotics can be spread and repetitive antibiotic usage. Also El-Moghazy and Aboutorkia [43,44] found that *P. aeruginosa* high sensitivity to Gentamicin and resistant to Moxifloxacin (fluoroquinolone member) and Trimethoprim/Sulfamethoxazole.

Conclusion

The present study explored that *Pseudomonas aeruginosa* isolation is more prevalent in diseased cultured Sea bream fish and PCR is a rapid, sensitive, specific assay in determining its pathogenicity by detecting molecular characterization of some virulence genes by 24 hrs-work time and this necessitate more rapid hygienic programs to minimize the possible infection to optimize the Sea bream aquaculture fisheries quality in Egypt, as well as the haphazard usage of broad-spectrum antibiotics in Sea bream aquacultures is the cornerstone of emergence of highly resistant strains of *P. aeruginosa* which narrow the zone of effective antibiotics and this highlighted the focus on the importance of determining the antibiotic susceptibility pattern of *P. aeruginosa* so that this aquaculture pathogen can be treated by narrow-spectrum and target-specific antibiotics as well as to calculate the minimal inhibitory concentration (MIC) before using antibiotic in cure. Following further studies should be followed to investigate more about pathogenicity of isolated *P. aeruginosa* and clarify the correlation between a DNA *gyr*A mutation and quinolone resistance. It would be helpful for further union of new quinolones that are powerful in treatment of Sea bream fish.

Compliance with Ethical Standards

Conflict of interest

All authors of this article have no conflict of interest.

Ethical approval

This article does not contain any studies with animals performed by any of the authors.

References

1. FAO (2006) FishStat.
2. Abisoye BF, Ojo SKS, Adeyemi RS, Olajuyigbe OO (2011) Bacteriological assessment of some commonly sold fishes in Lagos metropolis market Nigeria. Prime Journal of Microbiology Research 1: 23-26.
3. Abou Elatta ME (2013) Some Bacterial and Fungal affections Causing Disease Problems in Cultured Sea bream (Sparus aurata), in Damietta Governorate and Trials for Control. Journal of Arabian Aquaculture Society 8: 357-372.
4. Wang Y, Huang WM, Taylor DE (1993) Cloning and nucleotide sequence of the Campylobacter jejuni gyrA gene and characterization of quinolone resistance mutations. Antimicrob Agents Chemother 37: 457– 463.
5. Korten V, Huang WM, Murray BE (1994) Analysis by PCR and direct DNA sequencing of gyrA mutations associated with fluoroquinolone resistance in Enterococcus faecalis. Antimicrob Agents Chemother 38: 2091–2094.
6. Takiff HT, Salazar L, Guerrero C, Philipp W, Huang WM, et al. (1994) Cloning and nucleotide sequence of Mycobacterium tuberculosis gyrA gyrB genes and detection of quinolone resistance mutations. Antimicrob. Agents Chemother 38: 773– 780.
7. Vila J, Ruiz J, Marco F, Barcelo A, Goni P, et al. (1994) Association between double mutation in gyrA gene of ciprofloxacin resistant clinical isolates of Escherichia coli and MICs. Antimicrob Agents Chemother 38: 2477–2479.
8. Tokue Y, Sugano K, Saito D, Noda T, Ohkura H, et al. (1994) Detection of novel mutations in the gyrA gene of Staphylococcus aureus by nonradioisotopic single-strand conformation polymorphism analysis and direct DNA sequencing. Antimicrob Agents Chemother 38: 428–431.
9. Kureishi A, Diver JM, Beckthold B, Schollaardt T, Bryan LE (1994): Cloning and nucleotide sequence of Pseudomonas aeruginosa DNA gyrase gyrA gene from strain PAO1 and quinolone-resistant clinical isolates. Antimicrob Agents Chemother 38: 1944–1952.
10. Jalal S, Ciofu O, Høiby N, Gotoh N, Wretlind B (2000) Molecular mechanisms of fluoroquinolone resistance in Pseudomonas aeruginosa isolates from cystic fibrosis patients. Antimicrob Agents Chemother 44: 1410.
11. Yonezawa M, Takahata M, Matsubara N, Watanabe Y, Narita H (1995) DNA gyrase gyrA mutations in quinolone-resistant clinical isolates of Pseudomonas aeruginosa. Antimicrob Agents Chemother 39: 1970–1972.
12. Nakano M, Deguchi T, Kawamura T, Yasuda M, Kimura M, et al. (1997) Mutations in the gyrA and parC genes in fluoroquinolone-resistant clinical isolates of Pseudomonas aeruginosa. Antimicrob Agents Chemother 41: 2289–2291.
13. Gorgani N, Ahlbrand S, Patterson A, Pourmand N (2009) Detection of point mutations associated with antibiotic resistance in Pseudomonas aeruginosa. Int J Antimicrob Agents 34: 414–418.
14. Austin B, Austin DA (2007) Bacterial fish pathogens: Disease of farmed and wild fish (Fourth Revised Edition). Springer Praxis Publishing, Chichester, UK.
15. Suanyuk N, Sukkasame N, Tanmark N, Yoshida T, Itami T, et al. (2010) Streptococcus iniae infection in cultured Asian sea bass (Lates calcarifer) and red tilapia (Oreochromis sp.) in southern Thailand. Songklanakarin J Sci

Technol 32: 341-348

16. Snoussi M, Noumi E, Cheriaa J, Usai D, Sechi LA, et al. (2008) Adhesive properties of environmental V. alginolyticus strains to biotic and abiotic surfaces. New Microbiol 31: 489-500.
17. Eissa IAM, Badran AFH, Azza AMM, Somia AMM (2011) A contribution on motile Aeromonas septicemia caused by Aeromonas sobria in common carp (Cyprinus carpio) with trials for disease prevention. SCVMJ: 101-113.
18. Moeller B (2012) Bacterial Diseases of Fish, Cichlid- forum.com.
19. Seth-Smith HM, Dourala N, Fehr A, Qi W, Katharios P, et al. (2016) Emerging pathogens of gilthead seabream: characterisation and genomic analysis of novel intracellular β-proteobacteria. ISME J 10: 1791-803.
20. Amlacher E (1970) Text book of fish diseases. T.F.H. Publ, Hong Kong.
21. Conroy DA, Hermann LR (1981) Text book of fish diseases. T.F.H. publ. West Sylvania.
22. Austin B, Austin DA (1999) Bacterial Fish Pathogens – Disease of Farmed and Wild Fish, 3rd Ed., Springer-Praxis, Chichester, UK, 457.
23. Holt J, Krieg NR, Sneath PHA, Stanley JT, Williams ST (1994) Bergey's Manual of Determinative Bacteriology 9th edition. Williams and Wikins, Baltimore 527-558.
24. Xu J, Moore JE, Murphy PG, Millar BC, Elborn JS (2004) Early detection of P. aeruginosa: comparison of conventional versus molecular (PCR) detection directly from adult patients with cystic fibrosis (CF). Ann Clin Microbiol. Antimicrob 3: 21.
25. Stover CK, Pham XQ, Erwin AL, Mizoguchi SD, Warrener P, et al. (2000) Complete genome sequence of Pseudomonas aeruginosa PAO1, an opportunistic pathogen. Nature 406: 959-964.
26. Sambrook J, Russell DW (2001) Molecular cloning: a laboratory manual, 3rd edition. Cold Spring Harbor Lab. Press, Cold Spring Harbor, New York.
27. Barry AL, Thornsberry C (1991) Susceptibility tests: diffusion test procedures. American Society for Microbiology, Washington, DC 1117– 1125.
28. Öztürk RÇ, Altınok (2014) Bacterial and Viral Fish Diseases in Turkey. Turk J Fish Aquat Sci 14: 275-297.
29. Das BK, Sahu I, Kumari S, Sadique M, Nayak KK (2014) Phenotyping and Whole Cell Protein Profiling of Edwardsiella tarda strains isolated from infected Freshwater Fishes. Int J Curr Microbiol App Sci 3: 235-247.
30. Chatzigeorgiou KS, Sergentanis TN, Tsiodras S, Hamodrakas SJ, Bagos PG (2011) Phoenix 100 versus Vitek 2 in the identification of gram-positive and gram-negative bacteria: a comprehensive meta-analysis. J Clin Microbiol 49: 3284–3291.
31. Paim TGS, Cantarelli VV, d'Azevedo PA (2014) Performance of the Vitek 2 system software version 5.03 in the bacterial identification and antimicrobial susceptibility test: evaluation study of clinical and reference strains of Gram-positive cocci. Revista da Sociedade Brasileira de Medicina Tropical 47: 377-381.
32. Zorrilla M, Chabrillo´n S, Arijo P, Dı´az-Rosales E, Martı´nez-Manzanares MC, et al. (2003) Bacteria recovered from diseased cultured gilthead sea bream (Sparus aurata L.) in south western Spain. Aquaculture 218: 11 –20.
33. Hossain MMM, Ehsan A, Rahman MA, Haq M, Chowdhury MBR (2014) Transmission and pathology of Streptococcus inane in monosex Nile tilapia (Oreochromis niloticus) in aquaculture of Bangladesh. Journal of Fisheries 2: 90-99.
34. Yiagnisis M, Athanassopoulou F (2011) Bacteria Isolated From Diseased Wild and Farmed Marine Fish in Greece. Recent Advances in Fish Farms 153-168.
35. Elham IA (2007) Bacteriological studies on Pseudomonas microorganism in wide and cultured Oreochromis niloticus. J Egypt Vet Med Asso 67: 29-42.
36. Moustafa M, Eissa AE, Laila AM, Gaafar AY, Abumourad IMK, et al. (2014) Mass Mortalities in Mari-Cultured European Sea Bass (Dicentrarchus labrax) at Northern Egypt. Res J Pharm Biol Chem Sci 5: 95-109.
37. Eissa IAM, Derwa HI, El-Lamei M, Desuki A, Zaki MS, et al. (2013) Iron in water and some marine fishes in relation to vibriosis at Lake Temsah. Life Sci J 10: 2520-2528.
38. De Vos D, Lim A, Jr Pirnay JP, Struelens M, Vandenvelde C, et al. (1997) Direct detection and identification of Pseudomonas aeruginosa in clinical samples such as skin biopsy specimens and expectorations by multiplex PCR based on two outer membrane lipoprotein genes, oprl and oprL. J Clin Microbiol 35: 1295–1299.
39. Pirnay JP, De Vos D, Duinslaeger L, Reper P, Vandenvelde C, et al. (2000) Quantitation of P. aeruginosa in wound biopsy samples: from bacterial culture to 'rapid real-time' polymerase chain reaction. Crit Care 4: 255–261
40. Deschaght P, De Baere T, Van Simaey L, Van Daele S, De Baets F, et al. (2009) Comparison of the sensitivity of culture, PCR and quantitative real time PCR for the detection of Pseudomonas aeruginosa in sputum of cystic fibrosis patients. BMC Microbiol 9: 244.
41. Billard-Pomares T, Herwegh,S, Wizla-Derambure N, Turck D, Courcol R, et al. (2011) Application of quantitative PCR to the diagnosis and monitoring of P. aeruginosa colonization in 5–18-year-old cystic fibrosis patients. J Med Microbiol 60: 157–161.
42. Foti M, Giacopello C, Bottari T, Fisichella V, Rinaldo D, et al. (2009) Antibiotic Resistance of Gram Negatives isolates from loggerhead sea turtles (Caretta caretta) in the central Mediterranean Sea. Mar Pollut Bull 58:1363–1366.
43. El-Moghazy DF (2004) Studies on pseudomonas septicemia in cultured Oreochromis niloticus fish. Fish Disease and Management.
44. Abutorkia SM (2009) studies on some common fish diseases under different fish culture systems. Thesis, Fish Disease and Management, Egypt.

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