Egypt. J. Vet. Sci. **Vol. 54,** No. 5, pp. 895-905 (2023)

Egyptian Journal of Veterinary Sciences
https://ejvs.journals.ekb.eg/

Molecular Identification of 16S rRNA and Some Virulence Genes From Aeromonas spp. in Nasser Lake Fish Species, Aswan, Egypt

Aml Mokhtar[1]*, Mohamed Karmi[2], Yosra M. El Shery[3] and Marwa A. Ali[1]

[1]*Department of Microbiology and Immunology, Faculty of Veterinary Medicine, Aswan University, Egypt.*

[2]*Department of Food Hygiene, Faculty of Veterinary Medicine, Aswan University, Aswan 81528, Egypt.*

[3]*Department of Fish diseases, Faculty of Veterinary Medicine, Aswan University, Aswan, Egypt.*

FRESHWATER fish consider a major cheap source of animal protein in different parts of the world. In upper Egypt, Lake Nasser is the main source of fish production for human consumption. Fish can also be a cause of foodborne pathogens, such as the Aeromonas species, that pose a significant risk to public health. A total of 180 freshwater fish samples were represented, Nile tilapia, Nile perch, Pike perch, African sharp tooth catfish, Elephant-snout, and Fillet tilapia (30 of each) were randomly selected from Lake Nasser in Aswan province. Bacteriological examination reveals 117 (65%) occurrence of Aeromonas species. In isolated 60 Aeromonas species samples10 from each fish species, 16S rRNA genes were found with a percentage of 96.67% by using PCR. In Egypt and around the globe, the Aeromonas species has been linked to several foodborne outbreaks brought on by the consumption of raw fermented fish which having many sever virulence gene . Additionally, two genes; Aerolysin (aer) and cytotoxic enterotoxin (act) genes were selected to be screened by PCR. Results were 51.7% and 27.6%, respectively. So hygienic precautions must be taken to eradicate Nasser Lake Fish species contaminated with Aeromonas species. Molecular investigating of some virulence genes of Aeromonas spp. in Lake Nasser from different fish species was the aim of the present study.

Keywords:Aeromonas species, 16S rRNA, Aerolysin (aer) gene, Cytotoxic enterotoxin (act) gene, PCR.

Introduction

Aeromonas spp. are Gram-negative, facultative anaerobes, non-spore-forming rods, positive for oxidase and catalase, resistant to the vibrio static O/129, capable to reduce nitrates to nitrites and motile spp. have a single polar flagellum that usually lives water environment [1]. Aeromonas is divided into two clusters; the first one is non-motile species like A. salmonicida that cause disease in fish, another group includes motile species such as A. hydrophila which are implicated in human infections including gastroenteritis, peritonitis septicemia, urinary tract infection in neonates, osteomyelitis and wound infections [2] . Aeromonas species may spread through raw seafood [3]. Aeromonads have been associated with skin and soft-tissue infections, these pathogenic organisms have been recognized as a cause of foodborne and waterborne outbreaks

*Corresponding author: Aml Mokhtar, Email: amlmokhtar2011@hotmail.com Tel.: 01007088609
(Received 26/01/2023, accepted 15/06/2023)
DOI: 10.21608/EJVS.2023.189229.1435
©2023 National Information and Documentation Center (NIDOC)

[4] . Fish, however, can be a source of foodborne bacteria, such as the Aeromonas species, which have been identified as developing foodborne poisoning that pose a significant risk to community health [5]. Aeromonas members are related to foodstuff poisoning as well as certain human illnesses like abdominal and extra-intestinal disorders like skin and traumatic wound diseases, lower respiratory tract/urinary tract infections and soft-tissue illnesses [6] . Aeromonas is environmental microbe and their spread is global. Mesophilic motile aeromonads are common and indigenous aquatic microbes that can be found in both chlorinated and unchlorinated drinking water as well as sewage and brackish water [7]. The capability of A. hydrophila to produce diseases relates to a diversity of pathogenic factors which are compound and multifactorial [8]. A. hydrophila can produce a variety of virulence factors, especially toxins responsible for gastrointestinal infections including hemolysin, aerolysin, cytotoxin, enterotoxin, lipopolysaccharide and lipases, proteases gelatinase, DNases, and elastase enzymes [9,10]. Molecular identification is the most accurate identification tool of fish pathogens even in early infection phases. The rapid, confirmatory and accurate microbial identification in recent years is the molecular diagnosis, as well as the 16S rRNA gene, which is considered a stable molecular marker for identifying bacterial species since its distribution is universal and allows the comparison of microorganisms [11]. Some virulence genes were found in the isolated A. hydrophila, such as cytotoxic enterotoxins heat-stable (ast) and cytotoxic enterotoxin (act), while cytotoxic enterotoxin (alt) and hemolysin (hly) genes were absent. In contrast, the hly gene was present in A. hydrophila strains, and by amplification, the PCR products of 1500 bp were given [12,13]. Aerolysin and cytotoxic enterotoxin are an important and reliable molecular indicator for finding potentially pathogenicity of Aeromonas species . Aerolysin toxin lyses RBCs causing hemorrhagic lesions . Aerolysin toxin was found A. hydrophila, A. caviae, A. sobria, and A. veronii. Aer gene is absent in non-hemolytic A. hydrophila, but it can be absent in hemolytic A. caviae and A. sobria too [14] .The act gene encodes a type II secreted cytotoxic enterotoxin (Act) that is fatal to mice by having hemolytic, cytotoxic, and enterotoxic effects [15]. The current research focused on determining the occurrence of Aeromonas species isolated from various species of fish from Lake Nasser in Aswan governorate. A vital role in the pathogenesis of Aeromonas sp. infection in fish is played by virulence genes. They are connected to various human illnesses and food poisoning. Two genes; Aerolysin (aer) and cytotoxic enterotoxin (act) genes were selected to be screened in this study.

Material and Methods

Collection of samples

One hundred eighty fish samples in different species were collected from Nasser Lake, Aswan, Egypt, in 2020-2021. 30 of each were represented Nile tilapia, Nile perch, Pike perch, African sharp tooth catfish, Elephant-snout, and Fillet tilapia weighing between 150 to 250g. The specimens were packed in sterilized flexible bags and transported, in an icebox without delay, to the lab of Microbiology and immunology Department, Faculty of Veterinary Medicine, Aswan University, where microbiological examination were performed.

Samples preparation

Under complete aseptic condition, 25 g of each fish muscle and viscera samples were aseptically transported into a sterilized homogenizer at 14000 rpm for 3 min then mixed with 225 ml sterile Tryptic soy broth (Oxoid:CM0129) incubated at 37 °C for 24 hrs. [16].

Isolation of Aeromonas species

From each enriched homogenate, a loopful was streaked on duplicate sterile Petri plates of Aeromonas Agar media supplemented with ampicillin (5 mg/L) (Biolife: CN0801), after incubation of plates for 24 hs at 28 °C the appearance of green colonies with dark centers were theoretically considered to be Aeromonas spp. [17].

Morphological and biochemical identification of Aeromonas species

Phenotypic recognition of positive samples was shown as stated by Austin & Austin [18] according to (Table 1 and Fig.1) based on morphological, biochemical, and metabolic properties. By optical microscopy using Gram's stain, Confirmed the isolates by biochemical characteristics, catalase ,oxidase, growth at 4%, 10% NaCl, vibriostatic compound 0/129 (Thermo Scientific™, DD0015T) ,blood agar (Oxoid: CM0259) and Triple sugar iron (Oxoid: CM0277) esculin hydrolysis, indole, arginine hydrolysis, methyl red, Voges

Proskauer, hydrogen sulphide production, citrate utilisation, urease, nitrate reduction, gelatin liquefaction, ornithine decarboxylase, oxidation fermentation, L-lysine decarboxylase, arginine decarboxylase,-galactosidase, oxidation and sugar fermentation (Macfaddin, 2000). As shown in Table (2), suspected Aeromonas colonies were biochemically verified.

Molecular identification of selected isolates: Amplification of16S rRNA gene for the Aeromonas genus

According to the manufacturer's recommendations .The extraction of DNA was done by QIAamp DNA Gene JET Genomic DNA Purification Kit (Catalog No. #K0721, Thermo Scientific, USA). The reaction contained 25 µl of the following PCR master mix; 12.5 µl of COSMO PCR RED Master Mix (2x premix), 4.5 µl PCR grade water, 1 µl forward primer (20 pmol), 1 µl reverse primer (20 pmol), 6 µl template DNA. The amplification of the 16S rRNA gene for the Aeromonas genus was performed using primer pair F 5' AGAGTTTGATCATGGCTCA 3' and R 5'GGTTACCTTGTTACGACTT 3' amplifying 1502bp [19]. The amplification cycles (n=35) were carried out as follows: 5 min of primary denaturation at 94°C, 60 sec of secondary denaturation at 94°C, 56 sec of annealing at 60°C, 2 min of extension at 72°C, and 10 min of ultimate extension at 72°C. Electrophoresis in 1.5% agarose gel was used to identify the amplified products (Applichem, Germany, GmbH). Gel recording system (Alpha Innotech, Biomedia) was used for photography, and computer software was used for analysis.

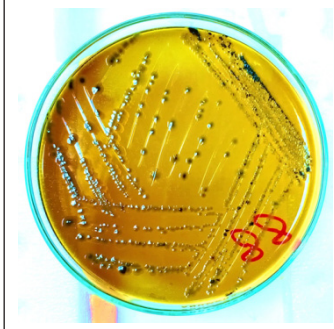
Amplification of Aerolysin and cytotoxic enterotoxin genes

For detection of virulence genes, amplification of Aerolysin (aer) gene primer with nucleotide sequences of F 5'-AACCGAACTCTCCAT-3' and R 5'-CGCCTTGTCCTTGTA-3' (product size of 301 bp) and Cytotoxic enterotoxin (act) gene primer with nucleotide sequences of F 5'-GAGAAGGTGACCACCAAGAACA-3' and R 5'-AACTGACATCGGCCTTGAACTC-3' (product size of 232 bp) were done according to Hu et al.[20] Table (4). All primers in this study were synthetized by Willow Fort Company (United Kingdom). Initial denaturation cycle was done at 94°C for 5 min, then 30 cycles at 94°C for 30 seconds, 54°C for 30 sec for aerolysin gene / 42°C for 30 seconds for Cytotoxic enterotoxin gene and then 72°C for 1 min followed by last extension at 72°C for 10 mins. Aliquots from PCR reactions were electrophoresed on 1.5% agarose gel and viewed under UV light.

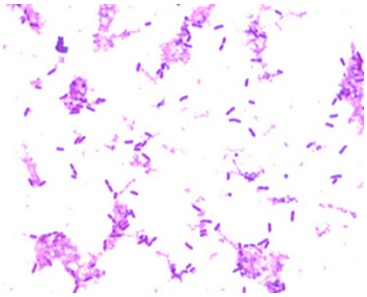
Statistical Analysis

TABLE 1. Conventional identification of *Aeromonas spp.*

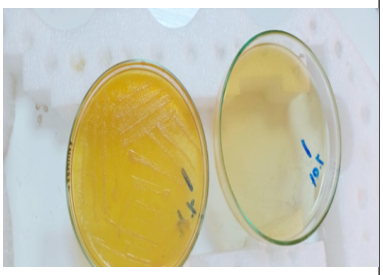
Fish sp.	No.	Cultural identification		Biochemical identification													
				Catalase		Oxidase		Blood agar		Growth on TSI		Growth with NaCl 4%		Growth with NaCl 10%		VSD	
		+Ve No.	-Ve No.	+Ve No.	-Ve No.	+Ve No.	-Ve No.	+Ve No.	-Ve No.	+Ve No.	-Ve No.	+Ve No.	-Ve No.	+Ve No.	-Ve No.	+Ve No.	-Ve No.
Nile Tilapia	30	21	9	20	10	19	11	18	12	16	14	20	10	19	11	19	11
Nile perch	30	19	11	15	15	13	17	13	17	12	18	17	13	13	17	13	17
Pike perch	30	17	13	14	16	15	15	13	17	13	17	16	14	15	15	15	15
African sharptooth catfish	30	21	9	18	12	17	13	16	14	17	13	18	12	17	13	17	13
Elephant-snout	30	12	18	10	20	12	18	11	19	11	19	12	18	10	20	10	20
Fillet Nile Tilapia	30	18	12	17	13	13	17	14	16	14	16	18	12	13	17	13	17
Total	180	108	72	94	86	89	91	85	95	83	97	101	79	87	93	87	93



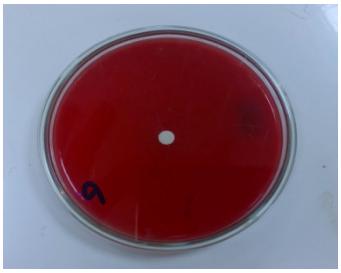
(A) Dark green, opaque with dark center colonies on ASA



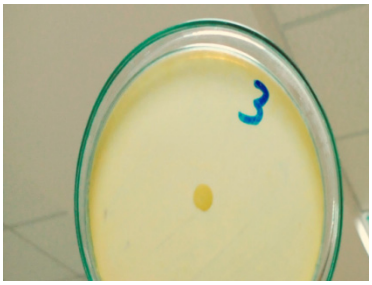
(B) Gram-negative rods by Gram's stain



(C) Growth on TSA with 4% and 10% NaCl



(D) Resistance to vibriostatic disc 0/129 on Blood agar



(E) Resistance to vibriostatic disc 0/129 on MHA

(F) Alkali slant/acidic butt with H_2S formation on TSI

Fig. 1. Conventional identification of the suspected isolates

According to Feldman et al. [21] Statistical Analysis of Variance (ANOVA) was done for significant differences between samples.

Results

Conventional identification of isolates biochemically : according to the data in Table (3) in the current study positive Aeromonas spp.117 (65%) isolates out of 180 fish samples were distributed as 24 (80%) Nile tilapia, 21 (70%) Catfish, 20 (66.7%) Pike perch, 19 (63%) Nile perch, 18 (60%) Fillet tilapia and 15 (50%) Elephant-snout respectively. According to the data there are 4 species of Aeromonas were represented by 117 (65%) isolates: A. hydrophila 78 (43.3%), A. caviae 26 (14.4%), A. sobria 7 (3.8%) and A. veronii 6 (3.3%), On the other hand, 10 samples biochemically identified Aeromonas spp. are taken from each fish species introduced in PCR findings showed that 16S rRNA was present in 58 (96.67%) out of a total of 60 isolates, distributed into 10 Nile tilapia, 10 Nile perch, 10 Pike perch, 10 Fillet tilapia, 9 Catfish and 9 Elephant-snout, respectively (Table 5 and Fig.2). Detection of other virulence genes, aerA gene was detected in 30 (51.7%) out of 58 isolates previously detect of Aeromonas spp. 16S rRNA gene distributed into 7 Nile tilapia, 3 Nile perch, 5 Pike perch, 4 Catfish, 5 Elephant-snout, and 6 Fillet tilapia, respectively (Fig.3). Furthermore, the act gene was detected in 16 (27.6%) out of 58 isolates contingent on the detection of A Aeromonas spp. 16S rRNA gene distributed into 4 Nile tilapia, 2 Nile perch, 2 Pike perch, 2 Catfish, 3 Elephant-snout, and 3 Fillet tilapia respectively (Table 6 and Fig.4).

Discussion

Nowadays microbiological examination of fish is very important for maintaining public health. In current study Aeromonas spp. was isolated from examined fish samples with a percentage of 65%, according to bacteriological analysis. Nile tilapia had the greatest isolation percentage (80%), followed by Cat fish (70%), pike perch (66.7%), Nile perch (63.3%) , Fillet tilapia (60%), and Elephanet snout (50%). The prevalence as, A. hydrophila (43.3%), A. caviae (14.4%), A. sobria (3.8%) and A. veronii (3.3%) (Table 3). In recent fish study in Egypt, the nearly similar result

TABLE 3. Prevalence of Aeromonas species in the examined fresh fishsamples (n=30 each)

Fish species (number 30)	*Aeromons sp.*(+)	*A. hydrophila*	*A. Caviae*	*A. veronii*	*A. sobria*
1-Nile Tilapia (*Oreochromis niloticus*)	24/30 (80%)	13/30 (43.3%)	7/30 (23.3%)	3/30 (10%)	1/30 (3.33%)
2- African sharptooth catfish *(Clariasgariepinus)*	21/30 (70%)	17/30 (56.7%)	3/30 (10%)	1/30 (3.33%)	-
3- Pike perch (*Sander lucioperca*)	20/30 (66.7%)	15/30 (50%)	3/30 (10%)	1/30 (3.33%)	1/30 (3.33%)
4- Nile perch (*Lates niloticus*)	19/30 (63.3%)	13/30 (43.3%)	4/30 (13.33%)	-	2/30 (6.7%)
5- Fillet Nile Tilapia	18/30 (60%)	10/30 (33.3%)	5/30 (16%)	1/30 (3.33%)	2/30 (6.7%)
6- Elephant-snout (*Mormyrus kannume*)	15/30 (50%)	10/30 (33.3%)	4/30 (13.3%)	-	1/30 (3.33%)
Total (180)	117/180 (65%)	78/180 43.3%	26/180 14.4%	6/180 3.3%	7/180 3.8%

TABLE 4. Primers used in the present study

Target gene	Primers sequences	Amplified segment	References
16S-rRNA	**F 5' AGAGTTTGATCATGGCTCA 3'** **R 5' GGTTACCTTGTTACGACTT 3'**	**1502 bp**	[19]
aerA	**F 5´ AACCGAACTCTCCAT 3´** **R 5´ TTGTCCGGGTTGTACTCGTC 3´**	**301 bp**	[20]
act	**F 5´ GAGAAGGTGACCACCAAGAAC 3´** **R 5´ AACTGACATCGGCCTTGAACTC 3´**	**232 bp**	

TABLE 5. Genotypic identification of 16S rRNA gene in the isolated Aeromonas species

Fish spp.	No. of examined samples	Identified 16S rRNA gene	
		No.	%
Nile tilapia	10	10	100
Nile perch	10	10	100
Pike perch	10	10	100
Catfish	10	9	90
Elephant-snout	10	9	90
Fillet tilapia	10	10	100
Total samples	60	58	96.67

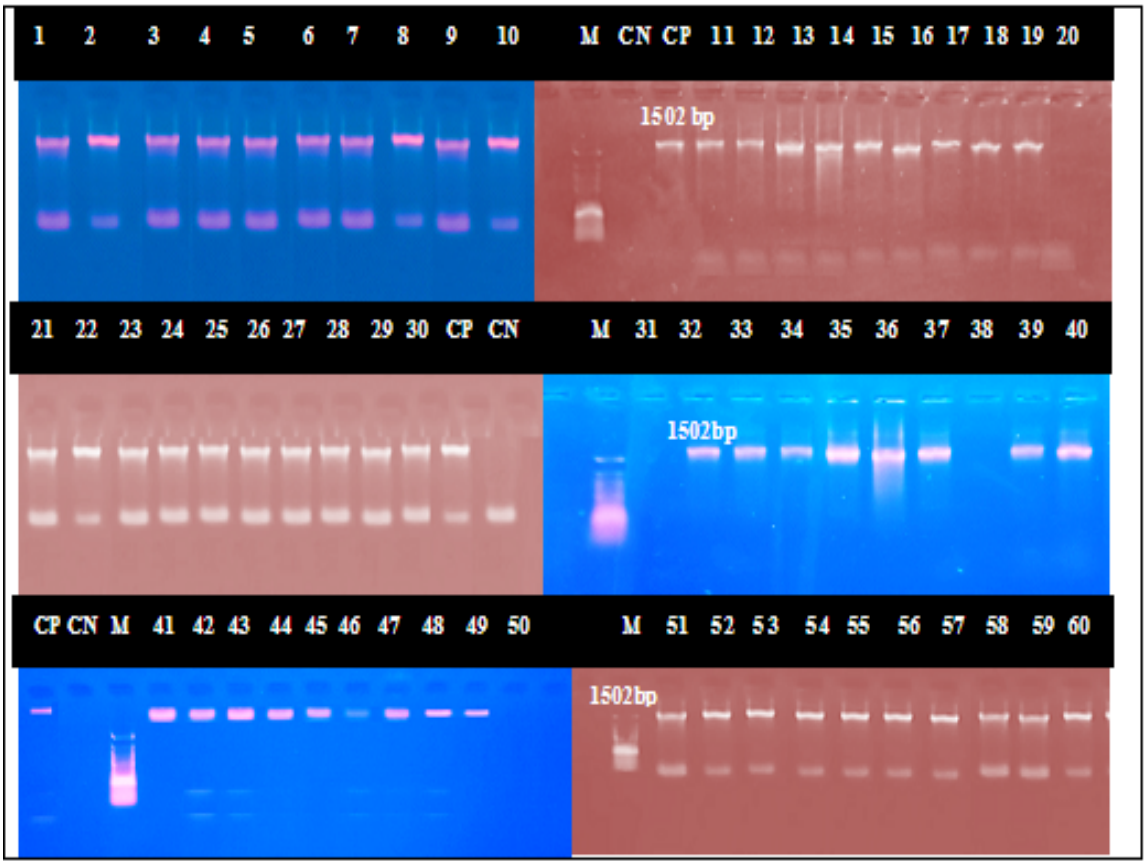


Fig. 2. Electrophoretic gel Fig.for detection of 16S rRNA gene in *A. hydrophila* detected by conventional PCR where CN= control negative, CP= control positive at 1500bp, M= Marker, Lane 1 to Lane 10: Nile tilapia, Lane 11 to Lane 20: Nile perch, Lane 21 to Lane 30: Pike perch, Lane 31 to Lane 40: Elephant-snout, Lane 41 to Lane 50: Catfish and Lane 51 to Lane 60 Fillet tilapia samples.

TABLE 6. Occurrence of virulence genes in the Aeromonas species isolated from the examined samples

	16S rRNA gene Identified A. hydrophila	Virulence genes			
		AerA		Act	
		No.	%	No.	%
Nile tilapia	10	7	70	4	40
Nile perch	10	3	30	2	20
Pike perch	10	5	50	2	20
Catfish	9	4	44.4	2	22.2
Elephant-snout	9	5	55.5	3	33.3
Fillet tilapia	10	6	60	3	30
Total samples	58	30	51.7	16	27.6

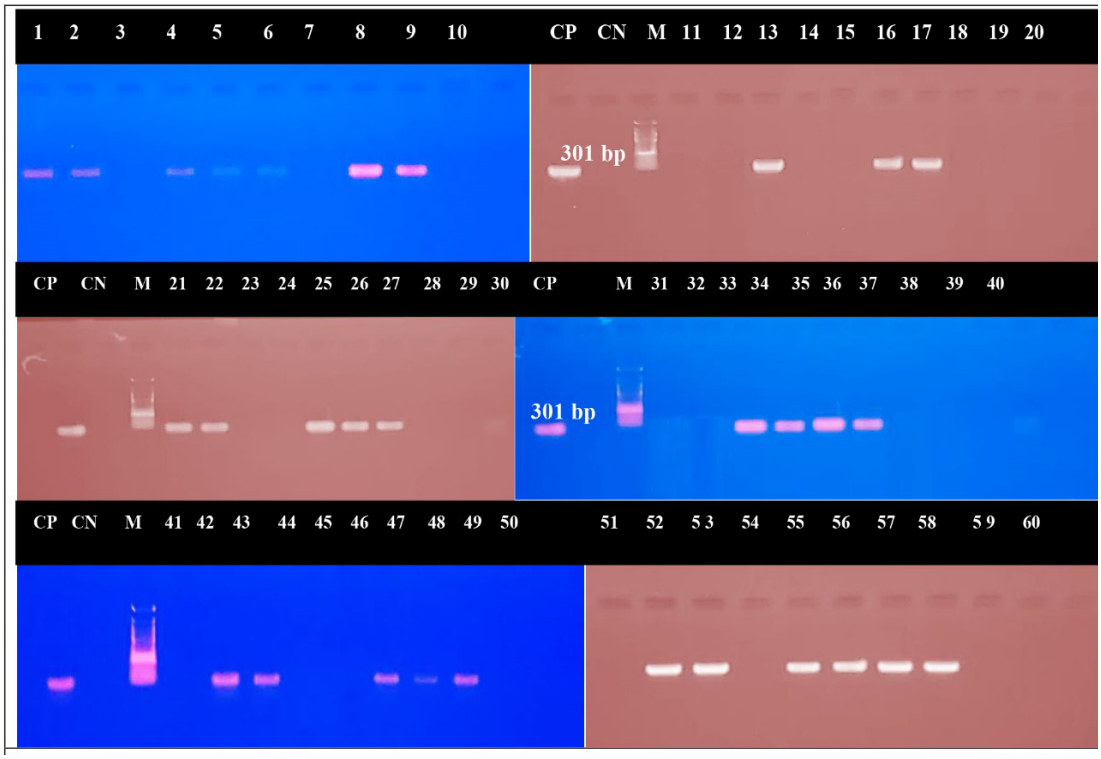


Fig. 3. Electrophoretic gel Fig.for detection of aerolysin (*aerA*) gene in *A. hydrophila* detected by conventional PCR where CN= control negative, CP= control positive at 303bp, M= Marker (100bp), Lane 1 to Lane 10: Nile tilapia, Lane 11 to Lane 20: Nile perch, Lane 21 to Lane 30: Pike perch, Lane 31 to Lane 40: Elephant-snout, Lane 41 to Lane 50: Catfish and Lane 51 to Lane 60 Fillet tilapia samples.

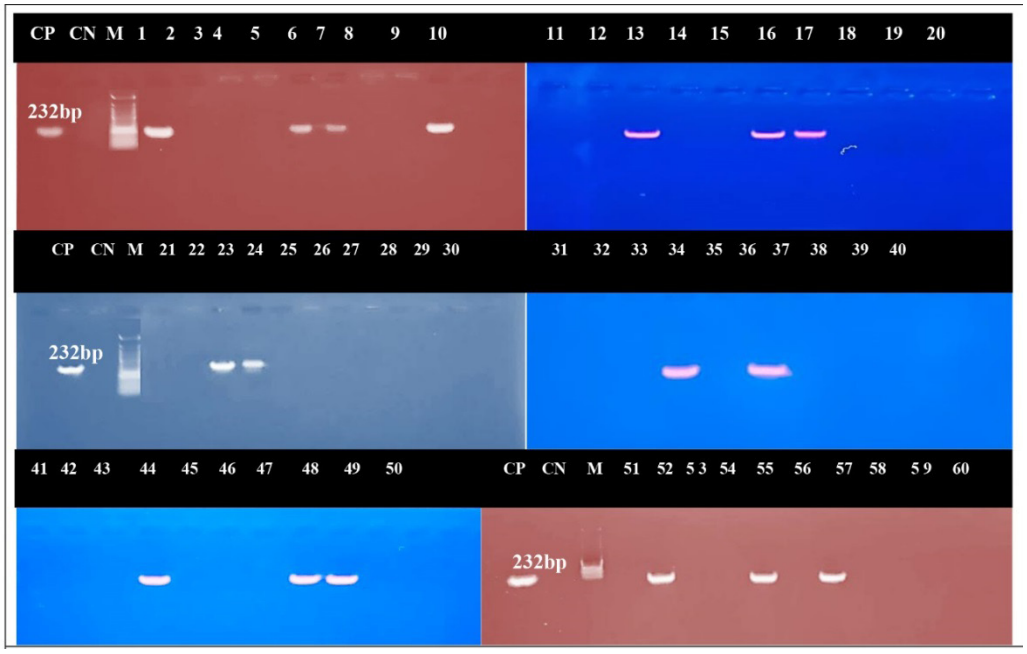


Fig. 4. Electrophoretic gel Fig.for detection of aerolysin enterotoxin (*act*) in *A. hydrophila* detected by conventional PCR where CN= control negative, CP= control positive at 232bp, M= Marker (100bp), Lane 1 to Lane 10: Nile tilapia, Lane 11 to Lane 20: Nile perch, Lane 21 to Lane 30: Pike perch, Lane 31 to Lane 40: Elephant-snout, Lane 41 to Lane 50: Catfish and Lane 51 to Lane 60 Fillet tilapia samples.

found by Morshdy et al.[22] Aeromonas spp. in 39.3% of the fish samples they investigated, Four Aeromonas species (A. hydrophila, A. caviae, A. fluvialis and A. sobria) were isolated from the tested fish samples (12%, 15.3%, 2.7% and 9.3%, respectively), with A. hydrophila is the most frequently found isolates from Nile tilapia . According to Kishk et al.[23], the incidence of Aeromonas species in Nile tilapia 32 (64%). The most prevalent Aeromonas species isolated from Nile tilapia was Aeromonas caviae 13 (40.6%), Aeromonas hydrophila 8 (25%), Aeromonas sobria 7 (21.9%), Aeromonas veronii 3 (9.4%), and Aeromonas fluvialis 1 (3.1%). Ebeed et al.[24], revealed that the incidence of Aeromonas spp. in Nile tilapia was 34 (68%).The most frequently identified Aeromonas species isolate from Nile tilapia was A. caviae 18 (36%), Aeromonas sobria 14 (28%). But, Ismail et al.[25] revealed 20.3% A. hydrophila is the most frequently found isolates (20.3%) from Clarias gariepinus fish (Catfish). Additionally fish study in Dhanapala et al. [26] identify eight distinct Aeromonas spp. from freshwater fish as A. veronii (75.8%), A. hydrophila (9.3%), A. caviae (5%), A. jandaei (4.3%), A. dhakensis (3.7%), and 0.6% for each of A. sobria, A. media and A. popoffii. Our results of the PCR indicated that 58 (96.67%) out of a total of 60 isolates biochemically identified Aeromonas spp , the 16S rRNA distributed into 10 Nile tilapia, 10 Nile perch, 10 Pike perch, 9 Catfish, 8 Elephant-snout, and 10 Fillet tilapia, respectively. On the other hand, Aer gen detected in 30 out of 58 (51.7%) and Act gene detected in 16 out of 58 (27.6%) The isolation of 16S rRNA can be used to validate the isolation of Aeromonas spp. [27,28] 40.67% was confirmed as A. hydrophila by using PCR amplification based on16S rRNA gene analysis. [29] 42% of the fish samples have A. hydrophila depending on the occurrence of the 16S rRNA. [30-32], reported that 11.47% , 75.4% and 50.65% of freshwater fish were recognized as A. hydrophila using the 16S rRNA. These variances may be because of the altered species, geographical location, and selection time. Moreover, The high prevalence rate achieved by Elsheshtawy et al. [33] reported that 100% of tested samples recognized as Aeromonas spp. depend on the 16S rRNA detection. Aer gene was detected in 53.57% of isolates that was confirmed by the hemolysis occurred on the blood agar [14, 34,31] were recorded aerA gene has more commonly occurred between isolates with an incidence of 64.3% and 80.5% respectively. From another point, Lower results were reported in previous studies by Abdel-Latef [35] who indicated the aerA gene in examined fish with a lower value of 3.3%. Act gene was detected in 30.35% of isolates [15,36] documented that 80% of examined samples harbored act genes. As well Nhinh et al.[31] identified the acting gene in 80.1% of the examined fish samples. Furthermore, Ahangarzadeh et al. [37] reported that (74.19%) of isolates possessed act genes. 40% of Nile tilapia and 30% tilapia fillet Aeromonas isolates are carrying both aer and act gene together that makes them a great threat for human health via consumption of their meat. Also Cat fish (70%) and pike perch (78%) consider of great health concern as most of isolates carrying virulence genes.

Conclusion

In addition to affecting fish quality, shelf life, and acceptability, Aeromonas species infection in fishes is a risk to the public's health. Therefore, hygienic measures must be taken to control microbial contamination either in the aquatic environment or during the transportation of seafood to consumers. Using 16s rRNA primer together with aer and act genes can be used as fast screening methods for determination of virulence of Aeromonas sp.

Acknowledgement

The authors would like to express thanks and appreciation to the team work of Lake Nasser Fisheries In Aswan, Upper Egypt

Conflict of interest

No conflict of interest

Authors contribution

The research is extracted from a Master's thesis in the Department of Microbiology and Immunology for the fourth author

Funding statement

Personal researchers fund

References

1. Martin-Carnahan, A. and Joseph, S.W. Family Aeromonadaceae Colwell, MacDonell and DeLey the percentage of 4%. 1986. Bergey's Manual of Systematic Bacteriology, vol. **2**, part B, New York, Springer, 557–578(2005).

2. Daskalov, M. The importance of Aeromonas hydrophila in food safety. *Food Control,* **17**(6), 474–483(2006).

3. Jag Pal, B.N. Shukla, Ashish Kumar Maurya, Hari Om Verma, Gayatri Pandey and Amitha. A review on role of fish in human nutrition with special emphasis to essential fatty acid. *International Journal of Fisheries and Aquatic Studies,* **6**(2), 427-430(2018).

4. Moyer, N.P. Aeromonas. In: Anonymous. Waterborne pathogens (M48). 2nd ed. Denver, CO: *American Water Works Association*, p. 81-85(2006).

5. Igbinosa, I.H., Igumbor, E. U., Aghdasi, F., Tom, M. and Okoh, A.I . Emerging Aeromonas species infections and their significance in public health. *The Scientific World Journal,* **2012**, 625023(2012). doi: 10.1100/2012/625023. Epub 2012 Jun 4.

6. Batra, P., Mathur, P. and Misra, M.C. Aeromonas spp.: an emerging nosocomial pathogen. Journal of Laboratory Physicians, **8**(1), 1–4(2016). doi: 10.4103/0974-2727.176234

7. Abraham, T.J. Food safety hazards related to emerging antibiotic resistant bacteria in cultured freshwater fishes of Kolkata, India. *Advanced Journal of Food Science Technology*, **3**(1), 69-72 (2011).

8. Lee, H.J., Hoel, S., Lunestad, B.T., Lerfall, J. and Jakobsen, A.N. Aeromonas spp. isolated from ready-to-eat seafood on the Norwegian market: Prevalence, putative virulence factors and antimicrobial resistance. *Journal of Applied Microbiology,* **130**, 1380–1393(2020).

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

10. Umesha, D., Srinivasa Rao, P., Pani Prasad, K., Reddy, A.K. and Srinivas , K.N. Aerolysin and haemolysin virulence genes of Aeromonas hydrophila isolated from diseased ornamental freshwater Oscar fish and goldfish by Polymerase Chain Reaction. *International Journal Advanced Science and Technology,* **3**(1), 82–89 (2011).

11. Vega-Sanchez, V. Caracterization fenotipica, Y., genotipica de aislamientos de. Aeromonas spp. obtenidos de trucha arcoiris (Oncorhynchus mykiss); Universidad Autonoma del Estado de Mexico:Toluca, Mexico (2015).

12. Stratev, D.; Gurova, E.; Vashin, I. and Daskalov, H. Multiplex PCR detection of haemolysin genes in B hemolytic Aeromonas hydrophila strains isolated from fish and fish products. *Bulgarian Journal of Agricultural Science,* **22**(2), 308-314(2016).

13. Sherif, A.H., Prince, A., Adel Seide, A., Saad Sharaf, M., Eldessouki, E.A. and Harfoush, M.A. Moringa oleifera mitigates oxytetracycline stress in Oreochtomis niloticus. *Aquaculture Research,* **53** (5),1790-1799(2022).

14. Vijai Singh, Gaurav Rathore, Kapoor, D., Mishra, B. N. and Lakra1, W. S. Detection of aerolysin gene in Aeromonas hydrophila isolated from fish and pond water. *Indian Journal of Microbiology*, **48**, 453-458(2008).

15. Jian Sha, Lakshmi Pillai, Amin A. Fadl, Cristi L. Galindo, Tatiana E. Erova and Ashok K. Chopra. The type III secretion system and cytotoxic enterotoxin alter the virulence of Aeromonas hydrophila. *Infection and Immunity*, **73**(10), 6446-6457 (2005).

16. Buller, N.B. Bacteria from fish and other aquatic animals. A practical I18- Austin, B. (1999): Aeromonas: Detection by cultural and modern techniques. In In Encyclopedia of Food Microbiology, Academic Press, London (pp. 30-37) (2004).

17. Kamble, S.R., Meshram, S.U. and Shanware, A.S. PCR detection of aerolysin from Aeromonas sp. isolated from diseased and healthy fish. *J. Environ. Res. Develop.,* **5**, 758-763(2011).

18. Austin, B. and Austin, D.A. Aeromonadaceae representatives (Motile Aeromonads). Bacterial fish pathogens. Disease of farmed and wild fish, 6th edition, Springer Nature publishing pp. 161-214(2016).

19. Borrell, N., Acinas , S.G., Figueras, M. J . and Murcia, A.J.M. Identification of Aeromonas clinical isolates by restriction fragment length polymorphism of PCR-amplified 16S-rRNA genes. *Journal of Clinical Microbiology,* **35**, 1671-1674(1997).

20. Hu, M., Wang, N., Pan, Z.H., Lu, C.P., Liu, Y.J. Identity and virulence properties of Aeromonas isolates from diseased fish, healthy controls and water environment in China. *Letters in Applied Microbiology*, **55**, 224-233(2012).

21. Feldman, D., Ganon, J., Haffman, R. and Simpson, J. *The Solution for Data Analysis and Presentation Graphics*. 2nd ed., Abacus Lancripts, Inc., Berkeley, USA. (2003).

22. Morshdy A.M.A., Nehal S.A. Abdelhameed, Rasha M. El Bayomi and Karima Abdallah. Prevalence of Antibiotic Resistant Aeromonas and Molecular Identification of Aeromonas hydrophila Isolated from Some Marketed Fish in Egypt. *Journal of Advanced Veterinary Research,* **12** (6), 717-721(2022)

23. Kishk, D., Moustafa, N.Y. and Kirrella, G.A. Prevalence and virulence characteristics of Aeromonas species isolated from fish farms in Egypt. *Kafrelsheikh Veterinary Medical Journal*, **18**, 5-8(2020).

24. Ebeed, A.S., Alaa Eldin, M.A., Mohamed, A.M. and Basma, F.E. Prevalence of Aeromonas species and their herbal control in fish. *Global Veterinary*, **18**, 286-293 (2017).

25. Ismail, S. M., Mohamed W. A., Mahmoud, M. M. and Aml Mokhtar, M. A. Microbiology studies on the affections of skin in sharp tooth catfish (Clarias gariepinus). *Assiut Vet. Med. J. ,* **59** (137), 157(2013).

26. Dhanapala, P.M., Kalupahana, R.S., Kalupahana, A.W., Wijesekera, D.P.H., Kottawatta, S.A., Jayasekera, N.K. and Jagoda, S.D.S. Characterization and antimicrobial resistance of environmental and clinical Aeromonas species isolated from fresh water ornamental fish and associated farming environment in Sri Lanka. *Microorganisms,* **9**, 2016 (2021).

27. Venkataiah, P., Poojary, N.S. and Harshvardhan, B. A multiplex PCR for detection of haemolytic aeromonas hydrophila from vegetable sources in Karnataka, India. *Recent Research in Science and Technology,* **5**(3),19-23 (2013).

28. El-ghareeb, H., Zahran, E. and Abd-Elghany, S. Occurrence, Molecular Characterization and Antimicrobial Resistance of Pathogenic Aeromonas Hydrophila from Retail Fish. *Alexandria Journal of Veterinary Sciences*, **62** (1), 172-181(2019).

29. Rodrigues, M.V., Maria, F., Claire, J., Gianmarco, S., Reinaldo, J. and João, P. Aeromonas hydrophila in Nile tilapia (Oreochromis niloticus) from Brazilian aquaculture: a public health problem. *Emergent Life Sciences Research,* **5**(1), 48-55 (2019).

30. Fauzi, N., Hamdan, R., Mohamed, M., Ismail, A., Zin, A. and Mohamad, N. Prevalence, antibiotic susceptibility, and presence of drug resistance genes in Aeromonas spp. isolated from freshwater fish in Kelantan and Terengganu states, Malaysia. *Veterinary World,* **14**(8), 2064-2072(2021).

31. Nhinh, D.T., Le, D.V., Van, K.V., Huong Giang, N.T., Dang, L.T. and Hoai, T.D. Prevalence, Virulence Gene Distribution and Alarming the Multidrug Resistance of Aeromonas hydrophila Associated with Disease Outbreaks in Freshwater Aquaculture. *Antibiotics,* **10**, 532 (2021).

32. Otero-Olarra, J.E., Curiel-Quesada, E., Baltazar-Cruz, J., Aguilera-Arreola, M.G. and Perez-Valdespino, A. Low Cassette Variability in Class 2 and Class 1 Integrons of Aeromonas Spp. Isolated from Environmental Samples. *Microb. Drug Rsistance,* **26**, 794–801(2020).

33. Elsheshtawy, A., Yehia, N., Elkemary, M. and Soliman, H. Investigation of Nile tilapia Summer Mortality in Kafr El-Sheikh Governorate, Egypt. *Genetics of Aquatic Organisms*, **3**(1), 17-25 (2019).

34. Emeish, W.F., Mohamed, H.M. and Elkamel, A.A. Aeromonas Infections in African Sharptooth Catfish. *Journal of Aquatic Research Development,* **9**, 548 (2018).

35. Abdel-Latef, G.K. Detection of aerolysin, hemolysin genes and antimicrobial susceptibility of Aeromonas hydrophila isolated from retail foods and human stool samples. *Global Veterinaria*, **14** (4), 528-534(2015).

36. Noor El Deen, A.E., Dorgham, S.M., Hassan, A.H. and Hakim, A.S. Studies on Aeromonas hydrophila in cultured Oreochromis niloticus at Kafr El Sheikh Governorate, Egypt with reference to histopathological alterations in some vital organs. *World Journal of Fish and Marine Science*, **6**, 233-240(2014).

37. Ahangarzadeh, M., Masoud, Gh., Rahim, P., Hossein, H., Mostafa, Sh. and Mehdi, S. Detection and distribution of virulence genes in Aeromonas hydrophila isolates causing infection in cultured carps. *Veterinary Research Forum*, **13** (1), 55-60(2022).

التحديد الجزيئي لـ 16 S rRNAوبعض جينات الضراوه من Aeromonas spp في أنواع أسماك بحيرة ناصر ، أسوان ، مصر

أمل مختار [١] *، محمد كرمى [٢] ، يسرا محمد الشرى[٣] و مروه عبد السيد على [١]
[١] قسم الميكروبيولوجى والمناعه - كلية الطب البيطرى - جامعة اسوان.
[٢] قسم الرقابه الصحيه على اللحوم - كلية الطب البيطرى - جامعة اسوان.
[٣] قسم الاسماك - كلية الطب البيطرى - جامعة اسوان.

38.

تعتبر أسماك المياه العذبة مصدرًا رئيسيًا رخيصًا للبروتين الحيواني في أجزاء مختلفة من العالم. في صعيد مصر ، تعد بحيرة ناصر المصدر الرئيسي لإنتاج الأسماك للاستهلاك البشري. يمكن أن تكون الأسماك أيضًا عامل لمسببات الأمراض المنقولة بالغذاء ، مثل أنواع Aeromonas ، التي تشكل خطرًا كبيرًا على الصحة العامة. تم اختيار مجموعه ١٨٠ عينة من أسماك المياه العذبة مثل البلطي النيلي ، وسمك الفرخ النيلي ، وسمك البايك ، والقرموط الأفريقي ذو الأسنان الحادة ، وأنف الفيل ، وفليه البلطي (٣٠ عينة لكل منها) تم اختيارها عشوائياً من بحيرة ناصر في محافظة أسوان. يكشف الفحص البكتريولوجي عن حدوث ١١٧ (٦٥٪) من أنواع الأيروموناس. في ٦٠ عينة معزولة 10 من كل نوع سمكي ، تم العثور على جينات ١٦S rRNA بنسبة ٩٦,٦٧٪ باستخدام تفاعل البوليميراز المتسلسل. في مصر وحول العالم ، تم ربط أنواع Aeromonas بالعديد من الامراض المتفشيه المنقولة عن طريق الأغذية التي نتجت عن استهلاك الأسماك المخمرة النيئة التي تحتوي على العديد من الجينات الشديدة الضراوة. بالإضافة إلى ذلك ، تم اختياراثنان من الجينات المحدثه للسميه الجينات Aerolysin(aer) والسم المعوي السام للخلايا (Act) ليتم فحص تواجدها بواسطة PCR. كانت النتائج ٥١,٧٪ و ٢٧,٦٪ على التوالي ، لذلك يجب اتخاذ الاحتياطات الصحية للقضاء على أنواع أسماك بحيرة ناصر الملوثة بأنواع الأيروموناس. الفحص الجزيئي لبعض جينات الضراوه من Aeromonas spp. في بحيرة ناصر من أنواع مختلفة من الأسماك كان الهدف من هذه الدراسة.