Prevalence and Molecular Characterization of *Pseudomonas* Species Isolated From Fish Markets in Port-Said

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Abstract

Total number of 200 apparently healthy fish, *Mugil cephalus* and *Oreochromis niloticus*100 of each fish were collected randomly from different markets in Port-Said city to isolate and identify *Pseudomonas spp*. from it and the antibiotic sensitivity pattern of isolated strains. *Pseudomonas* was found in (66%) of *Mugil cephalus* and (80%) of *Oreochromis niloticus*. Four hundred and thirty six isolates identified as *Pseudomonas spp*. and classified into *P. fluorescens* (29.13%), *P. aeruginosa* (27.06%), *P. putida* (11.01%), *P.cepacia* (10.55%), *P. stutzeri* (8.49%), *P. anguilliseptica* (5.73%), *P. alcaligenes* (4.13%) and *P. acidovorans* (3.90%). The identification of *Pseudomonas* isolates were confirmed by Polymerase Chain Reaction which revealed that tested isolates were *Pseudomonas* and produced specific band at 618 bp, *P. fluorescens* produced specific band at 850 bp and *P. aeruginosa* produced electrophoresis at 956 bp. Antibiogram results showed that all *Pseudomonas* isolates were highly sensitive to Ciprofloxacin, Gentamicin and Chloramphenicol while were highly resistant to Ampicillin/sulbactam, Penicillin and Amoxicillin.

Introduction

Pseudomonas considered as one of the most common bacterial pathogens in fish and classified as a stress related disease that can only cause disease when fish subjected to improper management and environmental stressors as poor water quality, sudden change in water temperature, overcrowding or other stressors, ***Nagasawa and Cruz-Lacierda (2004)***.

The genus *Pseudomonas* is ubiquitous Gram-negative, rod shape bacterium belonged to the family Pseudomonadanceae which capable of surviving in variety of environments including aquaculture environment ***Roberts (1989)***. *Pseudomonas spp.* considered as the most common and dominant bacterial pathogen associated with various fresh water fish ***Darak and Barde (2015)***.*Pseudomonas* septicemia is very difficult in

treatment due to wide varsities in *Pseudomonas* strains and their resistance toward different antibiotics ***Abdullahi et al. (2013)***.
This study aimed to investigate the propagation of *Pseudomonas spp*. in *Mugil cephalus* and *Oreochromis niloticus*, discuss the typing of the most important *Pseudomonas* strains by plasmid profile analysis and summarize the resistance pattern of isolated *Pseudomonas spp*. against various antibiotics.

Material and Method:

Fishes:

A total of 200 fish of *Mugil cephalus* and *Oreochromis niloticus* 100 of each, 150-250g in weight and collected randomly from different markets in Port-Said city. Each individual sample was placed separately into sealed sterile plastic bag, thoroughly identified and delivered to the laboratory in icebox.

Bacteriological examination:

One gram from surface, muscle, intestine, liver and kidneys were taken aseptically and enriched on Trypticase soya broth (Oxoid) at room temperature for 24 hours. Two loop-full from Trypticase soya broth of each sample were streaked over the surfaces of two different plates; a plate of *Pseudomonas* base medium with CN supplement for isolation of *Pseudomonas aeruginosa* and another plate of *Pseudomonas* base medium with CFC supplement for isolation of other *Pseudomonas* species. The plates incubated at 30°C for 24-48 hr as described by ***APHA (1992)***.The bacterial isolates identified according to ***Macfidden (1976)*** and ***Lopez Romalde et al. (2003)***.

Polymerase Chain reaction:

For accurate identification of *Pseudomonas spp*., universal primers for 16S rDNA gene of eubacteria were used Table (1). DNA extraction had been done by following manufacturer's instructions of QIAamp DNA mini kit as shown in Table (2). *Pseudomonas spp*. and *P. aeruginosa* primers designed by ***Spilker et al. (2004)*** and *P. fluorescens* primer designed by ***Machado et al. (2013)***.These primers were used to produce a 618bp, 956bp and 850bp 16S rDNA products for *Pseudomonas spp*. *P. aeruginosa* and *P. fluorescens* respectively. 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 soft ware according to ***Sambrook et al. (1989)***.

Antimicrobial sensitivity test:

The antimicrobial sensitivity test of *Pseudomonas spp*. isolates were performed by disc diffusion test according to ***Bauer et al. (1966)*** and interpreted according to **NCCLS/CLSI (2007)**.

Results and Discussion:

Pseudomonas colonies were circular, smooth, moist, convex surface, glistening, about 1-2 mm in diameter and spreading with increase the incubation period. Some colonies showed iridescent sheen in reflected light while others were non-iridescent sheen. Microscopically, isolates were Gram negative rods curved with round ends.

The prevalence of *Pseudomonas spp*. in *Mugil cephalus* was (66%) which is higher than ***El-Hady and Samy (2011)*** who recorded that prevalence of *Pseudomonas spp.* in *Mugil cephalus* is (36%).

On the other hand, prevalence of *Pseudomonas spp.* in *Oreochromis niloticus* was (80%) which is in agreement with that recoded by ***Azza (1994)*** who reported that *Pseudomonas spp.* was (82.9%) in *Oreochromis niloticus* and lower than ***Abd El-Aziz (2015)*** who found that *Pseudomonas spp.* existence was (100%) in Nile tilapia samples. In the meantime, this result is higher than ***Yagoub et al. (2009)*** who found the *Pseudomonas spp.* prevalence was (55.3%).

The variations in the incidence of *Pseudomonas* between both fish species may be due to environment, method of catching and extent to handling during catching ***Wang et al. (1994)***.The higher existence of *Pseudomonas* in Nile tilapia may be due to *Mugil* fish is immunologically protected than *Tilapia* fish. Culturing of fish farms on organic waste fertilizers including poultry manure fertilized ponds may also considered as a cause of high incidence of *Oreochromis niloticus* to *Pseudomonas spp*.

Four hundred and thirty six of *Pseudomonas spp*. were recovered from different organs of examined fish and classified as shown in Table (3) into 8 different species; *P. fluorescens*(29.13%), *P. aeruginosa* (27.06%), *P. putida* (11.01%), *P. cepacia* (10.55%), *P. stutzeri* (8.49%), *P. anguilliseptica* (5.73%), *P. alcaligenes* (4.13%) and *P. acidovorans* (3.90%). ***Azza et al. (2002)*** reported that the genus *Pseudomonas* contains five species that described as etiological agents of fish diseases in Egypt.

Dealing with the prevalence of *Pseudomonas* species in *Mugil cephalus*, tabled in Table (4) the present results confirmed that *P. fluorescens* and *P. aeruginosa* were the most isolated strains in *Mugil* fish. This result is nearly similar to those recorded by ***Amany (1997)*** who recorded that prevalence of *P. fluorescens* (33.3%) and *P. aeruginosa* (30.4%). On the contrary, present results were higher than ***Enany et al. (2011)*** who isolated *P. fluorescens* from *Mugil* fish as (18.3%), ***El-Banna (2014)*** who reported that *P. fluorescens* and *P. aeruginosa* in *Mugil cephalus* was (15.25%) and (13.6%) respectively and higher than ***Beula Rani and Murugan (2015)*** who found that *P. aeruginosa* in *Mugil cephalus* was (15.38%).

In concern of the prevalence of *Pseudomonas spp.* from examined *Oreochromis niloticus* listed in Table (5), a study on Nile Tilapia by ***Abou El-Atta and El-Tantawy (2008)*** recorded that *P. fluorescens* existence was (29.63%) which is in agreement with the present results. In addition, results were nearly similar to ***Amany (1997)*** who discussed that prevalence of *P. fluorescens* and *P. aeruginosa* were (25.9%) and (29.3%) respectively and other *Pseudomonas* species as *P. stutzeri, P. cepacia* and *P. acidovorans* were recovered in relatively low rate. Moreover, results were lower than ***Hanna et al. (2014)*** who recorded the prevalence of *P. aeruginosa* was(34.4%). Results were in agreement with those reported by ***El-Hady and Samy (2011)*** who found that *P. fluorescens* represented the highest frequency of isolated *Pseudomonas* strains among fresh fish samples.

The highest *Pseudomonas* existence found in intestine, (25.53%) in *Mugil cephalus* and (24.19%) *Oreochromis niloticus*. This result supported by ***Hatai et al. (1975)*** who indicated that *Pseudomonas spp*. found normally in fish intestine and matched that recorded by ***Noga (2010)*** who considered *Pseudomonas spp*. as one of the normal flora of fish.

PCR protocol offers a rapid diagnostic tool to identify *Pseudomonas* members ***Scarpeillni et al. (2004)***. The 16S rDNA technique is an important tool for rapid and accurate detection of bacteria that can replace conventional, time-consuming biochemical identification method. ***Uma et al. (2007)***.

Species-specific primer employing PCR assay was more sensitive in the confirmation of the isolates and time consuming. PCR based methodologies are easy, fast and considered as one of the strongest tools for bacterial identification and specific protocols have been developed for many important bacterial pathogens in aquaculture ***Lopez et al. (2012)***.

In the presnet study, PCR was done for 14 isolates which showed electrophoesis with the specific band at 618 bp in Figure (1). This result confirmed by ***Spilker et al. (2004)*** who designed16S rDNA based PCR assays that provide rapid of *Pseudomonas spp*. and help in its differentiation from other phylogenetically closely related *Pseudomonas spp*. Seven out of the 14 isolates amplified single DNA fragment at 850 bp which is specific for *P. fluorescens* Figure(2). This result supported by ***Younes et al. (2015)*** *and* ***Machado et al. (2013)***.The electrophoresis of *P. aeruginosa* PCR product was shown with specific band at 956 bp Figure(3). This result was in agreement with ***Hanna et al. (2014)***.

Results of antibiotic sensitivity of isolated *Pseudomonas spp*. against 12 commercial antibiotic discs

showed in Figure (4,5,6,7,8,9,10 and 11) and Table (6). The antibiogram of isolated strains revealed that in general *Pseudomonas spp.* were highly sensitive to Ciprofloxacin, Gentamicin and Chloramphenicol while were intermediate sensitive to Rifampicine and Ceftriaxon. In addition, *Pseudomonas spp*. were varied in degree of sensitivity toward Tobramycin, Neomycin and Kanamycin. In the meantime, all samples were highly resistant to Penicillin, Amoxicillin, Erythromycin and Ampicillin/sulbactam expect *P. putida* were intermediate sensitive with Erythromycin.

These were results confirmed by ***Mesaros et al. (2007)*** who found that Ciprofloxacin was more effective antibiotic against *Pseudomonas spp*. than other antibiotics. Gentamicin was effective drug against *Pseudomonas spp*. **Khalil *et al.* (2010)**. Present results confirmed the highly sensitivity of all *Pseudomonas spp.* toward Chloramphenicol which propped by ***Iman (2004)*** *and* ***Darak and Barde, (2015)*** while conflicted with ***Akinbowale et al. (2007)***.

On conclusion, this study indicated the presence of *Pseudomonas spp.* in *Mugil* fish and Nile tilapia sold in Port Said markets. Thus, we recommended that there is need for farmers to adhere to good management practices to reduce the bacterial count in fish. *Pseudomonas* is highly pathogenic to human thus; it is advisable that fishes adequately subjected to proper boiling and cooking before human consumption. *Pseudomonas* isolates showed antibiotic resistance toward many antibiotics. Farmers must stop haphazard use of antibiotics, which lead to the presence of multi-drug resistant bacteria.

Table (1): *Oligonucleotide primers sequences:*

Primer	Sequence	Amplified product	Reference
***Pseudomonas* species 16SrDNA**	GACGGGTGAGTAATGCCTA	618 bp	**Spilker*et al.*, 2004**
	CACTGGTGTTCCTTCCTATA		
***P. aeruginosa* 16SrDNA**	GGGGGATCTTCGGACCTCA	956 bp	
	TCCTTAGAGTGCCCACCCG		
***P. fluorescens* 16SrDNA**	TGCATTCAAAACTGACTG	850 bp	**Machado *et al.*, 2013**
	AATCACACCGTGGTAACCG		

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

Gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
***Pseudomonas* 16SrDNA**	94˚C 5 min.	94˚C 30 sec.	50˚C 45 sec.	72˚C 45 sec.	35	72˚C 10 min.
***P. aeruginosa* 16SrDNA**	94˚C 5 min.	94˚C 30 sec.	52˚C 45 sec	72˚C 1 min.	35	72˚C 5 min.
***P. fluorescens* 16SrDNA**	94˚C 5 min.	94˚C 30 sec.	48˚C 45 sec.	72˚C 1 min.	35	72˚C 10 min.

Table (3): *Prevalence of different Pseudomonas strains isolated from examined fish.*

Pseudomonas	Number	Percentage
P. fluorescence	127	29.13
P. aeruginosa	118	27.06
P. putida	48	11.01
P.cepacia	46	10.55
P. stutzeri	37	8.49
P. anguilliseptica	25	5.73
P. alcaligenes	18	4.13
P. acidovorans	17	3.90
Total	436	100

Table (4): *Prevalence of Pseudomonas spp. among various organs of Mugil cephalus*

Pseudomonas	Intestine		Liver		Kidney		Surface		Muscle		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
P. fluorescens	14	25.45	10	18.18	7	12.73	15	27.27	9	16.36	55	29.26
P. aeruginosa	13	27.08	9	18.75	11	22.92	8	16.67	7	14.58	48	25.53
P. putida	5	45.46	2	18.18	1	9.09	2	18.18	1	9.09	11	5.85
P. cepacia	3	13.64	3	13.64	6	27.27	5	22.73	5	22.73	22	11.70
P. stutzeri	7	28.00	3	12.00	5	20.00	8	32.00	2	8.00	25	13.30
P. anguilliseptica	2	20.00	1	10.00	3	30.00	2	20.00	2	20.00	10	5.32
P. alcaligenes	2	22.22	2	22.22	3	33.33	2	22.22	0	0.00	9	4.79
P. acidovorans	2	25.00	3	37.50	1	12.50	1	12.50	1	12.50	8	4.26
Total	48	25.53	33	17.55	37	19.68	43	22.87	27	14.36	188	100

Table (5): *Prevalence of Pseudomonas spp. among various organs of Oreochromis niloticus*

Pseudomonas	Intestine		Liver		Kidney		Surface		Muscle		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
P. fluorescens	19	26.39	12	16.67	15	20.83	13	18.06	13	18.06	72	29.03
P. aeruginosa	15	21.43	16	22.86	13	18.57	12	17.14	14	20.00	70	28.23
P. putida	8	21.62	6	16.22	9	24.32	6	16.22	8	21.62	37	14.92
P. cepacia	7	29.17	6	25	3	12.5	2	8.33	6	25.00	24	9.68
P. stutzeri	3	25.00	2	16.67	1	8.33	1	8.33	5	41.67	12	4.84
P. anguilliseptica	4	26.67	3	20.00	3	20.00	1	6.67	4	26.67	15	6.05
P. alcaligenes	3	33.33	1	11.11	0	0.00	1	11.11	4	44.44	9	3.63
P. acidovorans	1	11.11	2	22.22	1	11.11	2	22.22	3	33.33	9	3.63
Total	60	24.19	48	19.35	45	18.15	38	15.32	57	22.98	248	100

Table (6): *Antibiogram of Pseudomonas isolates*

Antimicrobial agent	*P. fluorescens*	*P. aeruginosa*	*P. putida*	*P. cepacia*	*P. stutzeri*	*P. anguilliseptica*	*P. alcaligenes*	*P. acidovorans*
Ciprofloxacin(5µg)	S	S	S	S	S	S	S	S
Kanamycin(30µg)	S	S	I	S	I	I	S	I
Gentamicin (10µg)	S	S	S	S	S	S	S	S
Neomycin(30µg)	S	I	I	I	S	I	S	I
Rifampicine(5µg)	I	I	I	I	I	I	I	I
Chloramphenicol(30g)	S	S	S	S	S	S	S	S
Tobramycin(10µg)	I	I	I	S	I	S	I	S
Cefatriaxone(30µg)	I	I	I	I	I	I	I	I
Erythromycin(15µg)	R	R	I	R	R	R	R	R
Penicillin(10µg)	R	R	R	R	R	R	R	R
Amoxicillin(25µg)	R	R	R	R	R	R	R	R
Ampicillin/Sulbactam(20µg)	R	R	R	R	R	R	R	R

S: senstive **I: intermediate** **R: Resistant**

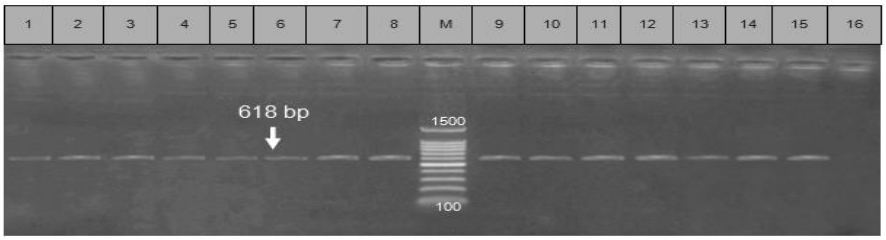

Figure (1): *Polymerase Chain Reaction of Pseudomonas isolates.*
Lane (1, 2, 3, 4, 5, 6, 7, 9, 10,11,12,13, 14, and 15): positive for ***Pseudomonas spp*.** with **618** bp band. **Lane (8):** Positive control. **Lane (16):** Negative control. **Lane (M):** Molecular marker.

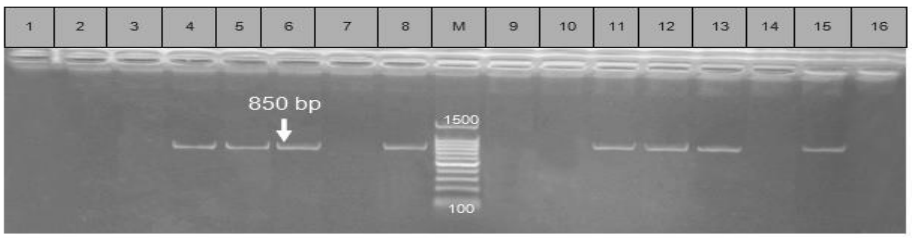

Figure (2): *Polymerase Chain Reaction of P. fluorescens species.*
Lane (4, 5, 6, 11, 12, 13 and 15) positive for ***P. fluorescens*** with **850** bp band. **Lane (8):** Positive control. **Lane (16):** Negative control. **Lane (M):** Molecular marker.

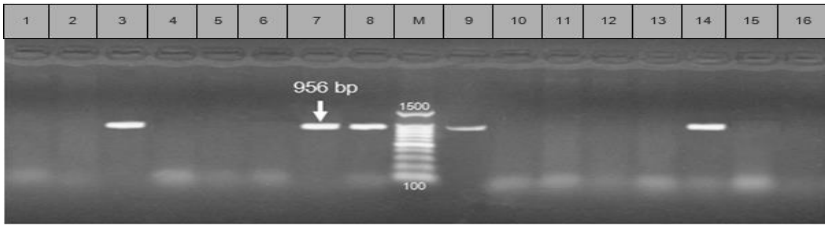

Figure (3): *Results of Polymerase Chain Reaction of P. aeruginosa species* **Lane (3, 7, 9 and 14):** positive for ***P. aeruginosa*** with **956**bp band. **Lane (8):** Positive control. **Lane (16):** Negative control. **Lane (M):** Molecular marker.

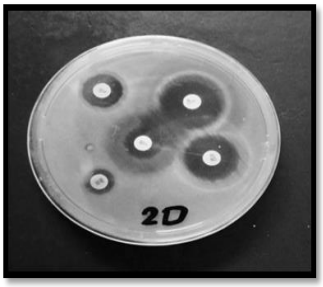

Figure (4): *P. fluorescens disc diffusion antibiotic sensitivity pattern*

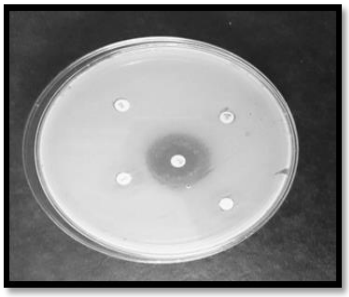

Figure (5): *P. aeruginosa disc diffusion antibiotic sensitivity pattern*

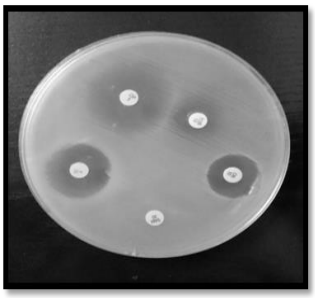

Figure (6): *P. putida disc diffusion antibiotic sensitivity pattern*

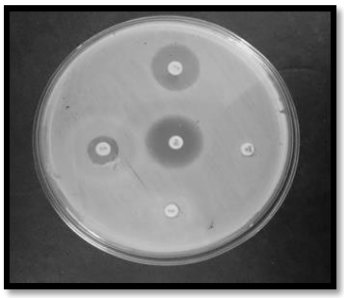

Figure (7*):* *P. cepacia disc diffusion antibiotic sensitivity pattern*

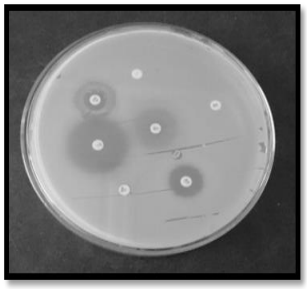

Figure (8): *P. stutzeri disc diffusion antibiotic sensitivity pattern*

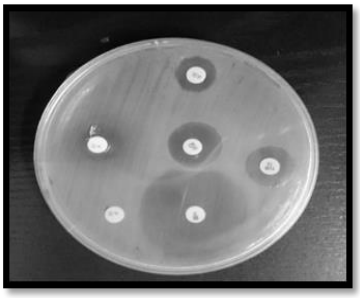

Figure (9): *P. anguilliseptica disc diffusion antibiotic sensitivity pattern*

Figure (10): *P. alcaligenes disc diffusion antibiotic sensitivity pattern*

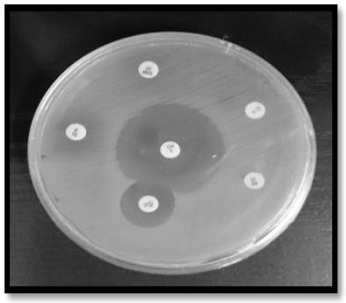

Figure (11): *P. acidovorans disc diffusion antibiotic sensitivity pattern*

Refrences:

Abd El-Aziz D.M.(2015): Detection of Pseudomonas *spp*. in chicken and fish sold in markets of Assiut City, Egypt. Journal of food Quality Hazards Control 2 (2015) 86-89.

Abdullahi, R.; Lihan S.; Carlos, B.S.; Bilung, M.L.; Mikal, M.K.; Abou El-Atta M.E. and El Tantawy M.M. (2008): Bacterial causes of skin ulcers affection in *Oreochromis niloticus* with special references to its control 8th International Symposium on Tilapia in Aquaculture 2008.

Akinbowale, O.L.; Peng, H. and Barton, M.D. (2007): Antibiotic and heavy metal resistance in motile *Aeromonas* and *Pseudomonas* from rainbow trout (*Oncorhynchus mykiss*) farms in Australia. Inter. J. Antimicrobial. Agents, 30(2):177.

Amany, A.M.S. (1997): Studies on virulence factors of some *Pseudomonas* and *vibrionaceae* microorganisms isolated from fresh and frozen fish. Ph.D. Thesis, Fac. Of Vet.Med., Suez Canal University.

APHA (1992):*Compendium of methods for the microbiological examination of food.* In C., method". Am. J. Clin. Pathol. 45. 493-496. 1966. Vanderzant, & D. F. Splittstoesser. (Eds.). Washington, DC: American Public Health Association.

Azza, E.H.A. (1994): Studies on the role of pseudomonas group of microorganisms in teleosts. Ph. D Thesis, Fac. Vet. Med., Cairo University.

Azza, M. M.; El-Nobi, G.A. and Moustafa, A. M. (2002): Studies on *Pseudomonas* Septicemia among *Tilapia Fish* in Abbassa Fish Farms. Zag. Vet. J. 30: (1), 25- 31.

Bauer, A.W., Kirby, W.M., Sherris, J.C., and Turck, M., (1966): "Antibiotic susceptibility testing by a standardized single disk

Beula Rani, K.R. and Murugan, M. (2015): Assessment of Microbial Diversity in Gastro Intestinal Tract of Estuarine Fishes. International

Collick, F. (2013): Detection of *opr*L gene and antibiotic resistance of *Pseudomonas aeruginosa* from aquaculture environment. Pelagia Research Library European Journal of Experimental Biology 2013; 3 (6):148-152.

Journal of Pharma and Bio Sciences 2015 July 6(3): (B) 31-36.

Darak O. and Barde R.D. (2015):*Pseudomonas fluorescens* associated with Bacterial Disease in Catla catla in Marathwada Region of Maharashtra International Journal of Advanced Biotechnology and Research (IJBR)Vol. 6, 2015, pp189-195.

El-Banna N.I. (2014): Prevalence of most pathogenic Gram-negative bacteria in marine fish. Thesis, M.V.Sc., Bacteriology, Mycology and Immunology, Fac. Vet. Med. Suez Canal Univ.

El-Hady, M.A. and Samy, A.A. (2011): Molecular Typing of *Pseudomonas* Species Isolated from Some Cultured Fishes in Egypt. Global Vetrerinaria 7 (6): 576-580, 2011.

Enany, M.El.; Ibrahim H.M.; Abou-ElAtta, M. and El.;ElAwady, M. M. (2011): Bacteriological and histopathological studies on some bacterial pathogens causing diseases in cultured mugil capito fish in Ismailia governorate. SCVMJ,XVI (1).

Hanna M.I.; El-Hady M.A.; Ahmed H.A.; Elmeadawy S.A. and Kenwy A.M. (2014): A contribution on *Pseudomonas aeruginosa* infection in African Catfish (*Clarias gariepinus*). Research Journal of Pharmaceutical, Biological and Chemical 5(5)575-588.

Hatai, K.; Egusa, S.; Nakajime, M. and Chikahata, H. (1975): *Pseudomonas chlorraphis* as a fish pathogen. Bull. Japanese Soc. Sci. Fish.41 (11), 1203.

Iman, M.M.M. (2004): Studies on pseudomonas infection in fish in Kafr El-Sheikh province. M.V.Sc. Thesis, Fac. Vet. Med., Tanta University.

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

Lo´pez, J.R.; Nanas, J.I.; Thanantong, N., R. de la Herran and Sparagano, O.A.E.(2012): Simultaneous identification of five marine fish pathogens belonging to the genra *Tenacibaculum, Vibrio, Photobacterium* and *Pseudomonas* by reverse line blot hybridization. Aquaculture 324-325 (2012) 33-38.

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 Aquat Anim 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.

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

PharmaceuticalSciences.

Nagasawa, K. and Cruz-Lacierda, E.R. (2004): Diseases of Cultured GroupersSoutheast Asian Fisheries Development Center, Aquaculture. Government of Japan TrustFund ISBN971-8511-70-9.

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.

Noga, E.J. (2010): Fish Diseases: Diagnosis and TreatmentAmes, IA: Iowa State University Press.

Roberts, R.J. (1989): Fish pathology, 2nd Ed. Bailliere, Tindall, London.

Sambrook, J.; Fritscgh, E.F.; and Mentiates (1989): Molecular coloning. A laboratory manual. Vol1., Cold spring Harbor Laboratory press, New York.

Scarpellini, M.; Ftanzetti, L. and Galli, A. (2004): FEMS Micobiol Lett 236:257-260.

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.

Uma, A.; Reddy, Y.K.; Meena, S.; Saravanabava, K. and Muralimanohar, B. (2007): Application of 16S rDNA amplification and sequencing for detection of fish and shrimp bacterial pathogens. Ind. J. Comp. Microbiol. Immunol. Inf. Dis.28:7-9.

Wang, Y.; Wilkie, M.P.; Heigenhaur, G. J. F. and Wood, C.M. (1994): The analysis of metabolites in rainbow trout white muscle: A comparison of different sampling and processing methods. J. Fish Biol., 45: 855-873.

Yagoub S.O. (2009): Isolation of Enterobacteriaceae and Pseudomonas *spp*. from raw fish sold in fish market in Khartoum stateJournal of Bacteriology Research Vol. 1(7), pp. 085-088, October, 2009.

Younes, A.M.; Laila, A. Mohamed; Eida, M.F. and Gaafar, A.Y. (2015): Characterization and pathogen challenge of Pseudomonas species from *Oreochromis niloticus* in Egypt. Research Journal of Pharmaceutical, Biology and Chemical Science. 6(1) 312-317.

مدى تواجد و التوصيف الجزيئي لانواع السيدوموناس في اسماك الاسواق ببورسعيد

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الملخص العربي

تم تجميع 200 سمكة (100 بوري , 100 بلطي) عشوائياً من أسواق بورسعيد لمعرفة مدى تواجد السيدوموناس بها. وُجِدَ أن 66% من اسماك البوري و 80% من اسماك البلطي ايجابية الفحص لميكروب السيدوموناس حيث أنتجت الفحوص البكتريولوجية للأمعاء الدقيقة و الكبد و الكلى و العضلات و سطح الأسماك 436 عتره بكتيرية من السيدوموناس;سودوموناس فلوريسينس و سودوموناس إيرجينوزا وسودوموناس بيوتيدا و سودوموناس سيباشيا و سودوموناس ستتزري و سودوموناس آنجيليسبتيكا و سودوموناس آلكاليجينز و سودوموناس آسيدوفورانس بنسب (29,13% و 27,06% و 11,01% و 10,55% و 8,49% و 5,73% و 4,13% و 3,90%) على التوالي. و قد انتج اختبار تفاعل انزيم البلمرة المتسلسل ل 14 عينة ان جميعها تنتمي لجنس السيدوموناس منها 7 فلوريسينس و 4 سودوموناس إيرجينوزا. و قد كانت جميع العترات حساسة للسيبروفلوكساسين و الجنتاميسين و الكلورامفنيكولو مُقاومة البنيسيلين و الأموكسيسيلين و مُركب الامبيسيلين و السلبكتام.