

Molecular characterization of *Aeromonas* species in mullet fish using PCR technique

Gihan M.O. Mohammed

Department of Microbiology, Port- Said Laboratory, Animal Health Research Institute, Dokki, Giza, Egypt.

Received in 20/1/2016

Accepted in 23/2/2016

Abstract

A total of 8 strains of *Aeromonas* species were isolated from 100 mullet fish samples which were collected from different sellers in Port-Said city fish markets. Biochemical characterization indicated that 8 strains were identified as *Aeromonas* species. *A. hydrophila* was the highest strains (62.5%) followed by *A. sobria* (25%), while the lowest strain was *A. caviae* (12.5%). All of *A. hydrophila*, *A. sobria* and *A. caviae* isolates were recovered from (liver, kidney and spleen) of examined samples. The antibiotic susceptibility profiles of 8 strains of *Aeromonas* spp. for to 11 antibacterial agents were investigated. The results showed that, all strains were resistant to amoxycillin and Penicillin G (100%), while half of tested stains were resistant to erythromycin (50%). The molecular identification of isolated aeromonads by conventional PCR using (16S rRNA for *Aeromonas* species) and the results revealed that the all 8 isolates were positive. Most of the isolates which were identified were positive for two virulence genes Aerolysin (*Aero*) and Haemolysin (*hly*). The genetic determinants revealed 8 acquired antibiotic resistance genes (*bla_{TEM}*) were represented in all of *Aeromonas* spp. while, *mph(A)* gene was detected only in 4 strains of *Aeromonas* species of tested strains which gave same results by disc diffusion method in Lab. The public health hazards of this microorganism, as well as recommended measures to improve quality status of market mullet fish were discussed.

Keywords: *Aeromonas* spp., Virulence genes resistance genes, PCR, mullet fish, Public health.

Introduction

The attention all over the world is directed to fish as rapid, highly desirable food and healthy compensatory source of good quality animal protein. Fish is a high-protein, low-fat food that provides a range of health benefits (Fitzsimmons, 2000). Worldwide people obtain about 25% of their animal protein from fin fish. The protein found in fish is of high biological value, which means that fish can be used as sole source of protein in the diet. The real importance of fish in the diet is not only its protein, but also the omega-3 fat. Omega-3 fatty acids are very important for normal growth and help preventing heart disease because they make the blood less likely to form clots that cause heart attacks. In Egypt, mullet

fish are economically very important because they have high market value and have been cultivated successfully by fish farmers (Mohamed *et al.*, 2009). The marine water mullet fish are the most common fish types (Cirillo *et al.*, 2009). It is an economically important species contributing to sizable fisheries of estuarine and coastal regions in many countries including Egypt (Saleh, 2008), India (Barman *et al.*, 2005), Italy (Luzzana *et al.*, 2005), Nigeria (Anyanwu *et al.*, 2007), Tunisia (Khérifi *et al.*, 2003) and Taiwan (Chang *et al.*, 2000).

Contamination of fish with pathogenic microorganisms reflected environment pollution (Adeyemo, 2003). So, the microbial flora associated with fish is a reflection of their aqueous

environment. If the fish habitats are contaminated by pathogenic bacteria, the consumption of these fish may possess risk to the human health.

Aeromonas spp. is ubiquitous, opportunistic bacterium and normal micro- flora in fish as well as other aquatic mammals. They have been reported in both marine and fresh water environments and can cause diseases in fish under stressful conditions (Conte, 2004). In humans, *Aeromonas* causes diarrhea, gastroenteritis and extra-enteric conditions such as septicemia, wound infection, endocarditis, meningitis and pneumonia (Ali and Hossein, 2010).

Antibiotic resistance has become a major public health concern because the organisms that cause infections are becoming less sensitive. However, antibiotic resistance can also arise in non-pathogenic bacteria as a result of horizontal gene transfer. Moreover, antibiotics are released daily into the natural environment with treated wastewater effluents and through use in animal husbandry. Antibiotic resistance and reduced efficacy of the drugs due to intense usage of antimicrobial agents has been identified in animal pathogens (Grim *et al.*, 2013).

Unlike conventional cultivation methods which are time consuming and labour intensive, molecular diagnosis tools being highly powerful, sensitive and rapid are gaining popularity for microbial identification (Böhme *et al.*, 2014).

Molecular approaches, especially those based on the use of rRNA genes and related techniques, have provided the opportunity to analyse complex communities on the basis of sequence diversity. Bacterial species can be identified by generating clone libraries of the 16S rDNA followed by sequencing and comparison with databases containing thousands of ribosomal sequences to allow a phylogenetic affiliation to cultured, as well as uncultured microorganisms (Ercolini, 2004). Since, laboratory isolation and identification of *Aeromonas* spp. are laborious and time consuming; PCR reaction was also standardized by Porteen *et al.*, (2007) for detection of *Aero-*

monas spp. from chicken and fish samples using primers 16S rRNA.

The pathogenesis of *Aeromonas* infection is complex and multifactorial. *Aeromonas* species secretes many extracellular proteins, including amylase, chitinase, elastase, aerolysin, nuclease gelatinase, lecithinase, lipase and protease. These proteins are known as virulence factors that cause disease in fish and humans. Aerolysin is a representative virulence factor of *Aeromonas* spp. and was reported to function as hemolysins and cytolytic enterotoxins (Bhowmik *et al.*, 2009). Several virulence associated genes have been targeted for detection of potentially pathogenic *Aeromonas* spp. by PCR including Aerolysin and hemolysins genes (Balakrishna *et al.*, 2010).

PCR assay used to detect *bla_{TEM}* genes in *Aeromonas* spp. which is an alarming public health concern (Igbinosa and Olcoh, 2012) while, Poole *et al.*, (2006) reported that the macrolide resistance operon encodes a macrolide phosphor transferase *Mph (A)* confers high level erythromycin resistance.

So the present study was carried out to assess the bacteriological and antibiotic susceptibility profiles of market mullet fish with special reference to *Aeromonas* spp. and to determine two virulence genes (*Aero* and *hly*) that may contribute to its pathogenicity, as well as to detect two antibiotic resistance genes *bla_{TEM}* and *Mph(A)* using specific primers that may act as alarming public health concern.

Materials and Methods

Samples collection:

A total of 100 mullet fish samples were collected randomly from different seller in Port-Said city fish market. The samples were transferred to the laboratory in an ice-box as soon as possible to be examined bacteriological for detection of *Aeromonas* species.

Bacteriological examination:

Isolation and identification of *Aeromonas* spp.:

Twenty-five grams of fish sample were weighed aseptically and homogenized for 2 min in stomacher bags containing 225 ml of

alkaline peptone water, then incubated for 24hr at 30°C (Villari *et al.*, 2000). A loopful from alkaline peptone water was subsequently plated on the surface of starch ampicillin agar plates (SAA) and incubated for 48 hr at 30°C. Typical yellow colonies of *Aeromonas* species were purified on tryptone soya agar then stained by Gram stain (A.P.H.A., 1992) and confirmed by the esculin hydrolysis, sugar fermentation and gas production, indole production and voges- proskaur test. Identification was performed on isolates according to the criteria of Castro *et al.*, (2003).

Antimicrobial susceptibility test:

The antimicrobial susceptibility test was performed using agar disc diffusion assay as described by Clinical and Laboratory Standards Institute (NCCLS, 2004). The used antimicrobials agents were amikacin 30 mcg ; Amoxicillin 30mg; ceftriaxone 30mg; chloramphenicol 30 µg; colistin sulphate 10mg, doxycycline hydrochloride 30 µg; erythromycin 15 µg; nalidixic acid 30 µg; Penicillin G 10 µg, streptomycin 10 µg and trimethoprim / sulphamethoxazole (1.25+23.75). Pure colonies of isolated *Aeromonas* were emulsified in normal saline and turbidity was matched with 0.5

McFarland turbidity standards. Selected antimicrobial discs were placed on Mueller Hinton Agar plates seeded with bacteria. These plates were incubated at 37°C for 24 hours. The organisms were observed for antimicrobial sensitivity based on diameters of zones of inhibition on Petri dishes. Susceptible and resistant isolates were interpreted.

Molecular confirmation of *Aeromonas* species and detection of virulence genes and antibiotic resistant genes:

DNA extraction:

DNA extraction from samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56°C for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

Oligonucleotide Primer: Primers used were supplied from Metabion (Germany) are listed in table (1).

Table (1). Primers sequences, target genes, amplicon sizes and cycling conditions.

Target gene	Primers sequences	Amplified segment (bp)	Primary denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondary denaturation	Annealing	Extension		
16S rRNA	GAAAGGTTGATGCCT AATACGTA	685	94°C 10 min.	94°C 45 sec.	55°C 45 sec.	72°C 45sec.	72°C 20 min.	Nielsen <i>et al.</i> , (2001)
	CGTGCTGGCAACAAA GGACAG							
Aerolysin (<i>Aero</i>)	CA- CAGCCAATATGTCGG TGAAG	326	94°C 5 min.	94°C 30 sec.	52°C 30 sec.	72°C 30 sec..	72°C 7 min.	Singh <i>et al.</i> , (2008)
	GTCACCTTCTCGCTCA GGC							
Haemolysin (<i>hly</i>)	CTATGAAAAAAC- TAAAAATAACTG	1500	94°C 15 min.	94°C 1 min.	55°C 1 min.	72°C 1.5 min.	72°C 15 min.	Yousr <i>et al.</i> , (2007)
	CAGTA- TAAGTGGGGAAATGG AAAG							
<i>bla_{TEM}</i>	ATCAGCAATAAAC- CAGC	516	94°C 5 min.	94°C 45 sec.	54°C 45 sec.	72°C 45 sec.	72°C 10 min.	Colom <i>et al.</i> , (2003)
	CCCCGAA- GAACGTTTTTC							
<i>mph(A)</i>	GTGAGGAG- GAGCTTCGCGAG	403	94°C 5 min.	94°C 45 sec.	58°C 45 sec.	72°C 45 sec.	72°C 10 min.	Nguyen <i>et al.</i> , (2009)
	TGCCGCAG- GACTCGGAGGTC							

PCR amplification:

Primers were utilized in a 25- µl reaction containing 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentrations, 4.5 µl of water, and 6 µl of DNA template. The reaction was performed in an applied biosystem 2720 thermal cycler.

Analysis of the PCR Products:

The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem,

Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 15 µl of the products was loaded in each gel slot. 100 bp and 100 bp plus DNA Ladders (Qiagen, Germany, GmbH) were used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

Results**Table (2).** Prevalence of *Aeromonas* species in examined market mullet fish (Total no. of samples=100).

<i>Aeromonas</i> species	No. of isolates	%
<i>A. hydrophila</i>	5	62.5
<i>A. sobria</i>	2	25
<i>A. caviae</i>	1	12.5
Total no. of isolates	8	-

Percentage was calculated based on number of each strain.

Table (3). Frequency distribution of *Aeromonas* species from different organs in examined mullet fish samples.

<i>Aeromonas</i> spp.	No. of strain	Mullet fish									
		Body surface		Internal organs						Muscle (Flesh)	
				Liver		Kidney		Spleen			
		No.	%	No.	%	No.	%	No.	%	No.	%
<i>A. hydrophila</i>	5	0	0	2	40	2	40	1	20	0	0
<i>A. sobria</i>	2	-	0	2	40	0	0	0	0	0	0
<i>A. caviae</i>	1	-	0	1	20	0	0	0	0	0	0

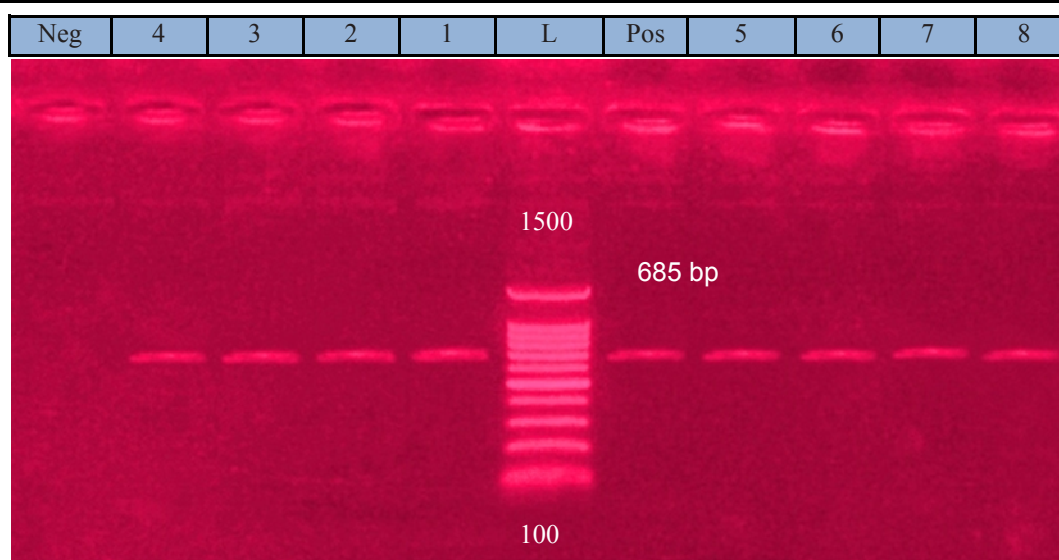
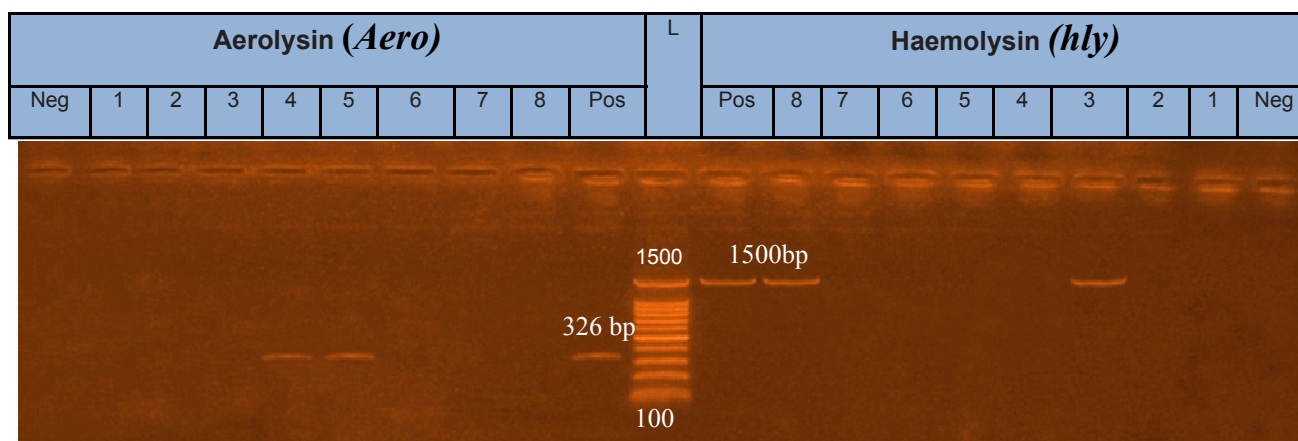
Percentage was calculated based on number of each strain

Table (4). Antibigram of (8) *Aeromonas* strains isolated from (100) examined mullet fish samples.

Antibiotic agents	Disc Potency	<i>Aeromonas</i> isolates			
		Sensitive		Resistant	
		No.	%	No.	%
Amikacin "Ak"	(30mg)	8	100	0	0
Amoxicillin "Amx"	(25 mg)	0	0	8	100
Chloramphenicol "C"	(30mg)	7	87.5	1	12.5
Ceftriaxone "CRO"	(30mg)	8	100	0	0
Colistin sulphate "CT"	(10mg)	6	75	2	25
Doxycycline "DO"	(30mg)	7	87.5	1	12.5
Erythromycin "E"	(15mg)	4	50	4	50
Nalidixic acid "NA"	(30mg)	6	75	2	25
Penicillin G "P"	(10 units)	0	0	8	100
Streptomycin "S"	(10mg)	7	87.5	1	12.5
Trimethoprim-sulfamethoxazole "SXT"	(1.25+23.75)	8	100	0	0

Table (5). Molecular characterization, virulence and antibiotics resistant genes present in *Aeromonas* isolates.

Sample	<i>Aeromonas</i> spp.	Results				
		16SrRNA	aerolysin	hemolysin	<i>Mph(A)</i>	<i>bla</i> _{TEM}
1	<i>A. caviae</i>	+	-	-	-	+
2	<i>A. caviae</i>	+	-	-	-	+
3	<i>A. hydrophila</i>	+	-	+	-	+
4	<i>A. hydrophila</i>	+	+	-	+	+
5	<i>A. hydrophila</i>	+	+	-	+	+
6	<i>A. sobria</i>	+	-	-	-	+
7	<i>A. hydrophila</i>	+	-	-	+	+
8	<i>A. hydrophila</i>	+	-	+	+	+

**Figure 1:** Agarose gel electrophoresis of PCR prod-ucts after amplification of: *Aeromonas* spp. 16SrRNA gene. MWM-molec-ular weight marker (100 – 1500 bp DNA ladder), + control (Positive, Negative) + different strains of *Aeromonas* spp. (*Aeromonas* spp. 16S rRNA gene products at 685 bp).**Figure 2:** Agarose gel electrophoresis of PCR prod-ucts after amplification of:
1.MWM-molec-ular weight marker (100 –1500 bp DNA ladder), + control (Positive, Negative) + different strains of *Aeromonas* spp.
2.Aerolysin gene. (Aerolysin gene products at 326 bp).
3.Hemolysin gene (Hemolysin gene products at 1500 bp).

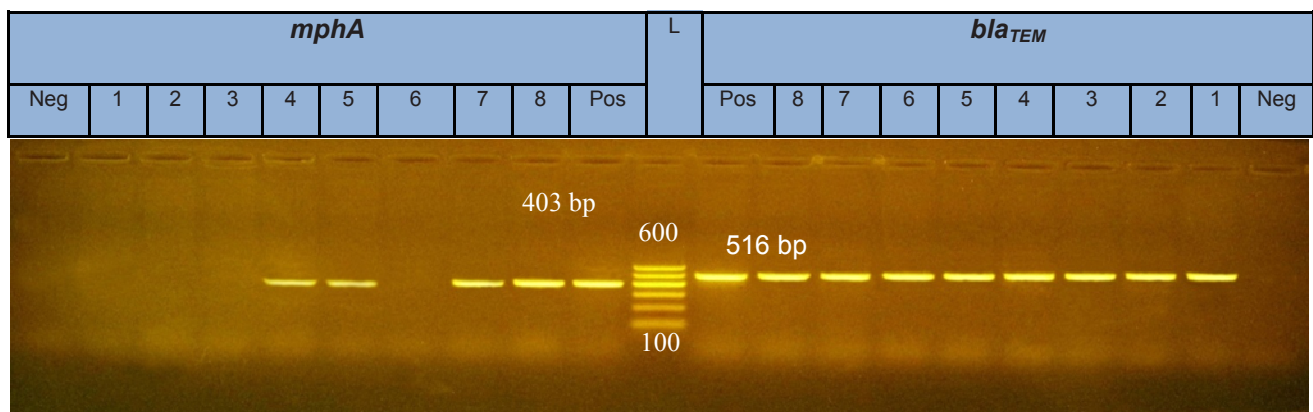


Figure 3: Agarose gel electrophoresis of PCR prod-ucts after amplification of:

1. MWM-molec-ular weight marker (100 – 600 bp DNA ladder), + control (Positive, Negative) + different strains of *Aeromonas* spp.
2. *mphA* gene. (*mphA* products at 403 bp)
3. *bla_{TEM}* gene (*bla_{TEM}* products at 516bp).

Discussion

The Port Said Governorate is fortunate to have the greatest water surface compared with other governorates in Egypt. It is bounded from the north by the Mediterranean Sea and in the southwest by Lake Manzala, which is the largest brackish water body in the country. In the circumstances, fishing activities sustain a large number of people in Port Said Governorate, and fish is a most favored dish to people in urban as well as rural communities. So, there is high ability of fish diseases to occur in this area especially bacterial one and its ability to affects the human as an agent of food borne diarrheal diseases in healthy people (Isonhood and drake 2002). The complex concept of fish quality consists of safety, nutritional value, availability, integrity, and freshness, eating quality and product size and type (Abbas *et al.*, 2008).

So total 100 samples of market mullet fish were examined bacteriological for presence of *Aeromonas* spp., the percentage of *Aeromonas* spp. was 8% (8 isolates out of 100 samples) as shown in Table (2). These results were nearly similar to that recorded by Ahmed (2013) who showed that *Aeromonas* spp. were isolated from mullet fish in a percentage of 9%. However; the current results were less than Neyts *et al.* (2000) who isolated aeromonads from fish and shrimps in proportion of 72% and Yucel *et*

al., (2005) who isolated *Aeromonas* spp. in a percentage of 80.3% from market fish. The obvious results resume to differences in sampling period, sampling types, geographical location, origin of the samples, total number of samples and methodology for analysis, these differences make the comparison a little bit difficult of *Aeromonas* spp. incidence published by different authors. However, the present data clearly confirm the presence of motile aeromonads in market mullet fish.

Table (2) cleared that the *A. hydrophila* was highest frequent isolates (62.5%) followed by *A. sobria* (25%) and *A. caviae* (12.5%). These results were agreed with Abou El-Atta (2003) and Sharma and Kumar (2011) who's recorded that the identification of *Aeromonas* spp. isolated from fish demonstrated the preponderance of *A. hydrophila*, followed by *A. sobria* and *A. caviae*. Also Hatha *et al.*, (2005) reported that, 100% of *A. hydrophila* 77.8% of *A. caviae* and 50% of *A. sobria* were isolated from fresh water fishes.

Table (3) showed that all of *Aeromonas* spp. isolates were recovered from internal organs (liver, kidney and spleen) of examined samples. These results were agreed with Enany *et al.*, (2011) who isolated *A. hydrophila* from internal organs (liver, kidney and spleen) of cultured mugil capito were collected from private fish farm (El-Kantara west) in Ismailia

Governorate.

Concerning to the results of antibiotic sensitivity, **Table (4)** revealed that *Aeromonas* spp. isolates were resist to Amoxycillin (100%) and erythromycin (50%) but sensitive to Amikacin, Ceftriaxone and trimethoprim- sulphamethoxazole (100%); Chloramphenicol, doxycycline and Streptomycin (87.5%); Colistin sulphate and Nalidixic acid (75%). The result agreed with **Guz and Kozinska (2004)** and **Adanir and Turutoglu (2007)** who founded that *A. sobria* strains were resistant to β -lactam antibiotics, such as amoxicillin. Also results were agreed with **Enany et al., (2011)** who recorded that *A. hydrophila* had been resistant to erythromycin. These results also agree with **Nifty and Hatha (2013)** who said that all the *Aeromonas* isolates from freshwater ornamental fish were found to be sensitive to chloramphenicol, ciprofloxacin and gentamicin and resistant to amoxicillin. Also the results agreed with **Hatha et al., (2005)** who recorded that motile aeromonads isolated from fish samples in India, showed relatively low level (<10%) of resistance to nalidixic acid, gentamicin and ciprofloxacin. Our results agreed with **(Krishnan et al., 2012)** who mentioned that all *Aeromonas* isolates were resistant to erythromycin. However, **Shuang-Hu et al., (2012)** reported sensitivity of *Aeromonas* strains to erythromycin. The development of antibiotic resistant strains is a great extent attributed to the misuse of antibiotics in culture systems for treatment and for better feed conversion, these levels are bound to vary according to local practice. These differences in resistance patterns to antibiotics of the isolates from author to other could be as a result of sampling locations, anthropogenic activities, and other environmental factors.

Results of agarose gel electrophoresis using **16S rRNA** gene for 8 tested *Aeromonas* strains in **table (5)** and **figure (1)** indicated that all tested strains were *Aeromonas* spp. with molecular weight 685bp. **Shuang-Hu et al., (2012)** used **16S rRNA** sequence for identified of *Aeromonas Veronii* bv. *Veronii* associated with Ulcerative syndrome from catfish. The

presence of plasmids may present a potential public health hazard. Thus, the presence of plasmids in clinically important bacteria increases their virulence. *Aeromonas* spp. exhibited very high levels of overall **16S rDNA** sequence similarity to each other (**Martinez-Murcia et al., 1992**).

PCR assays were developed with specific primers for the detection of different virulence genes (aerolysin and haemolysin) of *Aeromonas* species, which may be considered a significant in food safety threat. *Aeromonas* spp. possesses a variety of virulence factors that may contribute to its pathogenicity. Our results showed that aerolysin genes were detected in strains 4 and 5 tested isolates. But haemolysin genes detected in 3 and 8 isolates **table (5)** and **figure (2)**. These results agreed with **Kingombe et al., (1999)** who recorded that the pathogenicity of aeromonads has been linked to exotoxins such as hemolysin and aerolysin. Also the results of hemolysin genes presence in this study were confirmed by several researchers **Seethalakshmi et al., (2008)** and **Sanaa et al., (2014)**. Otherwise several virulence factors have been studied in *Aeromonas* spp. including aerolysin / hemolysin (**Chacón et al., 2003**). **Bhowmilk et al., (2009)** mentioned that the pathogenesis of *Aeromonas* infection is complex and multifactorial. But **Yucel et al., (2003)** report of that *A. hydrophila* and *A. sobria* were stronger producer of hemolysin, while *A. caviae* strains were non haemolytic. **Yogananth et al., (2009)** reported that the PCR assay proved to be a useful tool for the detection of virulent *A. hydrophila* by detecting haemolysin and aerolysin genes as genetic virulence markers. The differences in the distributions of virulence factor genes in the populations strengthen the probability that some *Aeromonas* spp. are better adapted to the specific conditions found in specific infectious sites that may returned to the different environmental and geographical sources.

Table (5) and **figure (3)** showed the genetic determinants of resist genes which rerevealed 8 acquired antibiotic resistance genes (*bla_{TEM}*) were represented in all of *Aeromonas* spp.

while, *mph (A)* gene detected only in 4 strains of *Aeromonas* spp. 4,5,7 and 8 tested strains which gave the same result for disc diffusion method in Lab. These results were agreed with **Igbinsosa and Okoh (2012)** who reported that the presence of (*bla_{TEM}*) genes in *Aeromonas* spp. act as alarming public health concern. *Aeromonads* are known to carry plasmids encoding antibiotic resistance determinants (**Majumdar et al., 2007**).

The present work highlights an important incidence of *Aeromonas* spp. in market mullet fish intended for human consumption in Port- Said city. The share of *Aeromonas* spp. could pose a risk for human health after consumption of undercooked fish or cross-contamination. Collectively, results in current study clarified that, PCR is considered as a rapid, reliable and sensitive tool which will help in prevention and cure of diseases in various aquatic animals. These days modern epidemiologists have been using a variety of tools which provide good molecular differentiation and which can be tailored to fit the needs of the both laboratory and clinical study. Several of these molecular methods could enable the creation of large reference libraries of typed organisms to which new outbreak strains can be compared across the world in order to monitor change in bacterial populations. These techniques will be useful for species identification of bacteria and should be applicable in the studies of epidemiology, diagnosis, virulence and molecular taxonomy.

References

- Abbas, K.A.; Mohamed, A.; Jamilah, B. and Ebrahimian, M. (2008).** A review on correlations between fish freshness and pH during cold storage. *American Journal of Biochemistry and Biotechnology* 4 (4): 416-421.
- Abou El-Atta, M.E. (2003).** Efficiency of polymerase chain Reaction (P.C.R.) in diagnosis of some bacterial fish pathogens. Ph. D. Thesis in Vet. Sci. (Bacteriology, immunology and mycology) Suez Canal University.
- Adanir, D. and Turutoglu, H. (2007).** Isolation and antibiotic susceptibility of *Aeromonas hydrophila* in a carp *Cyprinus carpio* hatchery farm. *Bull. Vet. Inst. Pulawy*, 51: 361-364.
- Adeyemo, O.K. (2003).** Consequences of pollution and degradation of Nigerian aquatic environment on fisheries resources. *The Environmentalist* 23(4): 297-306.
- Ahmed, M.E.E. (2013).** Studies on major bacterial diseases affecting fish; Tilapia *Oreochromis niloticus*, Catfish, *Clarias gariepinus* and mullets in Port Said, Egypt with special references to its pathological alterations. *Researcher*, 5(2): 5-14.
- Ali, A. and Hossein, J. (2010).** A review on Occurrence and characterization of the *Aeromonas* species from Marine Fishes. *World J. Fish Marine Sci.*, 2: 519-523.
- Anyanwu, P.E.; Gabriel, U.U.; Akinrotimi, O.A.; Bekibele, D.O. and Onunkwo, D.N. (2007).** Brackish water aquaculture: a veritable tool for the empowerment of Niger Delta communities. *Scientific Research and Assays*, 2, 295-301.
- A.P.H.A. (1992).** Compendium of methods for the microbiological examination of foods. 3rd Ed., Vavderzant, C. and Splittstoesser, D. F (eds.) Washington.
- Balakrishna, K.; Murali, H.S.; Batra H.V. (2010).** Detection of toxigenic strains of *Aeromonas* species in foods by a multiplex PCR assay. *Indian J. Microbiol.* 50: 139-144.
- Barman, U.K.; Jana, S.N.; Garg, S.K.; Bhatnagar, A. and Arasu, A. R.T (2005).** *Aquaculture International*, 13: 241-256.
- Bhowmilk, P.; Bag, P.K.; Hajra, T.K.; Sarkar, R.P.; Ramamuthy, T. (2009).** Pathogenic potential of *Aeromonas hydrophila* isolated from surface waters in Kolkata, India. *J. Med. Microbiol.* 58: 1549-1558.
- Böhme, K.; Cremonesi, P.; Severgnini, M.; Tomás, G.; Villa, I.; Fernández, C.; Barros-Velázquez, J.; Castiglioni, B. and Calo-Mata, P. (2014).** Detection of food spoilage and pathogenic bacteria based on ligation detection reaction coupled to flow-through hybridization on membranes. *Bio. Med. Research International* 156323: 1-11.

- Castro, E.G.; Figueras, M.J.; Aguilera, A.G.; Soler, L. and Fernandez, R.E. (2003).** Characterization of *Aeromonas* sp. isolated from frozen fish intended for human consumption in Mexico. *Int. J. Food Microbiol.*, 84: 41-49.
- Chacón, M.R.; Figueras, M.J.; Castro-Escarpulli, G.; Soler, L. and Guarro, J. (2003).** Distribution of virulence genes in clinical and environmental isolates of *Aeromonas* spp., *Antonie Van Leeuwenhoek*, 84 (4): 269-278.
- Chang, C.W.; Tzeng, W.N. and Lee, Y.C. (2000).** Recruitment and hatching dates of grey mullet (*Mugil cephalus* L.) juveniles in the Tanshui Estuary of Northwest Taiwan. *Zoological Studies*, 39: 99-106.
- Cirillo, T.; Viscardi, V.; Fasano, E.; Farina A. and Amodio-Cocchieri, R. (2009).** Polychlorinated biphenyls, organochlorine pesticides and polycyclic aromatic hydrocarbons in wild, farmed and frozen marine seafood marketed in Campania, Italy. *Journal of Food Protection*, 72(8): 1677-1685.
- Colom, K.; Pérez, J.; Alonso, R.; Fernández-Aranguiz, A.; Lariño, E. and Cisterna, R. (2003).** Simple and reliable multiplex PCR assay for detection of *bla_{TE}*, *bla_{SHV}* and *bla_{OXA-1}* genes in Enterobacteriaceae. *FEMS Microbiology Letters* 223: 147-151.
- Conte, F.S. (2004).** Stress and the welfare of cultured fish. *Appl. Anim. Behav. Sci.* 86: 205-223.
- Enany, M.E.; Hamza, M.I., Mohamed, E.A. and Mahmoud, M.E. (2011).** Bacteriological and histopathological studies on some bacterial pathogens causing diseases in cultured mugil capi-to fish in Ismailia governorate. *SCVMJ*, XVI (1):1-12.
- Ercolini, D. (2004).** PCR-DGGE fingerprinting: novel strategies for detection of microbes in food: a review. *Journal of Microbiological Methods* 56, 297-314.
- Fitzsimmons, K. (2000).** Tilapia the most important aquaculture species of the 21st century. 5th Int. symposium on tilapia aquaculture in the 21st century. Brazil, 7: 3-8.
- Grim, C.J.; Kozlova, E.V.; Sha, J.; Fitts, E.C. and van Lier, C.J. (2013).** Characterization of *Aeromonas hydrophila* wound pathotypes by comparative genomic and functional analyses of virulence genes. *MBIO*, 4: 1-13.
- Guz, L. and Kozinska, E. (2004).** Antibiotic susceptibility of *A. hydrophila* and *A. sobria* isolated from farmed carp (*Cyprinus carpio* L). *Bulletin of Veterinary Institute in Pulawy*, 48, 391-395.
- Hatha, M.; Vivekanandhan, A.A.; Joice, G. and Christol, J. (2005).** Antibiotic resistance pattern of motile aeromonads from farm raised fresh water fish, *Int. J. Food. Microbiol.*, 98(2): 131-134.
- Igbiosa, I.H.; Okoh, A.L. (2012).** Antibiotic susceptibility profile of *Aeromonas* species isolated from wastewater treatment plant. *Sc. World J.* 764563: 1-6.
- Isonhood, J.H., and Drake, M. (2002).** *Aeromonas* species in foods. *Journal of Food Protection*, 65(3): 575-582.
- Khériji, S.; El Cafsi, M.; Masmoudi, W.; Castell, J.D. and Romdhane, M.S. (2003).** Salinity and temperature effects on the lipid composition of mullet sea fry (*Mugil cephalus*, Linne, 1758). *Aquaculture International*. 11: 571-582.
- Kingombe, C.I.B.; Huys, G.; Tonolla, M.; Albert M.J.; Swings, J. and Peduzzi, R. (1999).** PCR detection, characterization and distribution of virulence genes in *Aeromonas* spp. *Applied and Environmental Microbiology*, 65: 5293-5302.
- Krishnan, S.; Rosamma, P. and Isaac, S.B.S. (2012).** Virulence potential and antibiotic susceptibility pattern of motile Aeromonads associated with freshwater ornamental fish culture systems: A possible threat to public health. *Brazilian Journal of Microbiology* 43 (2): 754-765.
- Luzzana, U.; Valfré, F.; Mangiarotti, M.; Domeneghini, C.; Radaelli, G.; Moretti, V.M. and Scolari, M. (2005).** *Aquaculture International*. 13:291-303.
- Majumdar, T.; Datta, S.; Ghosh, D.; Dutta, S.; Chakraborty, A. and Goswami, R. (2007).** Role of virulence plasmid of *Aero-*

- monas hydrophila* in the pathogenesis of ulcerative disease syndrome in *Clarias batrachus*. Ind J Biochem Biophys, 44 (5): 401–406.
- Martinez-Murcia, A.J.; Benlloch S. and Collins M.D. (1992).** Phylogenetic interrelationships of members of the genera *Aeromonas* and *Plesiomonas* as determined by 16S ribosomal DNA sequencing: lack of congruence with results of DNA-DNA hybridizations. Int. J. Syst. Bacteriol. 42: 412–421.
- Mohamed, H.B.; Abdel, A.A.K. and Nadia, A.D. (2009).** Seasonal variations of heavy metals concentrations in mullet, *Mugil cephalus* and *Liza ramada* (Mugilidae) from Lake Manzala, Egypt. Egypt J. Aquat. Biol. & Fish, 13(2): 81-100.
- NCCLS (National Committee for Clinical Laboratory Standards) (2004).** Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; 2nd ed. NCCLS M100-S14. Clinical Laboratory Standards Institute (formerly NCCLS), Wayne, PA.
- Neyts, K.; Huys, G.; Uyttendaele, M.; Swingsm, J. and Debevere, J. (2000).** Incidence and identification of mesophilic *Aeromonas* spp. from retail foods. Letters in Applied Microbiology 31 (5): 359-363.
- Nguyen, M.C.P.; Woerther, P.; Bouvet, M.; Andreumont, A.; Leclercq, R. and Canu, A. (2009).** *Escherichia coli* as reservoir for macrolide resistance genes. Emerging infectious diseases 15(10): 1648-1650.
- Nielsen, M.E.; Høi, L.; Schmidt, A.S.; Qian, D.; Shimada, T. Shen, J.Y. and Larsen, J.L. (2001).** Is *Aeromonas hydrophila* the dominant motile *Aeromonas* species that causes disease outbreaks in aquaculture production in the Zhejiang Province of China? Diseases of aquatic organisms 46: 23–29.
- Nifty, J. and Hatha, A.A.M. (2013).** Distribution, Extracellular Virulence Factors and Drug Resistance of Motile *Aeromonas* in Fresh Water Ornamental Fishes and Associated Carriage Water. International Journal of Aquaculture, 3(17):92-100.
- Poole, T.L.; Callaway, T.R.; Bischoff, K.M.; Warnes, C.E.; Nisbet, D.J. (2006).** Macrolide inactivation gene cluster *mphA-mrx-mphR* adjacent to a class 1 integron in *Aeromonas hydrophila* isolated from a diarrhoeic pig in Oklahoma. J Antimicrob Chemother, 57: 31–38.
- Porteen, K.; Agarwal, R.K.; Bhilegaonkar, K.N. (2007).** Detection of *Aeromonas* species from chicken and fish samples by polymerase chain reaction. Am. J. Food Technol. 2: 30-37.
- Saleh, M. (2008).** Capture-based aquaculture of mullets in Egypt. In: Lovatelli, A., Holthus, P.F. (Eds.), Capture-Based Aquaculture, Global Overview. : FAO Fisheries Technical Paper, 508, FAO, Rome: 109–126. Barman, U.K.
- Sanaa, R.O.; Rashid, M.M.; Majeed, A.S.; and Farah, T.A. (2014).** Detection of hemolysins (*hlyA* and *aerA*) Genes in *Aeromonas hydrophila* isolated from raw and drinking water in Baghdad City. International Journal of Advanced Research 2(2): 468-474.
- Seethalakshmi, I.; Subashkumar, R.; and Saminathan, P. (2008).** Distribution of putative virulence genes in *Aeromonas hydrophila* and *Aeromonas salmonicida* isolated from marketed fish samples. Journal of Fisheries and Aquatic Science, 3: 145-151.
- Sharma I. and Kumar A. (2011).** Occurrence of enterotoxigenic *Aeromonas* species in foods of animal origin in North East India. Eur. Rev. Med. Pharmacol. Sci., 15, 883-887.
- Shuang-Hu, C.; Zao-He, W.; Ji-Chang, Jian.; Yi-Shan, L. and Ju-Feng, T. (2012).** Characterization of pathogenic *Aeromonas Veronii* bv. *Veronii* associated with Ulcerative syndrome from chinese longsnout catfish (*leiocassis longirostris* günther). Brazilian Journal of Microbiology, 43(1): 382-388.
- Singh, V., Rathore, G., Kapoor, D., Mishra, B.N. and Lakra, W.S. (2008).** Detection of aerolysin gene in *Aeromonas hydrophila* isolated from fish and pond water. Indian J. Microbiol. 48: 453–458.
- Villari, P.; Crispino, M.; Montiuori, P. and Stanzione, S. (2000).** Prevalence and mo-

molecular characterization of *Aeromonas* species in ready to eat food in Italy. Journal of food protection, 63: 1754-1757.

Yogananth, N.; Bhagyaraj, R.; Chanthuru, A.; Anbalagan, T. and Mullai Nila, K. (2009). Detection of virulence gene in *Aeromonas hydrophila* isolated from fish samples using PCR technique. Global Journal of Biotechnology & Biochemistry 4 (1): 51-53.

Yousr, A.H.; Napis, S.; Rusul, G.R.A. and Son, R. (2007). Detection of Aerolysin and Hemolysin Genes in *Aeromonas* spp. Iso-

lated from Environmental and Shellfish Sources by Polymerase Chain Reaction. ASEAN Food Journal 14 (2): 115-122.

Yucel, N. and Citak, S. (2003). The occurrence, hemolytic activity and antibiotic susceptibility of motile *Aeromonas* spp. isolated from meat and milk samples in Turkey. J. Food Safety, 23: 189-200.

Yucel, N.; Aslim, B.; Beyatli, Y. (2005). Prevalence and resistance to antibiotics for *Aeromonas* species isolated from retail fish in Turkey. J. Food Quality, 28: 313-324.

التصنيف الجزيئي لميكروب الايرومونات في أسماك البوري باستخدام اختبار تفاعل إنزيم البلمرة المتسلسل جيهان محمد عمر محمد

معهد بحوث صحة الحيوان- قسم البكتريولوجى بمعمل بورسعيد

الملخص العربي

في هذه الدراسة تم عزل ٨ عترات *Aeromonas* من ١٠٠ عينة من سمك البوري المجمع من باعة مختلفة في سوق السمك بمدينة بورسعيد، للتعرف عن الميكروب بيوكيميائياً، جزيئياً و أيضاً تحديد مدى حساسية الميكروب للمضادات الحيوية. حيث أفضت النتائج إلى أن نسبة العينات الايجابية لبكتيريا الايرومونات كانت بنسبة ٨% . و كان تصنيفها كالاتي *Aeromonas hydrophila* بنسبة (٦٢.٥%) ، *Aeromonas sobria* بنسبة (٢٥%) و *Aeromonas caviae* بنسبة (١٢.٥%). وكانت كل العترات المعزولة من الأعضاء الداخلية (الكبد، الكلي و الطحال) للعينات تحت الفحص. و قد أجرى اختبار الحساسية للعترات المعزولة ل ١١ مضاد حيوى . و كانت اعلى مقاومة ل amoxycillin و Penicillin G بنسبة ١٠٠% و ٤ عترات فقط مقاومة لل erythromycin بنسبة ٥٠%. وباستخدام اختبار البلمرة المتسلسل (PCR) للتأكد من العترات المعزولة بانها *Aeromonas* وذلك باستخدام (16 SrRNA of *Aeromonas* spp.) والذي اعطى نتائج ايجابية بنسبة ١٠٠% . و أيضاً باستخدام (PCR) لتحديد جينات الضرواه (*Aero* and *hly*) فى عترات الـ *Aeromonas* المعزولة من سمك البوري، قد كشفت النتائج عن وجود كل من (*Aero*) جين و (*hly*) جين في بعض العترات. وباستخدام اختبار البلمرة المتسلسل للكشف عن تواجد جينات المقاومة للمضادات الحيوية بعدد ممثل للمعزولات، اظهرت النتائج تواجد ١٢ جين مكتسب مقاوم للمضادات الحيوية (*bla_{TEM}*) و (*mph(A)*) وهى المسؤولة عن مقاومة كل من Amoxicillin , Penicillin G و Erythromycin بأعداد مشابهة لاختبار الحساسية. وقد تم مناقشة النتائج وبيان أهمية الميكروب المعزول و خطورته علي الصحة العامة للمستهلك وعمل التوصيات للتقليل من مخاطره.