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Occurrence, Seasonal Variations and Virulence of *Aeromonas hydrophila* and *Aeromonas caviae* in Fish Farms at East Delta, Egypt

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Abstract: This study was conducted to evaluate the occurrence and seasonal variations of *Aeromonas hydrophila* and *Aeromonas caviae* in two large fish farms at East Delta, Egypt. The correlation between the obtained *Aeromonas* isolates from different sources and their virulence factors were also determined. For this purpose, a total of 702 samples of water (input and pond water), fish feed, bottom mud and fish, (intestinal contents and surface swabs) were collected from El-Abbassa fish farm located at Abo-Hamad, Sharkia governorate and from El-Warwary fish farm at El-Tall El-Kabir, Ismailia governorate. Samples were collected on monthly basis for 9 months during the period from June to November 2012 and from March to May 2013, meanwhile, winter season was excluded due to harvesting and marketing of fish in investigated farms. The results showed that, *A. hydrophila* and *A. caviae* were detected with the total values of 23.2% (163 out of 702 samples) and 31.5% (221 out of 702 samples), moreover, *A. hydrophila* was more frequently isolated from pond water and fish intestinal contents (25.9% each), whereas, *A. caviae* occurred more frequently in input water (55.6 %) and pond water (46.9%). On farm level, comparatively higher occurrence for *A. hydrophila* was detected in El-Warwary fish farm (29.1%) than in El-Abbassa fish farm (20.3%), however, *A. caviae* was more prevalent in El-Abbassa fish farm (33.1%) than in El-Warwary fish farm (28.2%). Analysis of seasonal variations of recovered *Aeromonas* isolates in examined fish farms revealed that occurrences of *A. hydrophila* and *A. caviae* were highest in summer season and were lowest in autumn. Studying the correlations between *Aeromonas* spp. isolates from different sources prospects to that *Aeromonas* in intestinal contents and surface swabs of fish might derived from pond and input water. In addition, PCR investigation of 20 *Aeromonas* isolates clarified detection of aerolysin and haemolysin genes in 100% and 50% of examined *A. hydrophila* isolates and in 60 and 30% of examined *A. caviae* isolates.

Key words: Fish farms · East Delta · *Aeromonas hydrophila* · *Aeromonas caviae* · Seasonal variations · Virulence genes

INTRODUCTION

In developing countries having over population problems like Egypt, fish is considered as one of the most important sources of protein for man. For this reason, aquaculture and fish farming become a prominent industrial activity in Egypt and produce about 62% of total fish production [1]. Nowadays, Egypt ranked as 7th of top ten countries in the inland fish production and the 2nd biggest Tilapia producer after China [1, 2].

In aquaculture, the aquatic environment is very important and is considered the main factor controlling fish health and diseases [3]. *Aeromonas* microorganism is ubiquitous in the aquatic environment and is one of most common bacteria in freshwater habitats throughout the world [4]. The genus *Aeromonas* comprises important fish and human opportunistic pathogens causing hemorrhagic septicemia and great losses in fish and wound infections, gastroenteritis, traveler's diarrhea and septicemia in humans [5].

The taxonomy of *Aeromonas* is complex and has undergone numerous changes. To date, the genus includes 25 well-recognized species [6], among them *A. hydrophila* and *A. caviae* comprise the most predominant clinical isolates that are typically associated with fish [7]. The pathogencity of these *aeromonads* has been linked

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to a number of putative virulence factors such as aerolysin, hemolysin, proteases, lipases and DNases that play an important role in the development of diseases either in humans or in fish [8]. In recent years several molecular methods, particularly PCR based methods have been developed for detection of such putative virulence genes of *Aeromonas* spp. [9].

The distribution of *Aermonas* spp. in aquatic environment during fish aquaculture was significantly related to levels of water pollution. *A. caviae* predominated in water with high degree of faecal and sewage pollution, however, in less polluted water, *A. caviae* and *A. hydrophila* were almost equally distributed [4]. Moreover, in fish farms, pond water, bottom sediment and fish may become reservoir for such organism [10].

The wide spread distribution of certain *Aeromonas* species like *A. hydrophila* and *A. caviae* in freshwater fish farms is probably a consequence of their high capacity to adapt to various environmental stressors including turbidity, PH, salinity and water temperature [11]. Even more, there seems to be a seasonal variation in densities of *Aeromonas* spp. within the environment of fish farms with higher frequency during high water temperature in summer season than cold months [12].

The aim of this study was to evaluate the occurrence and seasonal variations of *A. hydrophila* and *A. caviae* in two large fish farms at East Delta, Egypt. A second aim was to investigate the correlations between *Aeromonas* isolates from different sources and to determine their virulence factors.

MATERIALS AND METHODS

A total of 702 samples were collected from two large fish farms at East Delta, Egypt. The first farm is El-Abbassa fish farm located at Abo-Hamad, Sharkia governorate (governmental fish farm) and the second is El-Warwary fish farm located at El-Tall El-Kabir, Ismailia governorate (private fish farm). From each farm, water, fish feed, bottom mud and fish samples were collected on monthly basis for 9 months during a period from June to November 2012 and from March to May 2013 (summer, autumn and spring), while winter season was excluded due to harvesting and marketing of fish in investigated farms. The collected samples were subjected to bacteriological examination to determine occurrence, seasonal variations and virulence of *A. hydrophila* & *A. caviae* in the examined fish farms, even more to study the correlations between *Aeromonas* isolates from different sources in the investigated fish farms.

Topographical Examination: From the records of each farm, all information were obtained and data of topographical examination at farm level was described in Table (1), meanwhile data of topographical examination at pond level was shown in Table (2).

Samples Collection

Sampling Protocol: Distribution of collected samples from the investigated fish farms in relation to locality, sources and season is recorded in Table (3).

Sampling Procedures

Water Sampling from Input and Ponds: The technique of water sampling was conducted according to the recommendation of APHA [13]. Each sample was collected by sterile glass bottle of one liter capacity. The bottle has two cords; one attached to the neck and the other to the stopper and was caged with a load. The bottle was immersed closed to a depth of 50 cm

Table 1: Data of topographical examination at farm level

Fish farm	Farm area /feddan	Type	No. of rearingponds	Rate of water exchange	Pond disinfection
El-Abbassa	128	Earthen ponds	7-22	Triple /week	Dryness for 15 day + removal of upper layer
El-Warwary	80	Earthen ponds	8	Twice/week	Removal of upper layer

Table 2: Data of topographical examination at pond level

Fish farm	Examined ponds	Pond depth (m)	Pond area (feddan)	Reared species	Density of Tilapia /feddan	Density of Catfish/feddan	Mortality rate (%)
El-Abbassa	A	1.5-2	4	Tilapia & Catfish	11000	500	3.5%
	B		4	Tilapia & Catfish	11000	500	
	C		6	Tilapia & Catfish	11000	500	
	D		1	Tilapia only	11000	--	
	E		1.25	Tilapia only	11000	--	
	F		1	Tilapia only	11000	--	
El-Warwary	A	1-1.5	5	Tilapia & Catfish	15000	1500	5%
	B	1-1.5	6	Tilapia & Catfish	15000	1500	
	C	1.3-1.8	6	Tilapia & Catfish	15000	1500	

Table 3: Distribution of collected samples from examined fish farms

	No. of samples / month			Total no. of samples.		
Source	El-Abbassa farm	El-Warwary farm	Total	El-Abbassa farm	El-Warwary farm	Total
Input water	2	1	3	18	9	27
Pond water	6	3	9	54	27	81
Fish feed	2	1	3	18	9	27
Bottom mud	6	3	9	54	27	81
Fish surface swabs	18	9	27	162	81	243
Fish intestinal contents	18	9	27	162	81	243
Total	52	26	78	468	234	702

below water surface and filled by jerking out the cord attached to the stopper. From each water sample, 100 ml was taken in a sterile screw capped colorless glass bottle for bacteriological examination.

Fish Feeds: Fish feed samples were taken by sterile spatula from each feed store in examined farms and packed separately in sterile polyethylene bags.

Bottom Mud: The technique of bottom mud sampling was carried out according to Miles [14] using a homemade samplers consisted of a steel can 8.5 inches diameter and 4.5 inches depth, attached to end of 4 meter aluminum pole. The can was permitted to settle to the bottom of the pond in an inverted position. On rotation of 180°, the can sample 6 cm depth portion of bottom mud. Five samples of mud were taken from different parts of each pond and mixed thoroughly in one pooled sample. Samples packed separately in sterile polyethylene bags.

Fish: Fish samples were collected by fish nets (Nylon from 2-3 cm) and packed separately in sterile polyethylene bags. From each fish surface swab and intestinal content sample was obtained at the laboratory.

All collected samples were labeled, transported to the laboratory under cooled conditions with minimum delay and processed at the same day.

Isolation and Identification of *Aeromonas*

Preparation of samples: (Cruickshank *et al.* [15]):

i) Water samples:

25 ml of each water sample was thoroughly mixed with 225 ml of Buffered Peptone Water (BPW).

ii) Fish feeds:

25 g of each feed sample was homogenized well with 225 ml of B.P.W.

iii) Bottom mud:

Each sample was centrifuged at 8000 rpm for 5 minutes and 1 g of homogenous samples was mixed well with 9 ml of BPW.

iv) Fish:

* Surface swab:

Each swab was placed aseptically into a tube containing 9 ml of BPW.

* Intestinal content:

1 g of each intestinal content sample was mixed thoroughly with 9 ml of BPW.

Resuscitation and Enrichment: After preparation of collected samples, all samples were incubated at 37°C for 6 hours. Then for enrichment, 1 ml of each pre-enriched broth was transferred to 9 ml of nutrient broth and incubated at 37°C for 24 hours.

Isolation: (Ashiru *et al.* [16]): 0.1 ml of each enriched broth was streaked aseptically onto *Aeromonas* agar plate (LBA, 167) and then incubated at 37°C/ 24 hours. After incubation, the plates were examined and green colonies with opaque center and yellow colonies were selected and subcultured on nutrient agar and incubated at 37°C/24 hours. A loopfull of pure culture was inoculated onto agar slant for further identification.

Identification: The suspected *A. hydrophila* & *A. caviae* isolates were identified using morphological characters (microscopical examination and motility test) & biochemical reactions according to Collins and Lyne [17] and Koneman *et al*. [18].

Detection of Aerolysin (aer) and Haemolysin (hly) Genes in *Aeromonas hydrophila* and *Aeromonas caviae* Isolates: Twenty *Aeromonas* isolates comprising 10 *A. hydrophila* and 10 *A. caviae* recovered from pond water and fish were investigated with PCR for detection of aer and hly genes at Biotecnology Unit in National Laboratory for Veterinary Quality Control on Poultry, Animal Health Research Institute, Doki, Giza, Egypt. Genomic DNA was extracted and prepared by proteinase K method using QIA amp® DNA Mini Kit (Cat. No. 51304-Qiagen). Amplifications were carried out using PCR 1.1 x Ready-Mix TM master mix (Thermo Scientific, Cat. no. AB0575/LD-A) and primer pairs synthesized by NWG-

Table 4: Primer pairs used for detection of aerolysin and haemolysin genes

Gene	Sequence (5`-3`)	Amplicon size (bp)	Reference
Aerolysin (aer)	F: CACAGCCAATATGTCGGTGAAG R: GTCACCTTCTCGCTCAGGC	326	[19]
Haemolysin (hly)	F: CTATGAAAAAACTAAAAATAACTG R: CAGTATAAGTGGGGAAATGGAAAG	1500	[20]

Table 5: Cycling program of PCR for detection of aerolysin and haemolysin genes

Amplified DNA	PCR condition
Aerolysin (aer)	a) Initial denaturation: 95 °C for 5 minutes b) Actual cycles: 30 cycles of i- 94 °C for 1 minute ii- 52 °C for 1 minute iii- 72°C for 1 minute c) Final extension: 72 °C for 10 minutes
Haemolysin (hly)	a) Initial denaturation: 95 °C for 5 minutes b) Actual cycles: 30 cycles of i- 94 °C for 1 minute ii- 55 °C for 1 minute iii- 72 °C for 3 minute c) Final extension: 72 °C for 10 minutes

Biotech AC (Table, 4) and under cycling conditions shown in Table (5). The PCR products then electrophoresed for one hour at 80 v on a 1% horizontal agarose gel in Tris-Boric acid-EDTA buffer stained with 0.5 µg/ml ethiduim bromide solution. Eight µL of 100 bp DNA ladder marker was load into the wells in gel just before electrophoresis. The gel was photographed by a gel documentation system and data was analyzed by computer.

Statistical Analysis: Pearson correlation for *Aeromonas* spp. inters relationship from different sources in examined farms was carried out for exploration of significant values using SPSS 16.0 [21].

RESULTS

A. hydrophila and *A. caviae* were detected in the examined farms with the total percentages of 23.2% (163 out of 702 samples) and 31.5% (221 out of 702 samples) respectively. *A. hydrophila* was more frequently isolated from pond water & intestinal contents of fish (25.9%, each) and was not isolated from fish feeds. However, *A. caviae* occurred more frequent in input water (55.6%) and pond water (46.9%), meanwhile was lowest in fish feeds (11.1%). On farm level, comparatively higher occurrence for *A. hydrophila* was determined in El-Warwary farm (29.1%) than in El-Abbassa farm (20.3%), whereas, *A. caviae* was more prevalent in El-Abbassa (33.1%) than in El-Warwary farm (28.2%).

Investigating the occurrence of both *Aeromonas* spp. in examined fish farms at different seasons clarified that prevalence of *A. hydrophila* and *A. caviae* were highest in summer season (33.3% and 35.0%, respectively), followed by spring (21.8% & 33.3, respectively) and were lowest in autumn (14.5% & 26.1%, respectively). Assessing the correlations between *Aeromonas* spp. isolates from different sources revealed that in El-Abbassa farm, there were positive significant correlations at ($P<0.01$) between isolates of *Aeromonas* spp. in pond water and that in fish intestinal contents and also between isolates from fish surface swabs and that from fish intestinal contents.

PCR analysis of 20 *Aeromonas* isolates for detection of virulence genes clarified occurrence of aer and hly genes with the total percentage of 80% and 40%, respectively, where all of examined *A. hydrophila* and 60% of examined *A. caviae* isolates possessed aer gene, meanwhile, 5 out 10 *A. hydrophila* isolates and 3 out of 10 *A. caviae* isolates possessed hly gene.

DISCUSSION

Table (6) presents occurrence of *A. hydrophila* and *A. caviae* in different source from examined fish farms. It was evident that *A. hydrophila* and *A. caviae* were detected with the total percentages of 23.2% (163 out of 702 samples) and 31.5% (221 out of 702 samples) respectively. These results are in agreement with those of

Table 6: Occurrence of *Aeromonas hydrophila* and *Aeromonas caviae* in different sources from examined fish farms.

	El-Abbassa farm.					El-Warwary farm					Total.				
		A. hydrophila		*A. caviae*			*A. hydrophila*		*A. caviae*			*A. hydrophila*		*A. caviae.*	
Source	No. of examined samples	Positive samples	%	Positive samples	%	No. of examined samples	Positive samples	%	Positive samples	%	No.of examined samples	Positive samples	%	Positive samples	%
Input water	18	2	11.1	10	55.6	9	1	11.1	5	55.6	27	3	11.1	15	55.6
Pond water	54	12	22.2	26	48.1	27	9	33.3	12	44.4	81	21	25.9	38	46.9
Fish feed	18	0	0.0	1	5.6	9	0	0.0	2	22.2	27	0	0.0	3	11.1
Bottom mud	54	11	20.4	19	35.2	27	9	33.3	5	18.5	81	20	24.7	24	29.6
Fish intestinal contents	162	38	23.5	61	37.7	81	25	30.9	29	35.8	243	63	25.9	90	37.0
Fish surface swabs	162	32	19.8	38	23.5	81	24	29.6	13	16.0	243	56	23.0	51	21.0
Total	468	95	20.3	155	33.1	234	68	29.1	66	28.2	702	163	23.2	221	31.5

Goci-Urriza *et al.* [22], the author found that *A. caviae* (75%) was more prevalent in European rivers than *A. hydrophila* (9%); however percentages of occurrence were quality different. In Turkey, species differentiation of 60 typical *Aeromonas* isolates from Porsuk River in Eskisehir city, revealed 37% *A. caviae* and 13% *A. hydrophila* [23]. On contrast, Hatha *et al*. [24] recovered *A. hydrophila* and *A. caviae* from freshwater fish with the percentages of 61% and 30%, respectively. Even more, in Brazil, 59% and 27% of isolated *Aeromonas* spp. from water being *A. hydrophila* and *A. caviae* respectively [25].

Table (6) showed also that, *A. hydrophila* was more frequently isolated from pond water & intestinal content of fish with the percentage of 25.9% in both sources, followed by bottom mud (24.7%), fish surface swabs (23.0%) and input water (11.1%), meanwhile was not isolated from fish feeds. On the other hand, *A. caviae* occurred more frequently in input water (55.6%) followed by pond water (46.9%), intestinal content of fish (37.0%), bottom mud (29.6%), fish surface swabs (21.0%) and was lowest in fish feeds (11.1%). In Saudi Arabia, *A. hydrophila* was recovered from pond water, sediment and intestine of hybrid Tilapia with the values of 11.11%, 20.09% and 14.54%, respectively and was found predominant in pond water and sediment [10]. In addition, in Kerala India, examination of fish and water samples from aquaculture clarified *A. caviae* as a dominant *Aeromonas* spp. with the percentages of 31.43% and 16.57% in these sources, respectively [26]. However, in another study, *Aeromonas* could detected only in water, meanwhile was absent in sediment and intestine of Tilapia fish [27].

On farm level, in El-Abbassa fish farm, *A. hydrophila* was detected with the total percentage of 20.3% (95 out of 468 samples), where there were comparatively higher estimates for this microorganism in fish intestinal contents (23.5%), pond water (22.2%), bottom mud (20.4%), followed by fish surface swabs (19.8%) and input water (11.1%), mean while, no evidence for such organism was found in fish feeds. Whereas, in El-Warwary fish farm, *A. hydrophila* was detected with the total percentage of 29.1% (68 out of 234 samples), where the organism was more prevalent in pond water and bottom mud (33.3% for each), fish intestinal contents (30.9%), followed by fish surface swabs (29.6%) and input water (11.1%). The comparatively higher occurrence for *A. hydrophila* in El-Warwary fish farm in this study might be attributed to the lower rate of water exchange (twice /week), application of pond disinfection without complete water dryness and higher fish stocking density observed in this farm in comparison with El-Abbassa farm. Furthermore, these findings could explain the recorded higher mortality rate in El-Warwary fish farm (5%) than those reported in El-Abbassa farm (3.5%). In Greece, *A. hydrophila* was found to be indigenous in polluted aquatic environment [28]. Also, level of fish contamination with bacterial pathogens was found to be directly proportional to their levels in the overlying water and bottom mud [29].

Concerning *A. caviae*, in El-Abbassa fish farm such organism was determined with the total value of 33.1% (155 out of 468 samples) where was more frequently recovered from input water (55.6%), pond water (48.1%), then fish intestinal contents (37.7%), bottom mud (35.2%), fish surface swabs (23.5%) and feeds (5.6%). However, in El-Warwary fish farm, *A.caviae* was isolated with the total value of 28.2% (66 out of 234 samples), where its occurrence was highest in input water (55.6%), pond water (44.4%), then fish intestinal contents (35.8%), fish feeds (22.2%), bottom mud (18.5%) and surface swabs (16.0%). It is noticeable that *A. caviae* was widely distributed and frequently detected in all examined sources from both fish farms, despite its total occurrence was slightly higher in El-Abbassa farm (33.1%). This may postulated to the high prevalence of *A. caviae* in input water (55.6%) in both farms which subsequently

Table 7: Seasonal variations of *Aeromonas hydrophila* and *Aeromonas caviae* in examined fish farms.

	El-Abbassa farm[a].				El-Warwary farm[b].				Total[c].			
	A. hydrophila		*A. caviae*		*A. hydrophila*		*A. caviae*		*A. hydrophila*		*A. caviae*	
Season	Positive samples	%	Positive samples	%	Positive samples	%	Positive samples	%	Positive samples	%	Positive samples	%
Summer	46	29.5	61	39.1	32	41.0	21	26.9	78	33.3	82	35.0
Autumn	21	13.5	40	25.6	13	16.7	21	26.9	34	14.5	61	26.1
Spring	28	17.9	54	34.6	23	29.5	24	30.8	51	21.8	78	33.3

a: No. of samples per season= 156 b: No. of samples per season= 78 c: Total no. of samples per season= 234

Table 8: A Pearson correlation coefficient for *Aeromonas* spp. inter-relationship from different sources in El-Abbassa and El-Warwary fish farms

Farm	Input water	Pond water	Bottom mud	Fish feed	Fish surface swabs
I- El-Abbassa farm:					
Pond water	0.057				
Bottom mud	0	-0.091			
Fish feed	0.171	-0.02	-0.108		
Fish surface swabs	-0.123	0.178	0.092	-0.084	
Fish intestinal contents	-0.045	0.386**	0.085	-0.138	0.363**
II- El-Warwary farm:					
Pond water	-0.189				
Bottom mud	-0.21	0.376			
Fish feed	0.378	0.071	-0.02		
Fish surface swabs	0.414*	-0.301	-0.017	-0.024	
Fish intestinal contents	0	0.267	0.222	-0.134	-0.202

* Correlation is significant at 0.05 ** Correlation is significant at 0.01

contaminate the other sources in fish aquatic environment. Input water contamination may attributed to the heavy human and animal usage of water streams in such places with frequent activities of grazing, agricultural works and sewage disposal with continuous loading of water with microbial contaminates [29]. The high isolation rate of *A. caviae* from fish feed store in El-Warwary farm is of great importance and prospects to a dangerous role for fish feeds in transmission and maintenance of *A. caviae* infection in fish farms. In addition, the hygienic level during fish feed formulation and storage conditions especially storage temperature and humidity are important factors affecting microbial quality of fish feeds [30].

Regarding seasonal variations of *A. hydrophila* and *A. caviae* in the examined fish farms, Table (7) showed that the highest occurrences for the two *Aeromonas* spp. were recorded during summer season (33.3% & 35.0%, respectively), followed by spring (21.8% & 33.3%, respectively) and were lowest in autumn (14.5% & 26.1%, respectively). *A. hydrophila* was isolated with higher values from El-Warwary fish farm (41.0%, 16.7% and 29.5%) than those from El-Abbassa fish farm (29.5%, 13.5% and 17.9%) in summer, autumn and spring, respectively. Whereas, *A. caviae* was isolated with higher percentages from El-Abbassa farm (39.1% and 34.6%) than those from El-Warwary farm (26.9% and 30.8%) in summer and spring, respectively, meanwhile, in autumn *A. caviae* was more prevalent in El-Warwary farm (26.9%) than El-Abbassa farm (25.6%). These results substantiate what has been previously reported in El-Abbassa, Egypt, where *A. hydrophila* predominates among Tilapia fish during summer season [12]. In Turkey, *Aeromonas* spp. occurred most frequently in Porsuk River during the dry season from June to October [23]. Moreover, Al- Harbi and Uddin [10] recorded that the bacterial load in pond water, sediment and fish intestine was varied with high bacterial load in warm months due to high temperature of water bodies. On contrast, 96% of *A. hydrophila* isolates recovered from the fresh water fish in Croatia were obtained during winter and spring but never during summer [31].

It is obvious from results recorded in Table (8) that in El-Abbassa farm, there was a positive significant correlation at ($P<0.01$) between the detected *Aeromonas* spp. in pond water and that in intestinal contents of fish ($r = 0.386$), also between *Aeromonas* spp. from surface swabs and that from intestinal contents of fish ($r = 0.363$). Moreover, in El-Warwary fish farm, *Aeromonas* spp. in input water showed a positive significant correlation at ($P<0.05$) with that from surface swabs of fish ($r = 0.414$). These findings prospects to that *Aeromonas* spp. in intestinal contents and surface swabs of fish most likely derived from pond and input water. In Brazil, *Aeromonas* in water medium was found represented in the internal fish organs [32]. Furthermore, in Abu ElAkhder fresh sea, Egypt, the level of fish contamination with microorganisms was found to be directly proportional to their levels in the overlying water [29].

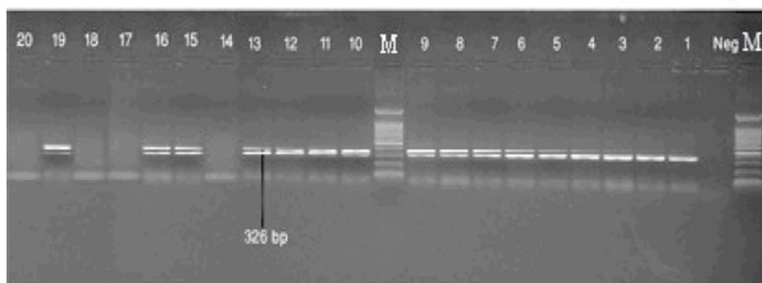

Fig. 1: Ethidium bromide-stained agarose gel showing PCR products obtained by PCR amplification of 10 *A. hydrophila* & *10 A. caviae* isolates. Lanes M: 100 bp ladder; lanes 1-10: 10 *A. hydrophila* isolates positive for aer gene at 326 bp; Lanes 11, 12, 13, 15, 16, 19: 6 *A. caviae* isolates positive for aer gene.

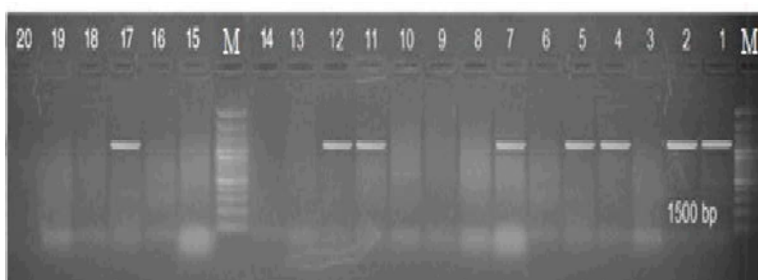

Fig. 2: Ethidium bromide-stained agarose gel showing PCR products obtained by PCR amplification of 10 *A. hydrophila* & 10 *A. caviae* isolates. Lanes M: 100 bp ladder; Lanes 1, 2, 4, 5, 7: 5 *A. hydrophila* isolates positive for hly gene at 1500 bp; Lanes 11, 12, 17: 3 *A. caviae* isolates positive for hly gene.

Table 9: Virulence genes of *Aeromonas hydrophila* and *Aeromonas caviae* from examined fish farms.

		Aerolysin gene (aer).		Haemolysin gene (hly).	
Aeromonas spp.	No. of examined isolates	Positive	%	Positive	%
A. hydrophila	10	10	100	5	50
A. caviae	10	6	60	3	30
Total	20	16	80	8	40

Virulence genes of *A. hydrophila* and *A. caviae* isolated from the examined fish farms are shown in Table (9) and Figs. (1 & 2). Regarding aerolysin gene (aer), it is clear that all (100%) of examined *A. hydrophila* possessed the gene, whereas, out of 10 *A. caviae* isolates only 6(60%) possessed such gene with of total occurrence of 80% (16 out of 20 isolates) for aer gene in *Aeromonas* spp. isolates. Closely similar results were reported by Singh *et al.* [19] where aer gene was detected in 85% of *Aeromonas* spp. recovered from fish and pond water. In Italy, 83.7% of *Aeromonas* spp. from fish, shellfish and water carried aer gene [33]. In addition, 100% of *A. hydrophila* recovered from diseased fish in Mumbai were found positive for aer gene [8]. Meanwhile, only 52.6% of *A. hydrophila* and 44.7% of *A. caviae* isolated from fish sources and fresh water in Malaysia possessed aer gene [34].

Concerning occurrence of haemolysin gene (hly) in isolated *Aeromonas* spp., it is noticeable that hly gene was detected in 50% (5 out 10 isolates) of *A. hydrophila* and 30% (3 out of 10 isolates) of *A. caviae* isolates with the total occurrence of 40% (8 out of 20 isolates) in *Aeromonas* spp. isolates. Nearly similar occurrence for hly gene (52.6% of *A. hydrophila* and 34.2% of *A. caviae*) was previously recorded [34]. Also, 50.5% of *Aeromonas* spp. isolated from water and fish in Malaysia were found positive for hly gene [35]. On the other hand, higher hly gene occurrence (77%) in *Aeromonas* spp. isolates was also reported [36].

CONCLUSION

From this study, it could be concluded that *A. hydriphila* and *A. caviae* are widely distributed in the aquatic environment and fish species of two fish farms at

East Delta, Egypt. A seasonal variation in occurrence of both *Aeromonas* spp. was recorded with the occurrence being higher in summer and lower in autumn. PCR analysis confirmed the high virulence as expressed by frequent detection of aerolysin and haemolysin genes in recovered isolates. The obtained results illustrate the need for regular examination of pond water and their input supplies for rapid detection of their contamination with *Aeromonas*, prohibition of sewage and faecal pollution of fish ponds and their surroundings. Usage of ditches and grading to divert polluted water away from fish ponds, together with regular pond disinfection. Furthermore, sanitary assessing of fish to clarify their hygienic condition and education program to fish handlers and consumers are essential to avoid the risk of human infection.

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