SVU- International Journal of Veterinary Sciences, 5(2): 106-116, 2022

Print ISSN: 2535-1826 **Online ISSN: 2535-1877**

Research Article Open Access

Incidence of *Aeromonas* species isolated from fresh fish, canned fish and shrimp in Sohag Governorate, Egypt

Tawfik Esmat Abd-Elhafeez Tawfik[1*], Hassan Mohammed Gad-Elrab[1], and Nahed Mahmoud Abd-ElAziz[1]

[1] Food Hygiene Department, Animal Health Research Institute, Agriculture Research center, Egypt,
[2] Food Hygiene Department, Faculty of Veterinary Medicine, Sohag University, Egypt

Abstract

A total of 180 samples of fish meat and canned fish were randomly collected from different markets and retail shops in Sohag city as the following; fresh water fish (Nile tilapia and catfish), marine water fish (mullet), shrimps, and canned fish (tuna and salmon) with 30 samples of each, to study the incidence of *Aeromonas* species *(Aeromonas* spp.) with special reference to *Aeromonas hydrophila* (*A.hydrophila*) and its virulence genes. The results of this study showed that the mean of *Aeromonas* counts were 0.122×10^2, 0.504×10^2, 0.124×10^2, 0.037×10^2 cfu/g for Nile tilapia, catfish, mullet and shrimps, respectively. While in canned fish it was uncountable. *Aeromonas* spp. were isolated from 60 of 180 examined samples with a percentage of 33.3%, 7 species were identified: *A. caviae*, *A. hydrophila*, *A. media*, *A. shubertii*, *A. sobria*, *A. veronii biovar sobria* and *A. veronii biovar veronii* were detected at a percentage of 5%, 7.8%, 2.8%, 7.2%, 5%, 2.8% and 2.8%, respectively. The results of PCR showed that, 12 isolates out of 14 were positive for *16S rRNA* gene of *A. hydrophila* with a percentage of 85.7 %. Virulence gene like, *Aerolysin AHA* was found in 41.6 % of the examined samples while, the *heat stable enterotoxin AST* gene was not detected. This study spots the lights on *Aeromonas* spp. especially *A. hydrophila* as potential biological hazard in fish meat and canned fish, as a foodborne pathogen.

Keywords:
Aeromonas spp., *A. hydrophila*, Canned fish, Fish meat, Virulence genes.

DOI: 10.21608/svu.2022.137345.1199 **Received:** May 11, 2022 **Accepted**: June 20, 2022 **Published**: June 30, 2022 ***Corresponding author:** Tawfik Esmat A. Tawfik **E-mail:** tawfik.esmat@vet.sohag.edu.eg

Citation: Tawfik et al., Incidence of *Aeromonas* species isolated from fresh fish, canned fish and shrimp in Sohag Governorate, Egypt. SVU-IJVS 2022, 5(2): 106-116.

Competing interest: The authors have declared that no competing interest exists.

Introduction

Fish is healthy and low-calorie food that provides essential macro and micronutrients as protein, vitamins and minerals (Abisoye et al., 2011). On the other side, fish may act as a vehicle for pathogenic bacteria leading to human gastroenteritis. Mesophilic aeromonads are one of the most common bacteria in water habitats throughout the world, and frequently cause disease in fish and causative agents of acute diarrheal disease in man. *Aeromonas* spp. are emergent food-borne pathogens, belong to the family *Aeromonadaceae*. They are Gram-negative bacteria ubiquitous in soil, aquatic environments, and food products. *Aeromonas* can survive and multiply at low temperatures (2-10°C) that is applied for cold storage of food products (Igbinosa et al. 2012; NCBI, 2020). The genus *Aeromonas* currently comprises 21 validated species, 11 of which are related to human clinical samples (Carnahan and Joseph, 2015). *Aeromonas* spp., as emerging pathogens to humans, cause a broad spectrum of infections, such as gastroenteritis, peritonitis, and hepatobiliary infections, myositis, bacteremia, septicemia, meningitis, soft-tissue and wound infections (Janda and Abbott, 2010; Bravo and Figueras, 2020).

A. hydrophila has been isolated from retail foods including fish, seafood, raw milk, poultry and red meat (Tahoun et al., 2016; Sreeremya, 2017; Wamala et al., 2018). *A. hydrophila* has been isolated previously from various fish in Egypt (Abd-El-Malek 2017; Ramadan et al., 2018). Fish can be contaminated with pathogenic bacteria either by polluted water or by handling, processing and unhygienic storage conditions (Sarkar et al., 2013). *A. hydrophila* were responsible for small outbreaks of food poisoning caused by ingestion of raw fermented fish (Igbinosa et al., 2012). To identify isolates of *Aeromonas spp.*, biochemical, morphological and molecular techniques are required (Figueras and Hidalgo et al., 2015). The *16S rRNA* gene is considered a stable molecular marker for identifying bacterial species, since its distribution is universal and allows comparison of microorganisms (Sánchez, 2015).

Pathogenicity of *Aeromonas* depends on several virulence factors which allow them to adhere, invade, and destroy the host cells, overcoming the immune host response, such as cytotoxins, adhesins, hemolysins, proteases and lipases, as well as their ability to form biofilms (Hidalgo and Figueras, 2013). *A. hydrophila* strains contain aerocytotoxin enterotoxin (*AST*) gene that releases a toxin (aerolysin) to cause tissue damage. Aerolysin *AHA* is a cytolytic and a hemolytic exotoxin, binds to specific glycoreceptors on the surface of eukaryotic cells before inserting into the lipid bilayer and forms holes, this plays a key role in the pathogenesis of *A. hydrophila* infection. Severe disease and watery diarrhea caused by strains with *AST* gene which produces a heat stable cytotonic enterotoxin. Cytotoxin and hemolysin activity increases when temperatures increase to 37ºC (Bravo and Figueras, 2020).

This study aimed to identify the incidence of *Aeromonas* species in fish meat and canned fish as well as the incidence of *A. hydrophila* and its virulence genes, which play important roles in human gastro-intestinal infections, by using cultural and molecular methods.

Materials and methods

Collection and preparation of samples:

A total of 180 samples of fish meat represented in freshwater fish (Nile tilapia and catfish), marine fish (mullet), shrimps as well as canned fish (tuna and salmon) with 30 samples of each, were collected randomly from different shops and supermarkets located in Sohag Governorate. Samples were prepared according to FDA, 2018.

Determination of *Aeromonas* counts:

Surface counting method was used according to (Austin, 2014). Inoculate 0.1 ml of the diluted samples and subsequent decimal dilutions onto plates of *Aeromonas* medium base (Himedia) supplemented with Ampicillin (Oxoid, SR0136), incubate plates at 30ºC for 24hr.Examine the plates and count typical colonies "dark green, opaque colonies with a darker center" (Fig.1). Subculture five typical colonies (or all if fewer than five) to a nutrient agar slope, then incubate at 30°C for 18–24 h. Perform an oxidase test. Retain oxidase-positive strains and identify by biochemical tests.

Isolation and identification of *Aeromonas spp*.

Samples were homogenized into Alkaline peptone water (APW) with 2.5mg/L Ampicillin selective supplement, Oxoid and incubated at 30º C for 18-24hr. A loopful of the enriched culture was inoculated on *Aeromonas* medium base plates, Himedia, then incubated at 30ºC for 24hr (Austin, 2014). Identification is made by morphological, and biochemical characteristics according to Carnahan and Joseph (2015)

Identification of *A. hydrophila* and its virulence genes by PCR

Suspected isolates examined for*16S rRNA* gene and then the positive isolates examined for virulence genes such as *AHA* and *AST*. Three pairs of primers were supplied from Metabion, Germany as shown in Table 1.

Table 1: Oligonucleotide primers sequences for *A. hydrophila* and its virulence genes

Gene	Primer sequence (5'-3')	Product size	Reference
16S rRNA	CTACTTTTGCCGGCGAGCGG TGATTCCCGAAGGCACTCCC	953 bp	Gordon et al., 2007
Aerolysin AHA	CACAGCCAATATGTCGGTGAAG GTCACCTTCTCGCTCAGGC	326 bp	Singh et al., 2008
AST	TCTCCATGCTTCCCTTCCACT GTGTAGGGATTGAAGAAGCCG	331 bp	Nawaz et al., 2010

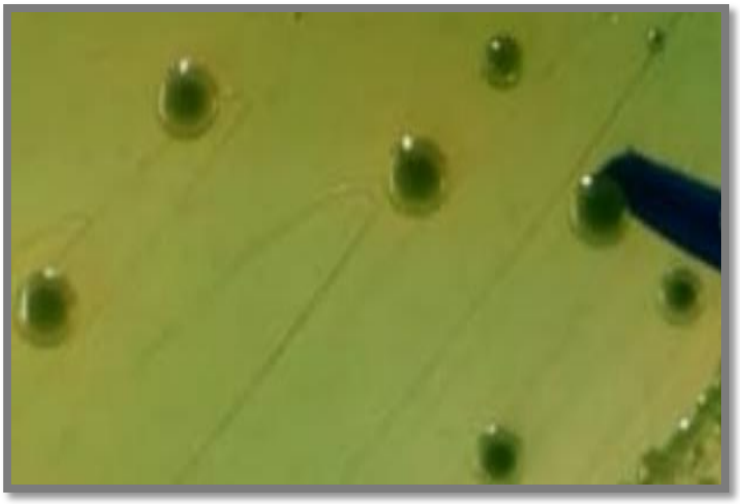

Fig. 1. *Aeromonas* spp. on *Aeromonas* medium base appears as green colony with dark green center

Molecular identification of *A. hydrophila* by PCR

DNA was extracted from the suspected isolates using QIAamp DNA mini kit, Qiagen. PCR was done for the detection of *16S rRNA* gene using specific primer for *A. hydrophila* using an applied biosystem thermal cycler. PCR condition

was initial denaturation at 94˚C for 5 minutes, 35 cycles at 94˚C for 30 sec., then 50 ˚C for 40 sec., 72˚ C for 45 sec. and final extension for 10 minutes 72˚ C according to Gordon et al. (2007). The products of PCR were examined in agarose gel electrophoresis (1.5%) with ethidium promide and photographed by light transilluminator (Biometra).

Detection of virulence genes by PCR

A. hydrophila strains were examined for the presence of two virulence genes such as *AHA* and *AST*. The aerolysin *AHA* gene cycling condition was primary denaturation at 94˚C for 5 minutes, then 30 cycles at 94˚C for 30 sec., 52˚C for 30 sec., 72˚ C for 30 sec. and final extension for 10 minutes 72˚ C according to Singh et al. (2008). The (*AST)* gene cycling condition was initial denaturation at 94˚C for 2 minutes, then 35 cycles at 94˚C for 30 sec., 50 sec at 1˚C, and 72˚C for 10 min. according to Nawaz et al. (2010).

Statistical analysis

The mean value and the standard error of *Aeromonas* spp. counts of the tested samples were analyzed by SPSS 18 software.

Results

Aeromonas spp. represented highly count in catfish with mean 0.504 ± 0.154, followed by mullet 0.124±0.042, Nile tilapia 0.122±0.032, and shrimp 0.037±0.008, and cannot be counted in canned fish samples (Table 2). The incidence of *Aeromonas* spp. in mullet samples was 63.3% which reported the highest percent, followed by catfish 60%, Nile tilapia 53.3%, shrimp 13%, tuna 10%, and cannot be detected in salmon samples (Table 3 and Fig.1). *A. hydrophilla* was identified in 14 (7.8 %) of the examined fish samples using biochemical tests (Figs. 2&3). PCR using specific 16S rRNA gene reported that 12 (85.7%) out of 14 samples were positive for *A. hydrophilla* (Fig. 4). Two virulence genes of *A. hydrophilla* of such as *AHA* and *AST* genes was examined by PCR and *AHA* was detected in 5 out of 12 samples, while *AST* gene cannot be detected (Fig. 5 and Fig. 6).

Table 2: *Aeromonas* counts (cfu/g) in the examined samples of fish, canned fish and shrimp

Sample	Min	Max	Mean±SE
Nile tilapia	0.01 X 10^2	0.25 X 10^2	0.122 ± 0.032
Catfish	0.05 X 10^2	2 X 10^2	0.504 ± 0.154
Mullet	0.05 X 10^2	0.5 X 10^2	0.124 ± 0.042
Shrimps	0.02 X 10^2	0.06 X 10^2	0.037 ± 0.008
Canned fish	**0**	**0**	**0**

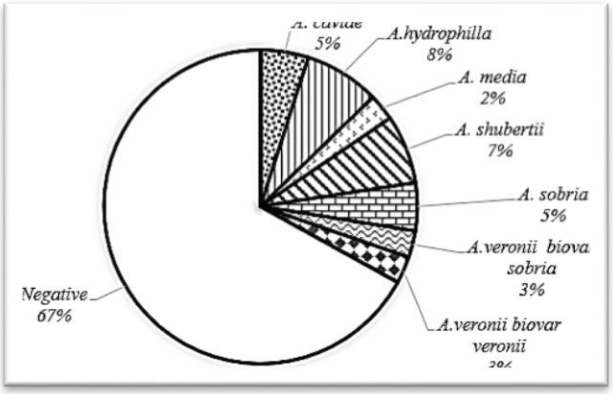

Fig. 2. Incidence of *Aeromonas* spp. in fish meat, canned fish and shrimp by using biochemical method

Table 3: Incidence of *Aeromonas* spp. isolated from fish meat, canned fish and shrimp by using biochemical method.

Species / Sample	Nile tilapia n=30		Catfish n=30		Mullet n=30		Shrimp n=30		Canned Tuna n=30		Canned Salmon n=30		Total (n=180)	
	No	**%**	**No**	**%**	**No**	**%**	**No**	**%**	**No**	**%**	**No**	**%**	**No**	**%**
A. caviae	3	10.0	2	6.6	3	10.0	1	3.3	0	0.0	0	0.0	**9**	**5.0**
A.hydrophilla	3	10.0	4	13.3	5	16.6	1	3.3	1	3.3	0	0.0	**14**	**7.8**
A. media	2	6.6	3	10.0	0	0.0	0	0.0	0	0.0	0	0.0	**5**	**2.8**
A. shubertii	1	3.3	4	13.3	8	26.6	0	0.0	0	0.0	0	0.0	**13**	**7.2**
A. sobria	2	6.6	3	10.0	1	3.3	1	3.3	2	6.6	0	0.0	**9**	**5.0**
A.veronii biovar sobria	2	6.6	0	0.0	2	6.6	1	3.3	0	0.0	0	0.0	**5**	**2.8**
A.veronii biovar veronii	3	10.0	2	6.6	0	0.0	0	0.0	0	0.0	0	0.0	**5**	**2.8**
Total	**16**	53.3	**18**	60.0	**19**	63.3	**4**	13.3	**3**	10	**0**	0.0	**60**	**33.3**

Table 4: PCR results of *A. hydrophilla* and its virulence genes

Sample	*16S rRNA* n=14	*AHA* gene n=12	*AST* gene n=12
Nile tilapia	3	1	0
Catfish	3	2	0
Mullet	5	2	0
Shrimp	1	0	0
Canned fish	0	0	0
Total	**12 (85.7%)**	**5 (41.7%)**	**0 (0.0%)**

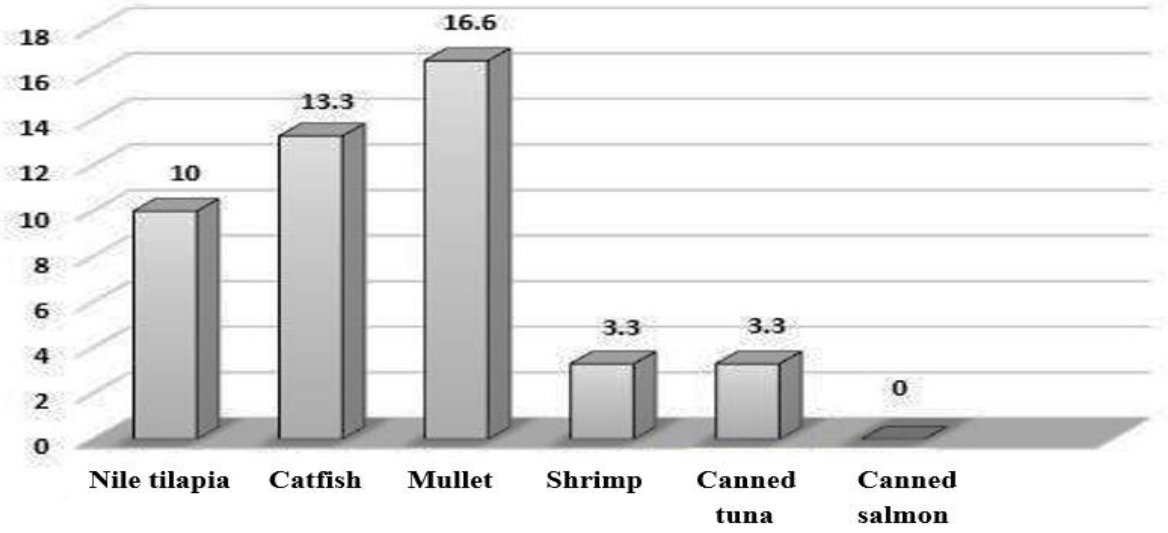

Fig. 3. Incidence of *A. hydrophila* in fish meat, canned fish and shrimp by using biochemical method

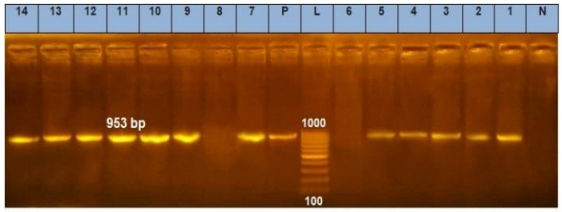

Fig. 4. PCR result for *16srRNA* gene of *A.hydrophila,* Lane L: 100 bp DNA marker. Lane P: Control positive (953 bp); Lane N: Control negative; Lanes 1-5: positive strains of *A. hydrophila* isolated from mullet. Lane 6: negative strains isolated from canned tuna. Lane 7: positive strains isolated from shrimp. Lanes 8: negative strains isolated from catfish. Lanes 9,10&11: positive strains isolated from catfish. Lanes 12, 13 & 14 positive strains isolated from Nile tilapia.

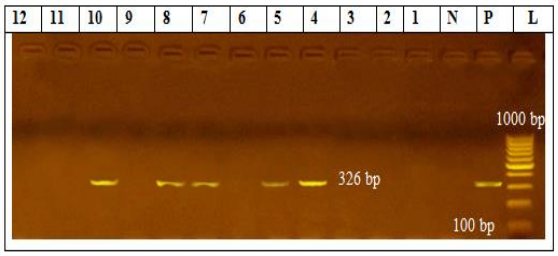

Fig. 5. PCR results for *AHA* gene, Lane L: 100 bp DNA marker; Lane P: Control positive; Lane N: Control negative; Lanes 1, 2& 3: negative results for AHA gene of *A. hydrophila* strains isolated from mullet. Lanes 4& 5: positive results from mullet. Lane 6: negative result from shrimp. Lanes 7& 8: positive results from catfish. Lane 9: negative results from catfish. Lane 10: positive result from Nile tilapia. Lanes 11&12: negative results from Nile tilapia.

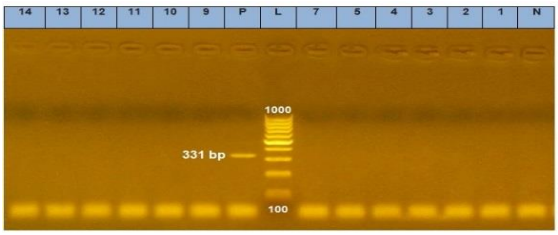

Fig. 6. PCR results for *AST* gene, Lane L: 100 bp DNA marker; Lane P: Control positive Lane N: Control negative; Lanes 1, 2, 3, 4, 5, 7, 9, 10, 11, 12, 13 &14: negative.

Discussion

Aeromonas counts

Aeromonas contamination was detected in Nile tilapia, catfish, mullet and shrimp samples with mean counts 0.122 ± 0.032, 0.504 ± 0.154, 0.124 ± 0.042 and 0.037 ± 0.008, respectively. While cannot be detected by direct plating in canned fish samples (Table 2). These results were lower than mean counts that was reported by Manna et al. (2013) where all or most of the samples of Indian major carps, tilapia and shrimp were contaminated with mean count 1.1 $\times 10^3$, 2.1 $\times 10^3$ and 1.6 $\times 10^4$ cfu/g and Ramadan et al. (2018) who mentioned that *Aeromonas sp.* were found with 3.35 log10 cfu/g in mullet samples. Variable counts and incidences between studies may be attributed to the difference in the examined samples, the status of fish prior to sampling time and place and geographical range. Also, fish may be contaminated with several pollutants during the production chain, transporting and retailing through bad hygiene, as well as absence of monitoring programs at farms (Eltholth et al., 2015; Hafez et al., 2018)

Incidence of *Aeromonas* spp. in fish meat and canned fish samples

Results in Table 3 and Fig. 2 showed that *Aeromonas spp.* incidence in fish meat and canned fish was 60 of 180 (33.3%). This result agrees with Elgohary et al. (2020) who detected *Aeromonas spp.* in 33.3 % of fish samples and disagrees with Yucel et al. (2005) and Yucel and Erdogan (2010) who reported 80.3% and18.4%, respectively. While, in Egypt, Attia et al. (2018) found *Aeromonas* spp. in 44.3% of raw fish. Our results revealed that highest incidence of *Aeromonas* spp. was found in mullet 19(63.3%) followed by catfish 18

(60%), tilapia 16 (53.3%), shrimps 4 (13.3%) and canned tuna 3 (10%) while couldn't be detected in canned salmon.

The incidence of *Aeromonas* spp. in Nile tilapia was 53.3% (Table 3). This result agrees with Ebeed et al. (2017) and El-ghareeb et al. (2019) who revealed that *Aeromonas* was isolated from Nile tilapia fish with a percentage of 51.4 % and 57.33%, respectively. However, our results were lower than that were reported by Kishk et al. (2020) who found *Aeromonas* spp. in farmed tilapia with a percentage of 68%, and higher than El-Gamal et al. (2018) and Salem et al. (2020) who found *Aeromonas* spp. in 25.9% and 29.84 % of Nile tilapia samples. The frequency distribution of isolated *Aeromonas* species of examined Nile tilapia samples was *A. hydrophila, A. caviae* and *A. veronii biovar veronii* 3 (10%) for each, followed by *A. media, A. sobria* and *A. veronii biovar sobria* 2 (6.6%) for each, and *A. shubertii* 1 (3.3%). These results disagree with El-Gamal et al. (2018) who detected *A. hydrophila* in (23.3%) and *A. caviae* in (2.6%), and Kishk et al. (2020) who found *A. caviae* 13 (40.6%), *A. hydrophila* 8 (25%), *A. sobria* 7 (21.9%), and *A. fluvialis* 1 (3.1%) while *A. veronii* 3 (9.4%) represented slightly similar results.

As shown in Table 3 and Fig. 3, *A. hydrophila* was detected in 16.6 % of the examined mullet, this result is lower than the results obtained in Kafr El-sheikh and Dakahliya Governorate by Ebeed et al. (2017) and Ramadan et al. (2018) who reported 62% and 37% contamination percentage in mullet samples, respectively. Frequency distribution of *Aeromonasspp.*in mullet samples were *A. shubertii* 8 (26.6%), *A. hydrophila* 5 (16.6%), *A. caviae* 3 (10%) and *A. veronii biovar sobria* 2 (6.6%) and *A. sobria* 1 (3.3%). These results disagree with Kishk et al. (2020) who found several species of *Aeromona*s as *A. sobria* 11 (44%), *A. caviae* 7 (28%), *A. hydrophila* 5 (20%), and *A. veronii* 2 (8%). *Aeromonas* spp. were detected in 6 shrimp samples at a percentage of 13.3%, of which *A. hydrophila* was 3.3%, this result disagreed with Khamesipour et al. (2014) in Iran who reported that incidence of *A. hydrophila* in shrimp was 13.89%. while, Kahraman et al. (2017) detected *A. hydrophila* in 15% of shrimp samples.

From previous results, it is denoted the ability of *Aeromonas* spp. to survive in freshwater and marine water environments with slight differences in incidence rates. The variations of *Aeromonas* species incidence could be attributed to various species, time and place of sampling, geographical area, and post-capture contamination, the type of water, fish species, handling, and manipulations during catching, storage, and transportation. and this agrees with Hafez et al. (2018). *Aeromonas* spp. were detected in 3(10%) canned tuna and not isolated in canned salmon. The identified species were 2 *A. sobria*(6.6%) and 1 *A. hydrophila* (3.3%), this may be attributed to treatments which applied to these products, as temperature, lack of Oxygen in vacuum packaging, salt or brine concentration and preservatives.

A. hydrophila has the highest incidence (14 out of 60) among the isolated *Aeromonas* spp. in the examined samples collectively; 5 mullet, 4 catfish,3 Nile tilapia, 1 shrimp and 1 canned tuna while it was not found in canned salmon (Fig. 3). These isolates were tested for *16S rRNA* gene as well as *AHA* and *AST* genes.

PCR results of *A. hydrophila* and its virulence genes

Results in Table 4 and Figure 4 showed that*16S rRNA* gene of *A. hydrophila* was detected in 12 of 14 examined samples with

a percentage of (85.7%), distributed as 25% (3/12) in each of freshwater (Nile tilapia and catfish) samples, 5 mugil (41.6%) and 1shrimp (8.3%). While, it was not detected in canned fish. A higher incidence was reported by Abd-El-Malek (2017) who detected *16S rRNA* gene in 35% of *A. hydrophila* isolated from tilapia samples.

Our findings revealed that the *AHA* gene (Fig. 5) was encoded in 5 (41.7%) of *A. hydrophila* isolates, with the highest incidence in catfish 2/3 (66.7%), mugil 2/5 (40 %) and Nile tilapia1/3 (33.3%). While, it was not detected in shrimp isolates. Wang et al. (2003) reported *A. hydrophila AHA* gene at a percentage of (37.5%). However, Blaszk (2014) reported that 85% *A. hydrophila* were encoding aerolysin gene. Also, Abd-Elall et al. (2014), Attia et al. (2018); Mansour et al., (2019) and Salem et al. (2020) reported that Aerolysin *AHA* gene was found in 100%, 55%,51% and 83.3 %, respectively. While, lower 13.15% incidence was reported by Sharma et al. (2010).

AST gene give negative results in all *A. hydrophila* isolates (Fig. 6), similar results were reported by Ghenghesh et al. (2014) and Silva et al. (2017). Opposite to De Jagoda et al. (2014) and Mansour et al. (2019) who reported that *AST* gene was encoded in 38% and 34%of *A. hydrophila* isolates, respectively.

Conclusion

The present study highlights the incidence of *Aeromonas sp.* in different fish species inhabit fresh and marine water and focused on *A. hydrophila* with its virulence genes as *AHA* and *AST* which may pose possible public health threats, given the importance of aeromonads as emerging human pathogens. Good hygienic practices are needed to provide safe and wholesome foods.

Conflict of interest

The authors declare that there is no conflict of interest.

References

Abd-Elall A, Abd-El-Kader M and Atia A (2014). Occurrence, seasonal variations and virulence of *Aeromonas hydrophila* and *Aeromonas caviae* in fish farms at East Delta, Egyptian Global Veterinary Journal, 13: 328-36.

Abd-El-Malek AM, (2017). Incidence and virulence characteristics of *Aeromonas* spp. in fish. Veterinary World, 10(1): 34-37.

Abisoye BF, Ojo SKS, Adeyemi RS, Olajuyigbe OO (2011). Bacteriological assessment of some commonly sold fishes in Lagos metropolis market Nigeria. Pakistan Journal of Medical Research, 1 (2): 23-26.

Attia ASA, Khedr MHE, Zaki MS (2018). Occurrence of potentially pathogenic *Aeromonas* species isolated from raw and ready-to-eat fish marketed in Sharkia Governorate, Egypt. Zagazig Veterinary Journal, 46(2): 154-159.

Austin B (2014). *Aeromonas*; Detection by cultural and modern techniques. Reference Module in Food Science, Encyclopedia in Food Microbiology, 2nd ed.: 31-37.

Blaszk FB (2014). Phenotypic and molecular characteristics of an *Aeromonas hydrophila* strain isolated from the River Nile. Microbiological Research, 169: 547-552.

Bravo FA, Figueras JM (2020). Review; An Update on the Genus *Aeromonas*: Taxonomy, Epidemiology, and Pathogenicity. Microorganisms, *MPDI*, 129(8):1-39.

Carnahan M A, Joseph SW (2015). *Aeromonas.* In: Bergeys manual of systemics of Archea and bacteria. Part B, The Gamma Proteobacteria Chapter*: Aeromonadaceae.* Whitman, W. B. (edt). John Wiley and Sons incorporation, *1st ed.,* 2: 556-560.

De Jagoda, SSS, Wijewardana TG, Arulkanthan A, Igarashi Y, Tan E, Kinoshita S, Watabe S, Asakawa S, (2014). Characterization and antimicrobial susceptibility of motile aeromonads isolated from freshwater ornamental fish showing signs of septicaemia. Diseases of Aquatic Organisms, 109(2): 127-137.

Ebeed AS, Alaa Eldin MA, Mohamed AM, Basma FE, (2017). Prevalence of *Aeromonas* species and Their Herbal Control in Fish. Global Veterinaria, 18(4): 286-293.

El-Gamal AM, El-Gohary MS, Gaafar AY (2018). Detection and molecular characterization of some bacteria causing skin ulceration in cultured Nile tilapia (*Oreochromis niloticus*) in Kafr El-Sheikh Governorate. Internatioal Journal of Zoology Research, 14: 14-20.

El-ghareeb HM, Zahranand E, Abd-Elghany SM, (2019). Occurrence, Molecular Characterization and Antimicrobial Resistance of Pathogenic *Aeromonas Hydrophila* from Retail Fish. Alexandria Journal for Veterinary Sciences, 62(1): 172-181.

El-Gohary FA, Zahran E, Abd El-Gawad EA, El-Gohary AH, Abdelhamid FM, El-Mleeh A, Elmahallawy EK, Elsayed MM (2020). Investigation of the prevalence, virulence genes, and antibiogram of motile aeromonads isolated from Nile tilapia fish farms in Egypt and assessment of their water quality. Animals (Basel), 10 (8): 1432-1448.

Eltholth M, Fornace K, Grace D, Rushton J, Häsler B (2015). Characterization of production, marketing and consumption patterns of farmed tilapia in the Nile Delta of Egypt. Food Policy, 51: 131-143.

Figueras MJ, Hidalgo RB, (2015). *Aeromonas* infections in humans. In *Aeromonas*; Academic Press: Norfolk, UK*:* 65-108.

Food and Drug Administration (FDA) (2018). Food sampling/preparation of sample homogenate. In: FDA, BAM; edt, Andrews, W. H. and Hammack, T. S., Ch. 1: 1-11.

Ghenghesh KS, Ahmed SF, Cappuccinelli P, Klena JD, (2014). Genospecies and virulence factors of *Aeromona*s species in different sources in a North African country. Libyan Journal of Medicine, 9: 1-5.

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

Hafez AEE, Darwish WS, Elbayomi RM, Hussein SM, (2018). Prevalence, antibiogram and molecular characterization of *Aeromonas hydrophila* isolated from frozen fish

marketed in Egypt. Slovenian Veterinary Research, 55: 445-454.

Hidalgo RB, Figueras MJ (2013). *Aeromonas* spp. whole genomes and virulence factors implicated in fish disease. Journal of Fish Diseases, 36: 371-388.

Igbinosa IH, Igumbor EU, Aghdasi F, Tomand M, Okoh AI, (2012). Emerging *Aeromonas* species infections and their significance in public health. The Scientific World Journal, 2012: 625023

Janda JM, Abbott SL (2010). The genus Aeromonas: Taxonomy, pathogenicity, and infection. Clinical Microbiology Reviews, 231: 35-73.

Kahraman BB, Dumen E, Issa G, Kahraman T, Ikiz S, (2017). Incidence of *Aeromonas hydrophila* and *Plesiomonas shigelloides* in seafoods. Turkish Journal of Fisheries and Aquatic Sciences, 17(6): 1309-1312.

Khamesipour F, Moradi M, Noshadi E, Shahraki MM (2014). Detection of the prevalence of *Aeromonas hyrophila* in shrimp samples by polymerase chain reaction (PCR) and cultural method in the Iran. Journal of Biology and Environmental Science, 4: 47-52.

Kishk DM, Moustafa NY, Kirrella GAK (2020). Prevalence and virulence characteristics of *Aeromonas* species isolated from fish farms in Egypt. Kafrelsheikh Veterinary Medical Journal, 18 (2): 5-8.

Manna SK, Maurye P, Dutta C, Samanta G (2013). Occurrence and virulence characteristics of *Aeromonas* species in meat, milk and fish in India. Journal of Food Safety, 33: 461-469.

Mansour A, Mahfouz NB, Husien MM, El-Magd MA, (2019). Molecular identification of *Aeromonas hydrophila* strains recovered from KafrEl Sheikh fish farms. Slovenian Veterinary Research, 56 (22): 201-208.

National Center for Biotechnology Information (NCBI) (2020). Taxonomy of *Aeromonas* species. Schoch, et al. edt., a comprehensive update on curation, resources and tools. Database (Oxford): baaa062.

Nawaz M, Khan SA, Khan AA, Sung K, Tran Q, Kerdahi K, Steele R (2010). Detection and characterization of virulence genes and integrons in *Aeromonas veronii* isolated from catfish. Food Microbiology, 27: 327–331.

Ramadan H, Ibrahim N, Samir M, Abd El-Moaty A, Gad T, (2018). *Aeromonas hydrophila* from marketed mullet (*Mugil cephalus*) in Egypt: PCR characterization of β-lactam resistance and virulence genes. Journal of Applied Microbiology, 124 (6): 1629-1637.

Salem M, Zharan E, Saad R, Zaki V, (2020). Prevalence, molecular characterization, virulotyping, and antibiotic resistance of motile aeromonads isolated from Nile tilapia farms at northern Egypt. Mansoura Veterinary Medical Journal, 21(1): 56

Sánchez VV (2015). Phenotypic and genotypic characterization of *Aeromonas* species isolated from rainbow trout "*Oncorhynchus mykiss*" Autonomous University of the State of Mexico :Toluca ,

Mexico. Microbiological Research, 169 (7-8): 483-642.

Sarkar A, Saha M, Roy P, (2013). Detection of 232bp virulent gene of pathogenic *Aeromonas hydrophila* through PCR based technique: (A rapid molecular diagnostic approach). Advanced Microbiology, 03(1): 83-87.

Sharma I, Kumar A, Pramanik AK (2010). Isolation and identification of mesophilic Aeromonas bacteria from meat and fish foods of North East India. Journal of Pure Applicable Microbiology, 3(2): 517-526.

Silva LC A, Balbino TCL, Melo BST, Marques CLM, Rezende AM, Almeida AMP, Leal NC (2017). Genetic diversity and virulence potential of clinical and environmental *Aeromonas* spp. isolates from a diarrhea outbreak. BioMed Central Microbiology, 17(1): 179-190.

Singh V, Rathore G, Kapoor D, Mishra BN, Lakra WS (2008). Detection of aerolysin gene in *Aeromonas hydrophila* isolated from fish and pond water. Indian Journal of Microbiology, 48: 453-458.

Sreeremya S (2017). Identification, characterization, antibiotic resistance of *Aeromonas hydrophila* in chicken intestine. International Review of Education Journal, 1(3): 19-22.

Tahoun AB, Ahmed HA, Abou Elez RM, El-Gedawy AA, Elsohaby I, Abd El-Ghafar AE (2016). Molecular characterization, genotyping and survival of *Aeromonas hydrophila* isolated from milk, dairy products and humans in Egypt. International Dairy Journal, 63: 52-58.

Wamala SP, Mugimba KK, MutolokiEvensen S, Mdegela R, Byarugaba DK, Sørum H (2018). Occurrence and antibiotic susceptibility of fish bacteria isolated from *Oreochromis niloticus* (Nile tilapia) and Clarias gariepinus (African catfish) in Uganda. Fish Aquatic Science, 21 (1): 6-10.

Wang CG, Clark CG, Liu C, Pucknell C, Munro CK, Kruk TMAC, Caldeira R, Woodward D L Rodgers FG (2003). Detection and Characterization of the hemolysin genes in *Aeromonas hydrophila* and *Aeromonas sobria* by multiplex PCR. Journal of Clinical Microbiology, 41(3):1048–1054.

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

Yucel N, Erdogan S (2010). Virulence properties and characterization of aeromonads isolated from foods of animal origin and environmental sources. Journal of Food Protection, 73(5): 855-860.