International Journal of Fisheries and Aquatic Research
ISSN: 2456-7248; Impact Factor: RJIF 5.44
Received: 04-11-2019; Accepted: 06-12-2019
www.fishjournals.com
Volume 5; Issue 1; January 2020; Page No. 24-29

Advanced Studies on Diagnosis of Pseudomonas septicemia in some Marine Fishes

Eissa IAM[1], Takwa Ismail[2], Shimaa Mansour[3]
[1] Department of Fish Diseases and Management, Faculty of Veterinary Medicine, Suez Canal University, Egypt
[2-3] Animal Health Research Institute, Ismailia branch, Egypt

Abstract
A total number of two hundreds (200) naturally infected and freshly caught marine fishes divided as 100 seabass *(Dicentrarchus labrax)* and 100 *Tilapia zillii* were collected randomly and seasonally from Lake Temsah in Ismailia governorate. Fishes subjected to clinical, postmortem and bacteriological studies to isolate and identify the bacteria causing the disease. The most isolated bacteria were *Pseudomonas aeruginosa* which identified by using traditional methods and Vitek 2 system. The identification was confirmed by detection of 16s rDNA gene and two virulence genes (*toxA* and *oprL*) of pathogenic *Pseudomonas aeruginosa* using PCR. The most prevalent and virulent bacteria isolated from naturally infected marine fishes was *Pseudomonas aeruginosa.* The experimentally infected *Tilapia zillii* by intraperitoneal route (I/P) with *Pseudomonas aeruginosa* showed the same signs recorded in the naturally infected marine fishes. The Mortality and morbidity rates were monitored daily for one week.

Keywords: seabass (*Dicentrarchus labrax*), *Tilapia zillii*, PCR, *Pseudomonas aeruginosa*, prevalence

Introduction
Bacterial diseases affecting marine fishes are numerous and cause high economic losses in marine culture sector in Egypt. Bacteria, the major group of pathogens, pose one of the most significant threats to successful fish and shellfish production throughout the world **(Klesius and Pridgeon, 2011) [14].**
Fish diseases due to bacterial infections were the major problems in aquaculture as it found naturally in the fish environment and under certain stress condition caused severe economic losses to fish farms **(Olsson *et al*., 1998) [20].**
Pseudomonades were opportunistic Gram negative pathogens, naturally occurring in aquatic environment and as a part of normal gut flora of healthy fish, it cause outbreak when the normal environmental conditions changed **(Roberts, 1989) [22].** *Pseudomonas fluorescens*, *P. angulliseptica*, *P. aeruginosa* and *P. putida* were identified in various species of fish as causative agents of Pseudomonas septicemia **(El-Nagar, 2010) [10].**
VITEK 2 compact system is an advanced method used for rapid identification of bacteria with avoiding false or weak positive and negative reaction and reducing the human faults so it improved the results for identification of Pseudomonas species **(Barry *et al*.,2003) [5].** Recently, PCR is an advanced method used for identification of bacteria and specific for the detection of virulence factors **(Eissa *et al*., 2015) [7].**
The present study was planned to investigate the clinical signs and the postmortem lesions of the most prevailing bacterial disease affecting marine fishes. Isolation and identification of the causative agents with traditional and advanced methods, experimental infection with the isolated bacteria and recording the total and seasonal prevalence of bacteria causing the disease.

Material and Methods
Naturally infected fish
Two hundreds (200) of naturally infected freshly caught marine fishes divided as 100 seabass *(Dicentrarchus labrax)* and 100 *Tilapia zillii* were collected randomly and seasonally from Lake Temsah in Ismailia governorate. The freshly caught fishes were transported in ice box with ice to the wet laboratory of Department of Fish Diseases and Management, Faculty of Veterinary Medicine, Suez Canal University.

Clinical picture
Clinical signs and postmortem alterations were adopted according to the methods described by **Austin and Austin (2007) [2].**

Bacterial isolation and identification
Under complete aseptic condition, fish specimens (lesions of gills and internal organs as liver, kidneys and spleen) of naturally infected alive or freshly dead fishes were inoculated over nutrient agar media and incubated at 37˚C for 24 hrs. Re-inoculation of cultured bacteria occurred until separated colonies appeared. The suspected purified colonies were picked up for further identification by inoculation of these bacteria on different media as MacConkey agar and Pseudomonas F agar according to **Austin and Austin (2007) [2].** Identification of bacteria was done by using biochemical tests and Vitek 2 system (bioMérieux, France). Confirmatory identification was done by detection of 16s rDNA gene and virulence genes of the isolated bacteria using PCR according to **Abd El Tawab *et al*., (2016) [1].**

Polymerase Chain Reaction
PCR was used to detect the presence of 16S rDNA and Two virulence genes (*toxA* and *oprL*) in the five isolates of the most prevalent and virulent bacteria (*Pseudomonas*

aeruginosa). The primers used (A16S F: 5`-GGGGGATCTTCGGACCTCA-3` and A16S R: 5`-TCCTTAGAGTGCCCACCCG -3` for 16S rDNA gene at 956bp **(Spilker *et al.*, 2004)** [23] and Two virulence genes as A*toxA* F: 5`- GACAACGCCCTCAGCATCACCAGC-3` and A*toxA* R: 5`- CGCTGGCCCATTCGCTCCAGCGCT-3` for exotoxin A gene at 396bp **(Matar *et al.*, 2002**) and A*oprL* F: 5`- ATG GAA ATG CTG AAA TTC GGC -3` and A*oprL* R: 5`- CTT CTT CAG CTC GAC GCG ACG -3` for lipoprotein L gene at 504bp **(Xu *et al.*, 2004).** Extraction of DNA of isolated *Pseudomonas aeruginosa* was performed according to QIAamp DNA mini kit instructions. The PCR Cycling conditions of *Pseudomonas aeruginosa* 16S rDNA was run for 35 cycles as initial denaturation at 94°C for 5 min, followed by secondary denaturation at 94°C for 30 sec., annealing of the primers at 52°C for 45 sec. and primer extension at 72°C for 1 min. with final extension at 72°C for 10 min while the PCR Cycling conditions of *Pseudomonas aeruginosa OprL* and *ToxA* were run for 35 cycles as initial denaturation at 94°C for 5 min, followed by secondary denaturation at 94°C for 30 sec., annealing of the primers at 55°C for 4o sec. and primer extension at 72°C for 1 min. with final extension at 72°C for 10 min. Agarose gel electrophoresis was performed in 1.5% agarose at 100 volt for 30 minutes and the gel was photographed by a gel documentation system and the data was analyzed through computer software.

Experimental infection

A total number of 40 apparently healthy *Tilapia zillii* with an average body weight 35±5 g were acclimated for two weeks before starting the experiment and fed on basal diet (commercial pelleted diet) of 4000 kcal/kg digestible energy and 32% protein twice daily at 3% feeding rate. The fish were divided into two replicate equal groups (1, 2 and 3,4). Each group contained 10 fish and kept in four fully prepared glass aquaria which supplied with marine water and conducted with electric air pump in a water temperature of 25±1°C. The first and second groups were served as control and injected by intra-peritoneal route (I/P) with 0.1 ml saline while the third and fourth groups were experimentally challenged by intra-peritoneal route (I/P) with 0.2 ml of a bacterial suspension of 3×10^7 cfu/ml (LD50) of *Pseudomonas aeruginosa* as adapted by **Ezzat *et al.*, (2018)** [12]**.** They were monitored and observed daily for any clinical signs and mortalities for one week post infection.

Results

Clinical picture

The examined naturally infected marine fishes including seabass (*Dicentrarchus labrax*) and *Tilapia zillii* showed clinically haemorrhages around eyes, at the operculum, at the base of the pectoral and anal fin in seabass (*Dicentrarchus labrax*). In naturally infected *Tilapia zillii*, there were hemorrhages distributed all over the external body surface Postmortem findings, were revealed congestion and haemorrhages in internal organs as liver, spleen, kidneys, gall bladder and intestines **(Plate. 1).**

Plate 1: Showing naturally infected seabass (*Dicentrarchus labrax*) with *Pseudomonas aeruginosa* a) suffered from hemorrhages at the operculum, at the base of the pectoral and anal fin and around eye b) suffered from haemorrhages in internal organs. Showing naturally

infected *Tilapia zillii* c) suffered from hemorrhages all over the body surface and fins d) septicemic lesions in different internal organs. Showing experimentally infected *Tilapia zillii* e) suffered from hemorrhages distributed all over the body surface and fins f) suffered from septicemic lesions in internal organs

Bacterial examination

The most isolated bacteria were identified as *Pseudomonas aeruginosa* in examined naturally infected marine fishes including seabass (*Dicentrarchus labrax*) and *Tilapia zillii* by using traditional method and Vitek 2 system. *Pseudomonas aeruginosa* was manifested as Gram-negative, rod shaped, non-spore forming and motile bacteria. *Pseudomonas aeruginosa* colonies were non-lactose fermenter and pale in color on Mac Conkey agar while it showed smooth, moist, convex surface and blue green colonies on nutrient agar. On Pseudomonas F aga, it was green colonies due to pigment production. Biochemically, The isolated bacteria showed motility and *Pseudomonas aeruginosa* was oxidative organism showed positive reactions for oxidase, catalase and citrate while gave negative reaction for methyl red, vogaus proskauer, indole production, urease and H_2S production on triple sugar iron (TSI).

Result of identification of 16S rDNA and two virulence genes (toxA and oprL) of pathogenic *Pseudomonas aeruginosa* by Polymerase Chain Reaction (PCR)

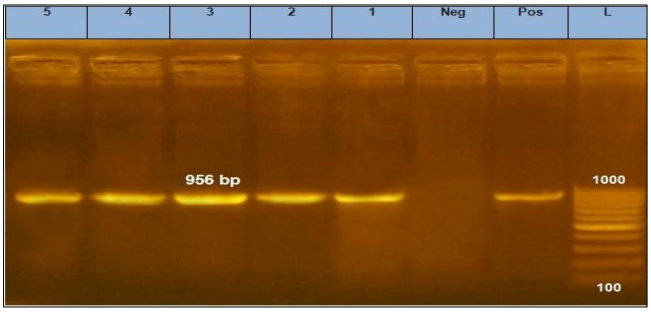

Fig 1: Detection of *Pseudomonas aeruginosa* 16s r DNA (956bp) gene by PCR. Lanes 0: negative control; lanes 1-5: *Pseudomonas aeruginosa* showing bands at 956bp.

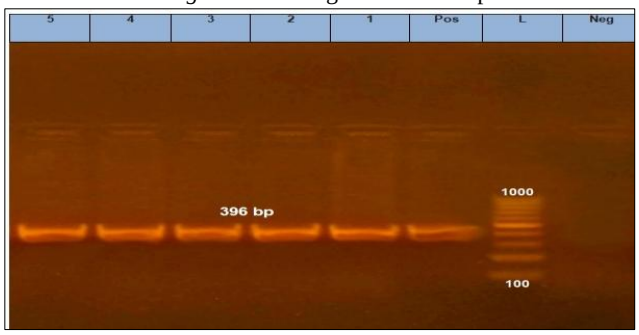

Fig 2: Detection of *Pseudomonas aeruginosa* virulence genes *toxA* (396bp) by PCR. Lanes 0: negative control; lanes 1-5: *Pseudomonas aeruginosa* showing *toxA* bands at 396 bp.

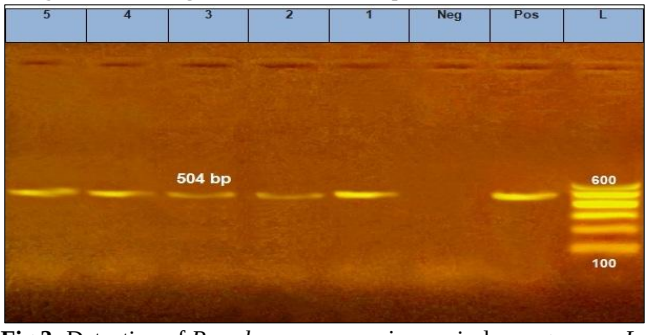

Fig 3: Detection of *Pseudomonas aeruginosa* virulence gene *oprL* (504 bp) by PCR. Lanes 0: negative control; lanes 1-5: *Pseudomonas aeruginosa* showing *oprL* bands at 504 bp.

The five isolates of *Pseudomonas aeruginosa* showed bands with 16s rDNA gene at 956 bp., *toxA* gene at 396 bp. and *oprL* gene at 504.

Prevalence of *Pseudomonas aeruginosa* in naturally Infected marine fishes

The total prevalence of suspected *Pseudomonas aeruginosa* in naturally infected seabass (*Dicentrarchus labrax*) and *Tilapia zillii* in the four seasons was 34 and 45% respectively with highest prevalence in Spring (48, 60%) followed by Summer (40, 48%) then Autumn (32, 40%) while the lowest prevalence was in Winter (16, 32%) respectively **(Fig.4).**

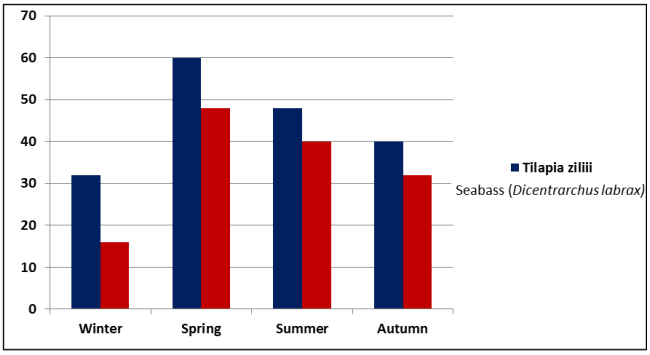

Fig 4: Prevalence of *Pseudomonas aeruginosa* infection in Seabass (*Dicentrarchus labrax*) and *Tilapia zillii* in different seasons.

Prevalence of *Pseudomonas aeruginosa* isolates from different organs and tissues of naturally infected marine fishes

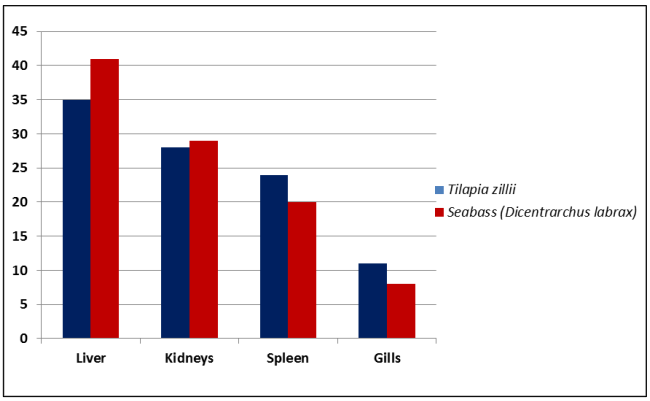

Fig 5: The prevalence of *Pseudomonas aeruginosa* isolates from different organs and tissues of naturally infected seabass (*Dicentrarchus labrax)* and *Tilapia zillii*

Fig. (5) showed that *Pseudomonas aeruginosa was* isolated from organs and tissues of naturally infected seabass (*Dicentrarchus labrax*) and *Tilapia zillii* with high prevalence from liver by (41.18, 35.56 %) followed by kidneys by (29.41, 28.89%) then spleen by (20.59, 24.44%) while the lowest prevalence from gills by (8.82, 11.11 %) respectively.

Experimental infection

The total mortality rates for the first and second groups which served as control and injected with saline were 0 and 10% respectively. While the total mortality rates for the third and fourth groups post infection with *Pseudomonas aeruginosa* were 80 and 70% respectively **(Table.1**).

Table 1: Mortality rates in experimentally infected *Tilapia zillii* with *Pseudomonas aeruginosa*

Group No.	Fish No.	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	total	%
1 (control)	10	0	0	0	0	0	0	0	0	0
2 (control)	10	0	0	1	0	0	0	0	0	10
3 (challenged)	10	0	3	2	1	1	1	0	9	80
4 (challenged)	10	0	3	2	1	1	0	0	7	70

The clinical picture in the experimentally infected *Tilapia zillii* was represented as hemorrhages distributed all over the body surface, lower jaw and base of fins. The internal organs appeared congested and haemorragic (liver, spleen, kidneys, gall bladder) **(Plate,1**).
The reisolation of isolated bacteria was identified as *Pseudomonas aeruginosa*.

Discussion
Fish diseases play a role as a limiting factor in fish production and causing mass mortalities and severe economic losses. The most observed clinical signs of naturally infected marine fish including seabass *(Dicentrarchus labrax)* and *Tilapia zillii* were congestion and haemorrahges distributed all over the body surface, at the operculum, at the base of the pectoral and anal fin as well as redness around eyes. The present results were nearly similar to that recorded by **Manal and Hal (2017) [16]; Aya *et al.* (2018); Ezzat *et al.* (2018) [12]** and **Fadel *et al.* (2018).**Such signs may be due to multiplication of bacteria inside the intestine during stress and changes in environmental conditions causing hemorrhagic mucous disquamative catarrhal exudates. Toxic metabolites of bacteria are absorbed from the intestine and traveled through the blood stream to the internal organs inducing toxemia.

In this study, postmortem examination showed varied lesions among the naturally infected fish of different species including seabass *(Dicentrarchus labrax)* and *Tilapia zillii* as congestion and hemorrhages in internal organs as liver, spleen, kidneys, gall bladder and intestines. These results were in agreement with **Manal and Hal (2017) [16]; Aya *et al.* (2018) [4]** and **Ezzat *et al.* (2018) [12]**.

This study revealed that all the isolates of *Pseudomonas aeruginosa* produced pale colonies on MacConkey agar due to they were non lactose fermenter. Also, on nutrient agar they produced blue green colonies due to production of pyoverdin and pyocynin pigments. Besides, they produced green colonies on Pseudomonas F agar and Pseudomonas agar base. These results were in agreement with **Rasheed *et al*. (2016); Aya *et al.* (2018) [4]** and **Noha *et al*. (2018) [19].**

The present study showed that all the isolates of *Pseudomonas aeruginosa* were Gram negative, rod shaped, motile, positive for oxidase test, catalase test, pyocyanin production, citrate utilization test, gelatin liquefaction test and negative for indole production, hydrogen sulfide (H2S) production, Vogues-Proskauer test, methyl red test, ornithine decarboxylase, lysine decarboxylase and arginine utilization test. These results agreed with **Rasheed *et al*. (2016); Aya *et al.* (2018) [4]** and **Noha *et al*. (2018) [19].**

The results of Vitek 2 compact sytem, in this study, revealed *P. aeruginosa* was positive for H2S production, Gamma-glutamyl transferase, D-mannose, Beta-alanine arylamidasepna, L-prolineary lamidase, Lipase, Tyrosine arylamidase, Urease, D-trehalose, Citrate (sodium), Malonate, L-lactate alkalinisation, Succinate alkalinisation, Alpha-galactosidase, Coumarate, O/1 29 resistance (comp.vibrio.) and L-malate assimilation while negative for Ala-phe-pro-arylamidase, Adonitol, LPyrrolydonyl-arylamidase, L-Arabitol, D-Cellobiose, Beta-galactosidase, Betan-acetyl glucosaminidase, Glutamylary lamidasepna, D-glucose, Fermentation/glucose, Beta-glucosidase, and L-lactate assimilation.These results nearly similar to those of **Austin and Austin (2012) [3]** and **Lopez *et al*. (2012) [15].**

In the present study, Polymerase Chain Reaction (PCR) was used for detection of 16s rDNA gene of *Pseudomonas aeruginosa* giving bands at 956bp. and used for detection of two virulence genes as *toxA (*exotoxin A) and *oprL* (Lipoprotein L) giving bands at 396 bp. and 504 bp. respectively in the selected five isolates of *Pseudomonas aeruginosa*. These results were agreement with **Abd El Tawab *et al*. (2016) [1]** who detected PCR could be used for detection of *Pseudomonas aeruginosa* virulence genes as *opr L, toxA, phz M* and *exo S* giving product at 504 bp., 396 bp, 875 bp and 118 bp respectively and **Eman *et al.* (2016) [11]** who reported polymerase chain reaction (PCR) can be used for detection of virulence genes of *Pseudomonas aeriginosa* giving positive results for outer membrane lipoprotein gene (*oprL*) at "504 bp" and exotoxin A gene (*toxA*) at "270 bp".

In the present study, the total seasonal prevalence of *Pseudomonas aeruginosa* in naturally infected marine fishes included seabass (*Dicentrarchus labrax*) and *Tilapia zillii* was 34 and 45% respectively, higher observation was recorded by **El-Hady and Samy (2011) [9]** who found the total seasonal prevalence of *Pseudomonas aeruginosa* in naturally infected marine fishes was 55.3% and **Ezzat *et al.* (2018) [12]** found that *Pseudomonas aeruginosa* in naturally infected *Tilapia zilli* in the four seasons was 60%. Lower observation was recorded by **Tanekhy (2013) [25]** who found that Pseudomonas species were isolated from naturally infected marine fishes as seabass and seabream with prevalence 18%. Also, **Zorrilla *et al.* (2003)** detected that low infection rates of Pseudomonas among the examined marine fish was 15.27 % and **Eman *et al.* (2016) [11]** reported that prevalence of *P. aeruginosa* isolates among naturally infected seabream fishes was 43.02%. This difference in results was attributed to fish species, nature of examined fish, age of examined fish, a total number of examined fish and time of the study.

The results of this study revealed that highest seasonal prevalence of *Pseudomonas aeruginosa* in naturally infected seabass (*Dicentrarchus labrax*) and *Tilapia zillii* was observed in spring followed by summer then autumn while the lowest prevalence was in winter. This may be due to increase in temperature which often occur in the summer and spring **(Tam *et al.* 2011) [24].** Increased temperature act

as stress factor on fish so decrease its resistance and increase virulence factors produced by bacteria leading to increase spread of disease during summer and spring **(Marcogliese, 2008**). These findings were in agreement with **Ezzat *et al.* (2018)** [12] who found that *Pseudomonas aeruginosa* was isolated only during spring and summer and **Thomas *et al.*(2014)** who isolated *Pseudomonas aeruginosa* with highest seasonal prevalence in spring (12.5%) followed by summer (9.09%) but not reported in winter and autumn. While, disagreed with **Wang *et al*. (2017)** [27] who reported that Pseudomonas were isolated in cold months.

In this study, the *Pseudomonas aeruginosa* was isolated from organs and tissues of naturally infected seabass (*Dicentrarchus labrax*) and *Tilapia zillii* with highest prevalence from liver followed by kidneys then spleen while the lowest prevalence from gills. These result was in agreement with result of **Tanekhy (2013)** [25] who reported that *Pseudomonas aeruginosa* in naturally infected marine fishes as seabass and seabream was isolated with highest prevalence from liver followed by kidneys, heart and spleen. These results might be due to the organ most associated with the detoxification and biotransformation process was the liver and due its function, position and blood supply, it was also one of the organs most affected by contaminants in the water **(Camargo and Martinez, 2007)** [6]**.** While, disagreed with **Ezzat *et al.* (2018)** [12] who reported that *Pseudomonas aeruginosa* in naturally infected marine fishes was isolated equally from liver and kidneys followed by spleen and gills.

In this study, the mortality rates in experimentally infected *Tilapia zillii* with *Pseudomonas aeruginosa* by intra-peritoneal route (I/P) representing 70 -80% of the total fish. These findings were higher than the results recorded by **Ezzat *et al.* (2018)** [12] who detected the mortality rate of experimentally infected *Tilapia zillii* with *Pseudomonas aeruginosa* was 60%.

The clinical signs observed in the experimentally infected *Tilapia zillii* with *Pseudomonas aeruginosa* in this study were hemorrhages distributed all over the body surface, lower jaw, base of fins, and redness around eyes. While the postmortem examination revealed congested to enlarged internal organs as liver, spleen, kidneys and gall blader. These results were similar to the results of **Eissa *et al.* (2017)** [8] and **Ezzat *et al.* (2018)** [12].

References

1. Abd El Tawab, Ahmed AA, Maarouf AA, Ahmed MG "Detection of Virulence factors of Pseudomonas species isolated from fresh water fish by PCR" Benha Veterinary Medical Journal. 2016; 30(1):199-207.
2. Austin B, Austin DA. "Characteristics of the diseases." Bacterial Fish Pathogens: Diseases of Farmed and Wild Fish, 2007, 15-46.
3. Austin B, Austin DA. "Bacterial fish pathogens, Diseases of farmed and wild fish" Fifth Edition, Springer Praxis in Aquaculture in Fisheries, Praxis Publication Ltd., Chichester, UK, 2012; 457.
4. Aya FM, Amel M, El Asely, Shaheen AA, Eman AA. Hiam E, *et al*."Phenotypic and molecular characterization of bacterial pathogens isolated from diseased freshwater fishes." International Journal of Fisheries and Aquatic Studies. 2018; 6(2):34-41.
5. Barry J, Brown A, Ensor V, Lakhani U, Petts D, Warren, C, *et al*. "Comparative evaluation of the VITEK 2 Advanced Expert System (AES) in five UK hospitals" J. of Antimicrobial Chemotherapy. 2003; 51:1191-1202.
6. Camargo MM, Martinez CB. " Histopathology of gills, kidney and liver of a Neotropical fish caged in an urban stream." Neotropical Ichthyology. 2007; 5(3): 327-336.
7. Eissa IAM, Maather El, Mona S, Fatma Y, Mona SZ, Bakry M. "Detection of Hemolysin gene and Antibiogramme of *Aeromonas veronii biovar sobria* Isolated From Mass Mortalities in Cultured Nile Tilapia in El-Sharkia governorate, Egypt" Life Science Journal. 2015; 12(5):1-23.
8. Eissa IAM, Derwa HIM, Mona M. Abdei Wahab, Khadiga KEM. " Role of some biological pollutants in Fish Disease Occurrence at Lake Temsah" M.V.Sc. thesis, Faculty of Veterinary Medicine, Suez Canal Univeristy, 2017.
9. El-Hady MA, Samy AA. "Molecular Typing of Pseudomonas Species isolated from Some Cultured Fishes in Egypt" Global Veterinaria. 2011; 7(6):576-580.
10. El-Nagar RMA. "Bacteriological studies on pseudomonas microorganisms in cultured." M.V.Sc. thesis, Faculty of Veterinary Medicine, Zagazig Univeristy, 2010.
11. Eman K, Khallaf M, Hashe M. "Molecular Study on some Virulence and Fluoroquinolone Resistance Genes of *Pseudomonas aeruginosa* Isolated from Naturally Infected Cultured Sea Bream Fish (*Sparus aurata*) in Egypt "Journal of Infectious Diseases and Preventive Medicine. 2016; 4(136):2.
12. Ezzat EM, Mahmoud E, Ibrahim SI, Mohamed A, Noha, E. " Studies on Bacterial Pathogens in Some Marine Fishes in EL Mansoura, Egypt." American Journal of Agricultural and Biological Sciences. 2018; 13(1):9-15.
13. Fadel A, Mabrok M, Aly S. "Epizootics of *Pseudomonas anguilliseptica* among cultured seabream (*Sparus aurata*) populations: Control and treatment strategies" Microbial Pathogenesis. 2018; 121:1-8.
14. Klesius PH, Pridgeon JW. "Live attenuated bacterial vaccines in aquaculture." In: Proceedings of the 9th International Symposium on Tilapia in Aquaculture. 2011; 8(1):18-26.
15. Lopez JR, Dieguez AL, Doce A, Roca EDl, Herran RDl. Navas JI, *et al*. Pseudomonas baetica sp. nov., a fish pathogen isolated from wedge sole, Dicologlossa cuneata (Moreau). International Journal of Systematic and Evolutionary Microbiology. 2012; 62(4):874-882.
16. Manal I, El-Barbary, Hal AM. Phenotypic and Genotypic Characterization of Some Pseudomonas sp. Associated with Burkholderia cepacia Isolated from Various Infected Fishes. Journal of Aquaculture Research and Development. 2017; 8(2):499.
17. Marcoglies DJ. "The impact of climate change on the parasites and infectious diseases of aquatic animals" Revue Scientifique et Technique-Office International desEpizooties. 2008; 27: 467-484.
18. Matar GM, Ramlawi F, Hijazi N, Khneisser I, Abdelnoor AM. "Transcription Levels of *Pseudomonas aeruginosa* Exotoxin A Gene and Severity of

Symptoms in Patients with Otitis Externa." Current Microbiology. 2002; 45 (5):350-354.
19. Noha M, Sabry Sahar TM, Abdel-Gawad F. "Monitoring some pathogenic bacteria in water and fish of Lake Qaroun" Egyptian Journal of Aquatic Biology and Fisheries. 2018; 22(5):73-85.
20. Olsson JC, Joborn A, Westerdahl A, Blomberg L Kjelleberg S, Conway PL, *et al*. "Survival, persistence and proliferation of *Vibrio anguillarum* in Juvenile turbot, Scophthalmus maximus (L.), intestine and faeces." Journal of Fish Diseases. 1998; 21(1):1-9.
21. Rasheed MN, Noor SL, Ashwak BJ, Nader MI. "Rapid Identification of *Pseudomonas aeruginosa* by Using Real Time PCR" Kufa Journal for Veterinary Medical Sciences. 2016; 7(1):80-86.
22. Roberts RJ. "Fish Pathology, Bailliere Tindall, London, Second edition. 1989; 263-274.
23. Spilker T, Coenye T, Vandamme P, LiPuma JJ. "PCR-based assay for differentiation of *Pseudomonas aeruginosa* from other Pseudomonas species recovered from cystic fibrosis patients." Journal of Clinical Micro biology. 2004; 42(5):2074-2079.
24. Tam B, Gough WA, Tsuji L. "The impact of warming on the appearance of furunculosis in fish of the James Bay region, Quebec, Canada" Regional Environmental Change. 2011; 11:123-132.
25. Tanekhy M. "Some Study on Bacterial Infection in some Cultured Marine Fish." Journal of the Arabian Aquaculture Society. 2013; 8(1):163-178.
26. Thomas J, Thanigaivel S, Vijaya kumar S, Acharya K, Shinge D. " Pathogenecity of *Pseudomonas aeruginosa* in Oreochromis mossambicus and treatment using lime oil nanoemulsion." Colloids Surf. B Bio inter faces. 2014; 116:372-377.
27. Wang YH, Salam N, Liu Q, Yang ZY, Cao LX. " Sym biotic bacteria associated with puffer fish *Gastrophysus spadiceus* and evaluation of their antimicrobial activities." 3 Bio tech. 2017; 7(6):366-366.
28. Xu J, Moore JE, Murphy PG, Millar BC, Elborn JS. "Early detection of *Pseudomonas aeruginosa* comparison of conventional versus molecular (PCR) detection directly from adult patients with cystic fibrosis (CF)." Annals of Clinical Microbiology and Antimicrobials. 2004; 3(1):21.
29. Zorrilla M, Chabrillon AS, Rosales PD, Manzanares E. M, Balebona MC, Morinigo MA, *et al*. "Bacteria recovered from diseased cultured gilthead sea bream Sparus aurata L. in south western Spain." Aquaculture. 2003; 218(1-4):11-20.