Microbial Causes of Summer Mortality in Farmed Fish in Egypt

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Abstract
Bacterial diseases are responsible for sever mortalities and morbidities in cultured Nile tilapia in Egypt in various fresh water fish farms and the increase of water temperature in summer has a significant effect on mortalities. A total number of 100 clinically diseased Nile Tilapia (*Orechromis niloticus)* were collected alive randomly from Abbassa, Elsharkia (Central Laboratory for Aquaculture Research) Egypt, to identify the bacterial pathogens affect that fish. Samples were isolated from liver, kidney, spleen, eye, ulcer and brain. The isolated fish pathogens were identified as *Aeromonas hydrophila, Ps.fluorescense, Ps.putida* and *V.cholera* by their morphological characteristics, biochemical tests, Antibiotic sensitivity test and polymerase chain reaction test as a confirmatory results. The results of Anmicrobial sensitivity of the isolated strains revealed that tetracycline (TE30), oxytetracycline (T30), nalidixic acid (NA30), norfloxacin (NOR10) and sulpha-Trimethoprim (SXT25) were the drugs of choice against ***Aeromonas hydrophila***, tetracycline (TE30),oxytetracycline (T30), tobramicin (TOB10) and kanamycin (K30) were the drugs of choice against ***Pseudomonas flurocence***, streptomycin (S10), tobramicin (TOB10), and kanamycin (K30) were the drugs of choice against ***Pseudomonas putida*** While oxytetracycline (T30) and nalidixic acid (NA30) were the drugs of choice against ***Vibrio cholera.*** The results of Polymerase Chain Reaction (PCR) confirmed the antibiotic sensitivity test results and also confirmed the incidence of *Aeromonas hydrophila, Pseudomonas flurocence* and *Vibrio cholera* in the infected fish.

Introduction

Fish represents a vital source of food for people also fish has economic importance. It is necessary for the financial and nutritional health of human ***(Tidwell and Allan, 2001).***

Egypt is the biggest aquaculture reproducer in Africa and eighth largest internationally in 2011, Fish

utilization in Egypt ascended from 8.5kg to 15.4kg/individual/year among 1996 and 2008.Nile Tilapia (*Oreochromis niloticus*) is the most cultured fish in Egypt. Unfortunately, intensive fish farming is associated with a number of challenges including diseases, which demotivate farmers due to the economical losses ***(ELTholth et al., 2015).***

Cultured fish diseases have a negative effect on the achievement maximum capacity in fish farming. Bacterial pathogens are the most critical problem in Tilapia production responsible for **80%** of fish mortalities**.** ***(EL-Refaee, 2004).*** Even though Tilapia is more resistant to a lot of pathogenic microorganisms, outbreaks of Aeromonas infection had been reported in cultured tilapia in various aquaculture farms ***Yambot (1998).***

Bacterial diseases are responsible for sever mortalities and morbidities in 2001 in cultured Nile tilapia in Egypt in various fresh water fish farms. Laboratory studies appeared the existence of *A.hydrophila* in 70% of examined fish ***(Aly, 2013).***

The proper management of fish health begins with prevention of disease better than treatment ***(Faruk et al. ,2004).***

Material and Method:

Fish:

A total number of 100 clinically infected Nile Tilapia were collected from Abbassa, Elsharkia (Central Laboratory for Aquaculture Research) Egypt for bacteriological examination. Each individual sample was placed separately into sealed sterile plastic bag, thoroughly identified and delivered to the laboratory in icebox.

Bacteriological examination:

A total number of 300 samples were collected from (fins, tails, skin ulcers, liver, kidney, spleen, brain and eyes) under complete aseptic conditions. The samples were inoculated into Tryptic soya broth and incubated at 29-30°c for 18-24 hours as described by ***APHA (1992)***, then subcultured on the selective media (Thiosulphate citrate bile salt sucrose agar, Aeromonas selective agar base and pseudomonas selective agar base) and incubated at 29-30°c for 24-48 hours. The suspected pure colonies were picked up and streaked onto the same specific media for further purification and isolated pure colonies were transferred into nutrient agar slant for further identification. The bacterial isolates were identified according to ***Macfidden (1976)*** *and* ***Lopez Romalde et al. (2003)***.

Antimicrobial sensitivity test:

The antimicrobial sensitivity test of the common isolated bacterial pathogens (*A.hydrophila, P.fluorescens, P.putida and V.alginolyticus*) were performed by disc diffusion test according to ***Bauer et al. (1966)*** and interpreted according to ***NCCLS/CLSI (2007)***.The antibiotic discs were

Streptomycin, tetracyline, oxytetracycline, tobramicin, nalidixic acid, rifampicine, kanamycine, gentamycin, novobiocin, neomycin, norfloxacin and sulpha-Trimethoprim**.**

Pathogenicity test:

Total number of 50 Nile Tilapia fish were collected alive an apparently healthy from private hatcheries in El Abbassa, Sharquia Governorate with an average body weight (25±5 g.). They were tested for susceptibility to experimental infection with *A.hydrophila, Ps.fluorescens, Ps.putida* and *V.cholera* isolated from naturally infected Nile Tilapia. All experimental fish were fed with commercial ration at rate of 5% body weight per day. Fish were divided into 5 groups (G1,G2,G3,G4 and G5 as control) 10 fish per group. (and the inocula of bacterial strains were prepared for I/P injection according to ***Austin and Austin (1999).***

The injected dose of *A.hydrophila* and *Ps.fluorescens* was (0.2 ml of 3x 10^7Cfu) while the dose in *V.cholera* (0.2ml of 2.5×10^8Cfu) according to ***(Austin and Austin 2007)*** and the control group injected with 0.2 ml of sterile saline.

All experimental injected fish were observed daily for 10 days to record any clinical or abnormal signs and the daily mortalities. Also postmortem examination was performed on dead fish to record gross lesions and re-isolation of injected pathogen.

Polymerase Chain reaction:

Polymerase Chain reaction were performed for accurate identification of *Aeromonas hydrophila*, Pseudomonas spp. and *Vibrio cholera* and for detection of resistant genes and the prevalence of virulence genes of *A. hydrophila* the oligonucleotide primers sequences of different genes **Table (1).** DNA extraction had been done by following manufacturer's instructions of QIAamp DNA mini kit. Temperature and time conditions of the primers during PCR are shown in **Table (2)** according to ***Machado et al. (2013)***. PCR products were electrophorized using 1% agarose gel using Gel casting apparatus (Biometra). The gel was photographed by a gel documentation system and the data analyzed through computer software according to ***Sambrook et al. (1989).***

Table (1): *Oligonucleotide primers sequences.*

genes	Gene	Sequence 5`-3`	Amplified product (bp)	Reference
P. fluorescens	16SrDNA	F: TGCATTCAAAACTGACTG	850	**Machado *et al.* 2013**
		R: AATCACACCGTGGTAACCG		
Pseudomonas species	16SrDNA	F: GACGGGTGAGTAATGCCTA	618	**Spilker *et al.*, 2004**
		R: CACTGGTGTTCCTTCCTATA		
A. hydrophila	16Sr RNA	F: GAAAGGTTGATGCCTAATACGTA	625	**Gordon *et al.*, 2007**
		R: CGTGCTGGCAACAAAGGACAG		
V. cholera	*omp*W	F: CACCAAGAAGGTGACTTTATTGTG	304	**De Menezes *et al.*, 2014**
		R: GGTTTGTCGAATTAGCTTCACC		
Pseudomonas species	*kan*	F: GTGTTTATGGCTCTCTTGGTC	621	**Frana *et al.*, 2001**
		R: CCGTGTCGTTCTGTCCACTCC		
All	*tet*A*(A)*	F: GGTTCACTCGAACGACGTCA	576	**Randall *et al.* 2004**
		R: CTGTCCGACAAGTTGCATGA		
V. cholera, P. fluorescens	*aad1*	F: TATCAGAGGTAGTTGGCGTCAT	484	
		R: GTTCCATAGCGTTAAGGTTTCATT		
A.hydrophila	*aad*B	F: GAGCGAAATCTGCCGCTCTGG	319	**Frana *et al.*, 2001**
		R: CTGTTACAACGGACTGGCCGC		
	Haemolysin (*hly*)	F: CTATGAAAAAACTAAAAATAACTG	1500	**Yousr *et al.*, 2007**
		R: CAGTATAAGTGGGGAAATGGAAAG		
	Aerolysin (Aero)	F: CACAGCCAATATGTCGGTGAAG	326	**Singh *et al.*, 2008**
		R: GTCACCTTCTCGCTCAGGC		

Table (2): *Cycling conditions of the different primers during cPCR*

genes	Gene	Primary denaturation	Amplification				Final extension
			Secondary denaturation	Annealing	Extension	No. of cycles	
P. fluorescens	*16S rDNA*	94˚C 5 min.	94˚C 30 sec.	48˚C 40 sec.	72˚C 1 min.	35	72˚C 10 min.
Pseudomonas species	*16SrDNA*	94˚C 5 min.	94˚C 30 sec.	57˚C 1 min.	72˚C 1 min.	35	72˚C 10 min.
A. hydrophila	*16S rRNA*	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 45sec.	35	72˚C 10 min.
V. cholera	*ompW*	94˚C 5 min.	94˚C 30 sec.	59˚C 30 sec.	72˚C 30 sec.	35	72˚C 7 min.
Pseudomonas species	*kan*	94˚C 5 min.	94˚C 30 sec.	54˚C 40 sec.	72˚C 45sec.	35	72˚C 10 min.
All	*TetA(A)*	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.
V. cholera, P. fluorescens	*Aada1*	94˚C 5 min.	94˚C 30 sec.	54˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.
A. hydrophila	*AadaB*	94˚C 5 min.	94˚C 30 sec.	58˚C 40 sec.	72˚C 40 sec.	35	72˚C 10 min.
	Haemolysin (*hly*)	94˚C 5 min.	94˚C 30 sec.	55˚C 1 min.	72˚C 1.5 min.	35	72˚C 12 min.
	Aerolysin (Aero)	94˚C 5 min.	94˚C 30 sec.	52˚C 40 sec.	72˚C 40 sec.	35	72˚C 10 min.

Results and Discussion:

A total number of 300 samples were collected from 100 clinically infected fishes. The bacteriological examination of samples that were collected during summer (2017) give rise to 160 bacterial isolates that were differentiated into *A.hydrophila, P.flurocence, P.putida* and *V.cholera* with the percentage of (50, 21.87, 15.62 and 12.5) respectively (**table 3**), while in winter (2018) *A.hydrophila, P.flurocence and V.cholera* were recovered from 100 samples with percentage of (50, 37, 13%) respectively **(table 4).** A total number of 40 samples were collected in summer (2018) where *A.hydrophila* isolated with percentage of 47.22%, *P.flurocence* 22.22%, *P.putida* (13.88%) and *V.cholera* (16.66%) **(table 5)**.

The results of seasonal prevalence of bacterial strains indicated that in summer the mortalities on naturally infected farmed fish was higher than that in winter.

The present study showed that Aeromonas spp. has been isolated from ulcerated tilapia fish in different seasons by 70% in summer and 50% in winter, this result is similar to ***McGarey et al. (1991)*** *and* ***Maimona and Sabiel*** **(2015).** On contrary, concerning *P. fluorescence*, in previous study in Egypt it was isolated from *O.niloticus* with skin ulcers in summer by 40% and in winter 37%. While ***Maimona and Sabiel (2015)*** in Sudan, didn't find Pseudomonas spp. in similar cases. This difference may be attributed to difference in seasonal or temperature variations during which the samples were collected, disagreement on these results can be explained as *Pseudomonads* prefer winter period as recorded by ***Castro-Escarpulli et al. (2003).***

The results of pathogenisty test revealed that the mortality rate of the experimental injected fish by dose of ***Aeromonas hydrophila*** (0.2 ml of 3x 10^7Cfu) was 90%, the injected fish by dose of ***Pseudomonas flurosence*** (0.2 ml of 3x 10^7Cfu) was 80%, ***Pseudomonas putida*** injected to healthy fish by dose (0.2 ml of 3x 10^7Cfu) was 70% while that injected by dose of ***Vibrio cholera*** (0.2 ml of 3x 10^7Cfu) was 30%.as shown in **table (6)** *Aeromonashydrophila* causes the highest mortality rate.

The results of Antibiotic sensitivity of the isolated strains revealed that tetracycline (TE30), oxytetracycline (T30), nalidixic acid (NA30), norfloxacin (NOR10) and sulpha-Trimethoprim (SXT25) were the drugs of choice against ***Aeromonas hydrophila***, tetracycline (TE30), oxytetracycline (T30), tobramicin (TOB10) and kanamycin (K30) were the drug of choice against ***Pseudomonas flurocence***, Streptomycin (S10), tobramicin (TOB10), and kanamycin (K30) were the drugs of choice against ***Pseudomonas putida*** While

oxytetracycline (T30) and nalidixic acid (NA30) were the drugs of choice against ***Vibrio cholera*.**

These results agreed with ***Kaznowski (1998), Salama (1999), Abou El-Atta and El-Tantawy (2008)*** but the results were partially agreed with **Sarma *et al.*, (1990)** who described that ampicillin, oxytetracycline, streptomycin and nalidixic acid were the most effective against *Aeromonas hydrophila*, ***Megahed (2002)*** who recorded chloramphenicol and Nalidixic acid were the most effective drugs against *Aeromonas hydrophila* and The results were disagreed with ***Hettiarachchi and Cheong (1994)*** who reported that all *Aeromonas hydrophila* were resistant to Tetracycline, Penicillin and Streptomycin.

The present results supported by gentamicin was effective drug against *Pseudomonas spp*. ***Khalil et al. (2010)***, ciprofloxacin was more effective antibiotic against *Pseudomonas spp*. than other antibiotics ***Mesaros et al. (2007)***. In a study by ***Enany et al. (2011)*** concluded that *P. fluorescens* was sensitive to Ciprofloxacin and rifampicine while resist amoxicillin and erythromycin.

In a recent study on *Pseudomonas spp*. by ***Younes et al. (2015)***, the most of isolates were sensitive to Choloramphenicol, Kanamycin and Gentamicin while resistant to Amoxicillin and Ampicillin. *P. putida* was the only *Pseudomonas spp*. that was sensitive to Erythromycin and this support the present result. In addition, this result is nearly in consonant with ***Eissa et al., (2010)*** who concluded that *P. putida* and *P. anguilliseptica* were sensitive to Erythromycin.

The results of Polymerase Chain Reaction (PCR) confirmed the incidence of *A.hydrophila*, *P.flurocence*, *P.putida* and *V.cholera* (nonpathogenic to fish) in the infected fish and also confirmed the results of antimicrobial sensitivity test where the examined isolates of these microorganisms were carrier to the resistant and sensitive gene that observed in the sensitivity test. (Fig. 1, 2, 3, 4, 5 and 6)

The most prevalent organism that cause summer mortalities was *A. hydrophila* so the detection of virulence genes was very important for confirmation of the results. The result of theprevalence of virulence gene (*aerolysin* and *haemolysin*) is shown in **Fig. (3).**

Table (3): *The isolated bacterial strains from the collected fish in summer (2017):*

Season	Total fish	Total isolates	Isolated strains	Total isolates	Percentage %
Summer (2017)	**35**	**160**	*Aeromonas hydrophila*	**80**	**50**
			Pseudomonas fluorensce	**35**	**21.87**
			Pseudomonas putida	**25**	**15.62**
			Vibrio cholera	**20**	**12.5**

Table (4): *The isolated bacterial strains from the collected fish in Winter (2018):*

Season	Total fish	Total isolates	Isolated strains	Total isolates	Percentage %
Winter 2018	**25**	**100**	*Aeromonashydrophila*	**50**	**50**
			Pseudomonas fluorensce	**37**	**37**
			Vibrio cholera	**13**	**13**

Table (5): *The isolated bacterial strains from the collected fish in Summer (2018):*

Season	Total fish	Total isolates	Isolated strains	Total isolates	Percentage %
Summer (2018)	**40**	**180**	*Aeromonashydrophila*	**85**	**47.22**
			Pseudomonas fluorensce	**40**	**22.22**
			Pseudomonas putida	**25**	**13.88**
			Vibrio cholera	**30**	**16.66**

Table (6) *Mortality rate of experimentally infected (Oreochromisniloticus) with isolated strains:*

Groups	No. Fish	Dose of isolated strains	Rout of injection	Dead fish post injection							No. of dead fish	Mortality rate
				1	2	3	4	5	6	7		
1	10	*A.hydrophila* (0.2 ml of 3x 10^7Cfu)	I/P	3	2	3	1	0	0	0	9	90%
2	10	*P.fluroscense* (0.2 ml of 3x 10^7Cfu)	I/P	2	2	3	1	0	0	0	8	80%
3	10	*P.putida* (0.2 ml of 3x 10^7Cfu)	I/P	0	2	2	3	0	0	0	7	70%
4	10	*V.cholera* (0.2ml of 2.5×10^8Cfu)	I/P	0	0	0	1	1	1	0	3	30%
5	10	0.2 ml sterile solution	I/P	_	_	_	_	_	_	_	0	0%

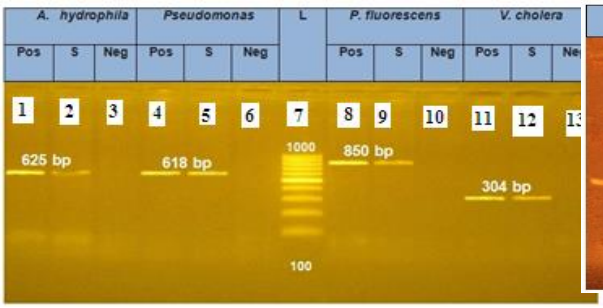

Figure (1) Gel electrophoresis of PCR amplification of the identification of Pseudomonas spp by 16SrDNA gene, *Aeromonas hydrophila* by 16SrRNA gene and *Vibrio cholera* by *omp*W gene showing positive amplification in *A.hydrophila* at 625 bp products at lane 2, positive control (lane 1) and negative control (lane 3), *Pseudomonas putida* at 618 bp products at lane 5, positive control (lane 4) and negative control (lane 6), *P.fluorescens* at 850 bp at lane 9, positive control (lane 8) and negative control (lane 10) *and V.cholera* at 304 bp at lane 12, positive control (lane11) negative control (lane 13), respectively. Lane 7 (L) is 100 bp DNA marker.

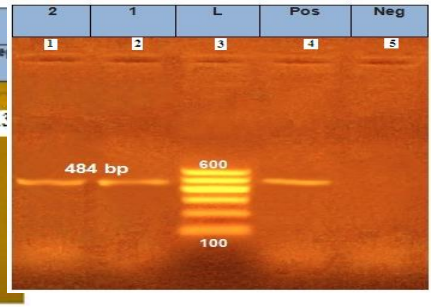

Figure (2).Gel electrophoresis of PCR amplification of the resistance gene (aada1) in *V. cholera* and *P. fluorescens*, showing positive amplification at 484 bp products at lanes 1-2, positive control (lane 4) and negative control (lane 5), respectively. Lane 3 (L) is 100 bp DNA marker.

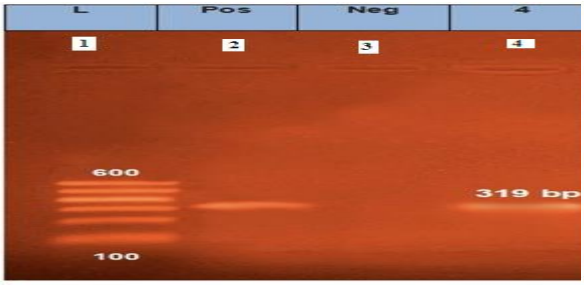

Figure (3). Gel electrophoresis of PCR amplification of the resistance gene (*aadB*) in *Aeromonas hydrophila*, showing positive amplification at 319bp products at lane 4, positive control (lane 2) and negative control (lane 3), respectably. Lane L is 100 bp DNA marker.

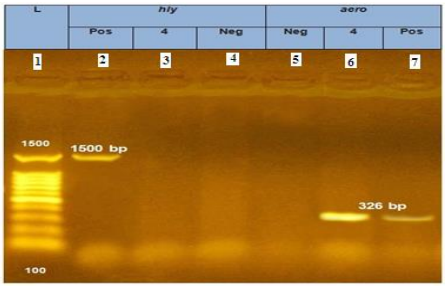

Figure (4). Gel electrophoresis of PCR amplification of the virulence gene (Haemolysin (*hly*)) in *Aeromonas hydrophila*, showing no amplification at lane 3, positive control (lane 2) and negative control (lane 4), respectively. Lane 1(L) is 100 bp DNA marker. Lane 6 showing positive amplification of 326 bp products of *Aerolysin (Aero)* gene. And positive control (lane 7) and negative control (lane 5).

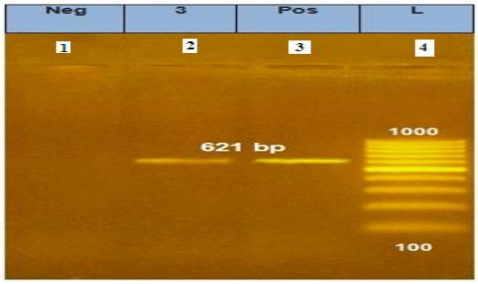

Figure (5). Gel electrophoresis of PCR amplification of the resistance gene (Kan) in *Pseudomonas species*, showing positive amplification at 621 bp products at lane 2, positive control (lane 3) and negative control (lane 1), respectively. Lane 4 (L) is 100 bp DNA marker.

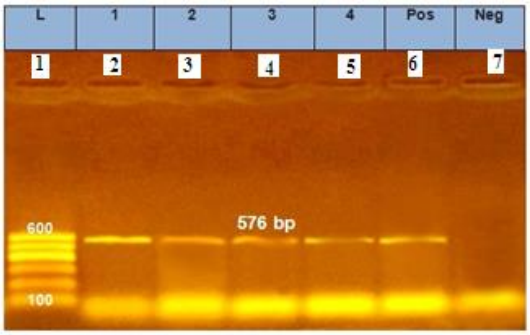

Figure (6). Gel electrophoresis of PCR amplification of the resistance gene (*tetA(A*) in all species (*Aeromomas hydrophila, Pseudomonas fluroscence, Pseudomonas putida and Vibrio cholera*), showing positive amplification at 576 bp products at lane 1, 2, 3, 4 and 5, positive control (lane 6) and negative control (lane 7), respectively. Lane 1 (L) is 100 bp DNA marker.

Refrences:

Aly, S. M. (2013): A review of fish diseases in the Egyptian aquaculture sector: Working report.

Austin, B. and Austin, D.A (1999): Third edit. chapter 2 ; characteristics of the diseases . In the bacterial pathogens. Diseases of farmed and wild fish . Springer-Praxis, Praxis Publishing,Ltd. Chichester, Cuk.PP 13-15.

Austin, B., & Austin, D. A. (2007): Characteristics of the diseases. Bacterial Fish Pathogens: Diseases of Farmed and Wild Fish, 15-46.

APHA (1992): *Compendium of methods for the microbiological examination of food.* In C., Vanderzant, & D. F. Splittstoesser. (Eds.). Washington, DC: American Public Health Association.

Bauer, A. W., Kirby, W. M., Sherris, J. C., &Turck, M. (1966): Antibiotic Susceptibility Testing by a Standardized Single Disk Method. American Journal of Clinical Pathology, 45(4_ts), 493-496. doi:10.1093/ajcp/45.4_ts.493.

Castro-Escarpulli, G., Figueras, M. J., Aguilera-Arreola, G., Soler, L., Fernandez-Rendon, E., Aparicio, G. O., ...& Chacon, M. R. (2003): Characterisation of Aeromonas spp. isolated from frozen fish intended for human consumption in Mexico. *International Journal of Food Microbiology*, *84*(1), 41-49.

De Menezes,F.G.R.; Neves,S.D.; De Sousa, O.V.; Vila-Nova(2), Rodrigo Maggioni, C.MV.M.; Theophilo, G.N.D.; Hofer, E. and Vieira, R.H.S.D. (2014): Detection Of Virulence Genes In Environmental Strains Of Vibrio

cholera From Estuaries In Northeastern Brazil. *Rev. Inst. Med. Trop. Sao Paulo, 56(5):427-432.*

Eissa, N. M. E., El-Ghiet, E. A., Shaheen, A. A., &Abbass, A. (2010): Characterization of Pseudomonas species isolated from tilapia "Oreochromisniloticus" in Qaroun and Wadi-El-Rayan lakes, Egypt. *Global Veterinaria, 5*(2), 116-121.

EL-Banna N.I. (2014): Prevalence of common bacterial pathogens in marine fishes. M.V.Sc. Theses in Microbiology. Department of Bacteriology-Immunology-Mycology. Fac.Vet.Med. Suez Canal Uni.

El-Refaee, A.M.E. (2004) Streptococcus infection in freshwater fish. Master's Thesis, Faculty of Veterinary Medicine, Alexandria University, Egypt.

Eltholth, M., Fornace, K., Grace, D., Rushton, J., &Häsler, B. (2015): Characterisation of production, marketing and consumption patterns of farmed tilapia in the Nile Delta of Egypt. *Food Policy, 51*, 131-143. doi: 10.1016/j.foodpol.2015.01.002.

Enany, M. E., Ibrahim, H. A. Abou EL Att. M. E. and El Adawy, M. M. (2011): Bacteriological and histopathological studies on some bacterial pathogens causing diseases in cultured Mugilcapito fish in Ismailia governorate. SCV MJ X VI (1) Pp 1-10.

Faruk, M. A. R., Alam, M. J., Sarker, M. M. R., &Kabir, M. B. (2004): Status of fish disease and health management practices in rural freshwater aquaculture of Bangladesh. *Pakistan J. Biol. Sci*, *7*(12), 2092-2098.

Finegold, S. M., & Martin, S. (1982). Diagnostic Microbiology 6th ed the CV Mosby Company, St. Louis Tranto, London. Wiener TierarstilichMschr, 6, 233-236.

Frana, T. S., Carlson, S. A. & Griffith, R. W. (2001). Relative distribution and conservation of genes encoding aminoglycoside-modifying enzymes in Salmonella enterica serotype Typhimurium phage type DT104. Applied and Environmental Microbiology 67, 445–8.

Gordon, L.; Giraud, E.; Ganiẽre, G.P.; Armand, F.; Bouju-Albert, A.; de la Cotte, N.; Mangion, C. and Le Bri, H. (2007): Antimicrobial resistance survey in a river receiving effluents from freshwater fish farms. Journal of Applied Microbiology; 102, 1167–1176.

Khalil, S. A.; Khalil, R. H.; Saad, T.T. and Safaa, M. H. (2010): Studies on *Pseudomonas* Septicemia among Cultured *Oreochromisniloticus*, Journal of the Arabian aquaculture society, vol. 5 no 1.

Lo´pez-Romalde , S.; Magarin˜ os, B.; Nu´n˜ez, S.; Toranzo, A.E. and Romalde, J.L. (2003): Phenotypic and genetic characterization of *Pseudomonas anguilliseptica* strains isolated from fish. J AquatAnim Health.2003a; 15:39–47.

MacFaddin, J.F. (1976): Biochemical tests for identification of medical

bacteria the Williams and Wilkins Company, Baltimore, USA.

Machado, S.G.; Bazzolli, D.M.S. and Vanetti, M.C.D. (2013): Development of a PCR method for detecting proteolytic psychrotrophic bacteria in raw milk. International Dairy Journal 29 (2013) 8e14.

Maimona, S. and Y.A. Sabiel (2015): Detection of the causative agents of bacterial fish septicemia of tilapia and clarais in Khartoum state. Int. J. Recent Scient. Res., 6: 4374-4377.

McGarey, D.J., L. Milanesi, D.P. Foley, B. Reyes, Jr., L.C. Frye and D.V. Lim (1991): The role of motile Aeromonads in the fish disease, Ulcerative Disease Syndrome (UDS). Experientia, 47:441-444.

Mesaros, N.; Nordmann, P.; Roussel-Delvallez, M.; Van Eldere, J.; Glupczynski, Y.; Van Laethem, R. (2007): *Pseudomonas aeruginosa* resistance and therapeutic options at the turn of the new millennium. International Journal of Pharmacy and PharmaceuticalSciences.

NCCLS/CLSI (National Committee for Clinical Laboratory Standards/Clinical and Laboratory Standards Institute), (2007): CLSI document GP 18- Laboratory design; approved guideline.2nd ed. Clinical and Laboratory Standards Institute, Wayne (PA); 2007.

Randall, L.P.; Cooles, S.W.; Osborn, M.K.; Piddock, L.J.V. and Woodward, M.J. (2004). Antibiotic resistance genes, integrons and multiple antibiotic resistance in thirty-five serotypes of Salmonella entericaisolated from humans and animals in the UK. Journal of Antimicrobial Chemotherapy.53, 208–216.

Sambrook, J.; Fritscgh, E.F.; andMentiates (1989). Molecular coloning. A laboratory manual.Vol !., Cold spring Harbor Laboratotry press, New York.

Singh, V., Rathore, G., Kapoor, D., Mishra, B.N. and Lakra, W.S. (2008): Detection of aerolysin gene in Aeromonashydrophila isolated from fish and pond water. Indian J. Microbiol. (December 2008) 48:453–458.

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. Journal of clinical microbiology, *42*(5),2074-2079.

Tidwell, J. H., & Allan, G. L. (2001): Fish as food: aquaculture's contribution: Ecological and economic impacts and contributions of fish farming and capture fisheries. *EMBO reports*, *2*(11), 958-963.

Yambot, A. V. (1998): Isolation of Aeromonashydrophila from Oreochromisniloticus during fish disease outbreaks in the Philippines. *Asian fisheries science*, *10*, 347-354.

Younes, A. M., Fares, M. O., Gaafar, A. Y., & Mohamed, L. A. (2016): Isolation of Vibrio alginolyticus and Vibrio vulnificus Strains from Cultured

Oreochromisniloticus Around Qarun Lake, Egypt. *Global Veterinaria*, *16*(1), 01-05.

Yousr, A.H., Napis, S., Rusul, G.R.A. and Son, R. (2007): Detection of Aerolysin and Hemolysin Genes in Aeromonasspp.Isolated from Environmental and Shellfish Sources by Polymerase Chain Reaction. ASEAN Food Journal 14 (2): 115-122 (2007).