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Original Research Article

PREVALENCE, ANTIBIOGRAM AND MOLECULAR CHARACTERIZATION OF *Aeromonas hydrophila* ISOLATED FROM FROZEN FISH MARKETED IN EGYPT

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Abstract: Fish is one of the most important foods because of its high nutritional value, high palatability and easy digestion. On the same time, it acts as a vehicle for many types of pathogenic microorganisms especially Aeromonas spp., which results in public health hazards. Therefore, the present study was conducted to evaluate the prevalence of Aeromonas spp. in frozen fish (mackerel, herrings and fish fillets) marketed in Zagazig city, Sharkia Governorate, Egypt. In addition, multiplex PCR was done to detect some virulence-associated genes in A. hydrophila isolates. Furthermore, antimicrobial susceptibility testing of A. hydrophila isolates to the commonly used antimicrobials in Egypt including cephalothin, ampicillin, chloramphenicol, sulphamethoxazol, oxytetracycline, cloxacillin, gentamicin, kanamycin, amikacin, ciprofloxacin, cefotaxime, erythromycin, streptomycin and neomycin was conducted using the disc diffusion method. The achieved results indicated contamination of frozen fish with different species of Aeromonas such as A. veronii, A. sobria, A. caviae and A. hydrophila. A. veronii was the predominant species isolated from the examined fish; its prevalence rates in mackerel, fish fillets and herrings were 62.5, 50 and 45%, respectively. A. sobria came second; it was isolated only from herrings (30%) and fish fillets (16.7%). The prevalence rates of A. hydrophila in mackerel, fillets and herrings were 12.5, 33.3 and 10%, respectively; while A. caviae was isolated only from mackerel (25%) and herrings (15%). The isolated A. hydrophila harbored some virulence attributes such as aerolysin (aerA) and haemolysin (ahh1). A. hydrophila isolates were resistant to different antimicrobial agents used in Egypt including cloxacillin, erythromycin and streptomycin (100% each); cefotaxime and sulphamethoxazol (80% each); and cephalothin, chloramphenicoland oxytetracycline (60% each); while it was sensitive to ampicillin (80%) and gentamicin (60%). Therefore, hygienic measures should be adopted to control the microbial contamination either in the aquatic environment or in fish markets.

Key words: fish; *A. hydrophila*; virulence genes; antibiotic sensitivity

Introduction

Fish is a healthy food, it plays an important role in human nutrition providing one third of the world's population by at least 20% of their

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protein intake (1). Fish protein is also a rich source of the omega-3 polyunsaturated fatty acids. In addition, fish contains some micronutrients as vitamin D, minerals (magnesium, calcium, iodine, zinc, and selenium) which provide a role in the deficiency eradication of human-related micronutrient diseases (2). Mesophilic *Aeromonas* spp., is an important fish pathogen that causes significant problems in aquaculture and leads to fish spoilage and food-borne disease (3). *Aeromonas* spp. is opportunistic microorganism, one of the normal microbial flora of many aquatic animals and widely distributed in the aquatic environments, including fresh, marine, and ground water. It is considered as a primary pathogen of fish and can be isolated from healthy or diseased ones (4). *Aeromonas* spp. is responsible for large economic losses because of high fish mortalities (5). Many *Aeromonas* spp. have been identified in fish diseases, including *A. hydrophila, A. allosaccharophila, A. salmonicida* and *A. veronii* biovar *sobria* (6). In human, motile *Aeromonas* spp. can cause food-borne gastroenteritis in addition to extra intestinal symptoms (septicemia, wound infections, meningitis, endocarditis and osteomyelitis) with a high mortality rate in immune-compromised persons (7).

The pathogenicity of *Aeromonas* spp. is attributed to the release of various virulent attributes, including enterotoxin, hemolysin, and adhesion-related factors which contribute to the adhesion of the bacteria into the host gastrointestinal tract and responsible for hemolysis and enterotoxin production; meanwhile, extracellular hemolysin and aerolysin potentially contribute to the occurrence of septicemia (8,9). Antimicrobial resistance in *Aeromonas* spp. is usually chromosomally mediated, but β-lactamases produced by aeromonads may occasionally be encoded by plasmids or integrons (6). However, the antibiogram of *Aeromonas* spp. isolated from fish in Egypt is less informed.

In developing countries, shortage and high price of animal proteins is the principle cause of the high demand on fish; so many trials have been developed by National Authorities during the last years in order to improve the fish quality**.**

There is little information about the prevalence of *Aeromonas* spp. in fish marketed in Egypt. Therefore, this study was conducted to detect the prevalence of *Aeromonas* spp. in different types of frozen fish retailed in fish markets in Zagazig city, Sharkia Governorate, Egypt. Due to the significant roles of *A. hydrophila* as food-borne pathogen, characterization of its virulence attributes and antibiotic resistance profile were further studied.

Material and methods

Collection of fish samples

A total of one hundred and fifty frozen fish samples of mackerel, herring and fish fillets (50 of each) were randomly collected from different fish markets at Zagazig city, Sharkia Governorate, Egypt. The collected samples were identified and packaged separately in a sterile plastic bag; then transported in cooled aseptic conditions without any delay to the Laboratory of Meat Hygiene, Faculty of Veterinary Medicine, Zagazig University, Egypt for bacterial isolation and identification.

Preparation of fish samples

After removal of the dorsal, pectoral and ventral fins by sterile scissors and forceps, the scales were removed from the body surface everywhere by a sterile scalpel. The surface was sterilized by a hot spatula and was removed. About 25 grams of the back muscles were aseptically homogenized with 225 ml of sterile 0.1% alkaline peptone water for 2.5 min and then were allowed to stand for 5 min (10).

Bacterial isolation

From the prepared homogenate, 1 ml was transferred into a sterile test tube containing 0.1% alkaline peptone water as an enrichment broth and incubated at 37°C for 18 h. After incubation, a loopful of the alkaline peptone water was streaked on the surface of Aeromonas agar medium (Himedia, Mumbai, India) and incubated at 37°C for 18-24 h (11). Typical colonies (Pale green with dark centers, their size is typically

between 0.5 and 1.5-mm) were sub-cultured on a non-selective medium (Nutrient agar, Himedia, Mumbai, India), and incubated for 24 h.

Primary characterization and identification of the isolates

Pure cultures of the isolates were morphologically, biochemically and physiologically identified (12). Morphological characters including shape, size, Gram staining, and motility of the isolated Aeromonas were confirmed (12,13). Biochemical identification was done using the following tests; oxidase, esculin hydrolysis, arginine hydrolysis, indole, methyl red, Voges–Proskauer test, citrate utilization, urease, hydrogen sulphide production, nitrate reduction, gelatin liquefaction, oxidation-fermentation, pigment formation, sugars fermentation and detection of ornithine decarboxylase, L- lysine decarboxylase, arginine decarboxylase and β- galactosidase (14). Biochemical reagents used were from Himedia, Mumbai, India. Physiological properties were investigated by observing the growth of each isolate at various temperatures (4, 24, 37 and 40°C) and different NaCl concentrations (0, 1, 2, 3 and 4%) (12,15).

Detection of virulence genes of A. hydrophila by multiplex PCR

The extraction of the bacterial DNA was carried using QIA amp kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Application of PCR for identification of *16SrRNA*, *aerolysin (aerA)* and haemolysin *(ahh1)* virulence genes of *A. hydrophila* was performed by using primers purchased from Pharmacia Biotech, Sweden. The 16SrRNA primers were sense 5′GGG AGT GCC TTC GGG AAT CAG A′3 and antisense 5′TCA CCG CAA CAT TCT GAT TTG′3 with a product size of 356 bp. while the primers used for detection of *aerA* were sense 5′ CAA GAA CAA GTT CAA GTG GCC A ′3 and antisense 5′ACG AAG GTG TGG TTC CAG T′3 with a product size of 309 bp. The primers used to detect *ahh1* were sense 5′GCC GAG CGC CCA GAA GGT GAG TT′3 and antisense 5′GAG CGG CTG GAT GCG GTT GT′3 with a product size of 130 bp (16).

The amplification reaction was performed on a thermal cycler (Master cycler, Eppendorf, Hamburg, Germany). The PCR reaction (10 µl) consisted of bacterial DNA (2µl), 10X EX Taq buffer (1µl), forward primer (1µl), reverse primer (1µl), 2.5 mM dntp (0.8 µl), EX Taq polymerase (TaKaRa, Japan) (0.05µl) and nuclease free water (4.15µl) (17). Amplification consisted of an initial denaturation at 95°C for 5 min, 50 cycles at 95°C for 30 sec., 59°C for 30 sec., 72°C for 30 sec., followed by final elongation at 72°C for 7 min. Amplified DNA fragments were analyzed by 2% of agarose gel electrophoresis (Applichem, Germany, GmbH). Finally, the gel was stained with ethidium bromide and captured as well as visualized on UV transilluminator. A 100 bp plus DNA Ladder (Qiagen, Germany, GmbH) was used to determine the fragment sizes (18).

Antibiotic resistance of the isolated A. hydrophila

Antimicrobial susceptibility of the isolated *A. hydrophila* strains was tested by Kirby-Bauer disc diffusion method (19) using the antibiotic discs *(*Oxoid Limited, Basingstoke, Hampshire, UK*)* with variable concentrations (µg) including cephalothin (CN, 30µg), ampicillin (AM, 10µg), chloramphenicol (C, 30µg), sulphamethoxazol (SXT, 25µg), oxytetracycline (T, 30µg), cloxacillin (Cl, 5µg), gentamicin (G, 10µg), kanamycin (K, 30µg), amikacin (AK, 30µg), ciprofloxacin (CIP, 5µg), cefotaxime (CF, 30µg), erythromycin (E, 15µg), streptomycin (S, 10 µg) and neomycin (N, 30µg). Interpretation of the results was carried out according to the guidelines stipulated by National Committee for Clinical Laboratory Standards (NCCLS) (20).

The tested isolates were evaluated as susceptible, intermediate and resistant and multiple antibiotic resistances (MAR) index for each strain was determined; MAR index = No. of resistance (isolates classified as intermediate were considered sensitive for MAR index) / Total No. of tested antibiotics (21).

Results

Prevalence of Aeromonas spp. in frozen fish

The prevalence of *Aeromonas* spp. in the examined mackerel, fish fillets and herrings was recorded in Table 1. Out of 150 fish samples analyzed, 68 (45.3%) were found to be contaminated with *Aeromonas spp*. High level of contamination by *Aeromonas* spp. was found in the herring fish (40/50; 80%), while the prevalence in mackerel and fish fillet was 32% (16/50) and 24% (12/50), respectively.

Identification of Aeromonas spp. isolated from the examined fish

Morphological and biochemical properties of the isolated species showed in Table (2) and revealed that the examined frozen fish samples were contaminated by *A. hydrophila*, *A. sobria*, *A. caviae* and *A. veronii*. Of the 68 identified isolates; *A. veronii* (34/68, 50%) was the predominant species isolated from the examined fish; its prevalence rates in mackerel, fish fillets and herrings were 62.5, 50 and 45%, respectively. *A. sobria* came second with 14 (20.6%) isolates; it was isolated only from herrings (30%) and fish fillets (16.7%). Ten isolates were identified as *A. hydrophila* and *A. caviae* (each). The prevalence rates of *A. hydrophila* in mackerel, fillets and herrings were 12.5, 33.3 and 10% respectively; while *A. caviae* was isolated only from mackerel (25%) and herrings (15%) (Table 1).

Table 1: Prevalence of *Aeromonas* spp in the examined frozen fish samples

Fish samples (No=50, each)	Positive samples*	*A. hydrophila***	*A. sobria***	*A. caviae***	*A. veronii***
Mackerel	16 (32)	2 (12.5)	0	4 (25)	10 (62.5)
Fillet	12 (24)	4 (33.3)	2 (16.7)	0	6 (50)
Herrings	40 (80)	4 (10)	12 (30)	6 (15)	18 (45)
Total	68 (45.3)	10 (14.7)	14 (20.6)	10 (14.7)	34 (50)

No: Number of examined fish samples. Data were represented by number (%)
*Percentages were calculated according to number of examined samples
**Percentages were calculated according to number of positive samples

Table 2: Morphological and biochemical characteristics of the isolated *Aeromonas* spp. from frozen fish

Test	*A. hydrophila*	*A. sobria*	*A. caviae*	*A. veronii*
Motility	+	+	+	+
Esculine hydrolysis	+	-	+	-
Oxidase	+	+	+	+
Arginine dihydrolase	+	+	+	+
Indole	+	+	+	+
Methyle red	+	-	-	V
Voges Proskuaer	+	-	-	-
Citrate utilization	V	+	+	+
Urease	-	-	-	-
H2S	+	+	-	V
Nitrate reduction	+	+	-	V
Gelatin liquefaction	+	+	+	+
Glucose fermentation (F)	+	+	+	+
Sucrose (F)	+	+	+	+
Rhamnose (F)	+	-	-	-
Mannose (F)	+	+	V	V
Arabinose (F)	+	-	+	V
Inositol (F)	-	-	V	-
Sorbitol (F)	-	-	-	-

F: Fermentation; (+): Positive response; (-): Negative response; V: variable in reaction

Molecular characterization of A. hydrophila using multiplex PCR

In the current study, a multiplex PCR was used as a reliable DNA based technique for detection of virulence associated genes in the isolated bacteria. It was found that 6 out of 10 (60%) of *A. hydrophila* isolated from the examined fish samples harbored *16S rRNA*, aerolysin *(aerA)* and haemolysin *(ahh1)* genes; while 2 (20%) harbored *16S rRNA* and aerolysin *(aerA)*; and 2 (20%) harbored *16S rRNA* and hemolysin *(ahh1)* genes (Table 3).

Antimicrobial susceptibility of A. hydrophila

Results achieved from the disc diffusion test in table 4 revealed that *A. hydrophila* isolates (n=10) were resistant to different antimicrobial agents used in Egypt including cloxacillin, erythromycin and streptomycin (100% each); cefotaxime and sulphamethoxazol (80% each); and cephalothin, chloramphenicoland oxytetracycline (60% each); while it was sensitive to ampicillin (80%) and gentamicin (60%).

The average MAR value was 0.614 for all *A. hydrophila* isolates (Table 3). Antimicrobial resistance varied among the obtained isolates. For instances, the two strains of *A. hydrophila* isolated from herrings, harbored *16S rRNA*, *aerA* and *ahh1* genes, differed in their antibiotic susceptibilities. The first strain was resistant to all tested antibiotics with MAR index 1; however, the other strain was resistant to cephalothin, chloramphenicol, sulphamethoxazol, oxytetracycline, cloxacillin, amikacin, cefotaxime, erythromycin, and streptomycin with MAR index value of 0.643 and sensitive to ampicillin, gentamicin, kanamycin, ciprofloxacin and neomycin.

While *A. hydrophila* isolated from fish fillets, harbored 16S *rRNA* and *aerA*, were resistant to cephalothin, chloramphenicol, sulphametho-xazol, oxytetracycline, cloxacillin, amikacin, cefotaxime, erythromycin, streptomycin, kanamycin, ciprofloxacin and neomycin with MAR index value of 0.857, and sensitive to ampicillin and gentamicin. The other strain that harbored *16S rRNA, aerA* and *ahh1* had a MAR index value of 0.357. It was resistant tostreptomycin, cloxacillin, erythromycin, sulphamethoxazol and cefotaxime; but sensitive to chloramphenicol, oxytetracycline, cephalothin, amikacin, kanamycin, ciprofloxacin, neomycin, gentamicin and ampicillin (Table 4). *A. hydrophila* isolated from mackerel fish that harbored *16S rRNA* and *ahh1* was resistant to only 3 of the tested antibiotics (streptomycin, cloxacillin and erythromycin) with MAR index value of 0.214; while sensitive to sulphamethoxazol, cefotaxime, chloramphenicol, oxytetracycline, cephalothin, amikacin, kanamycin, ciprofloxacin, neomycin, gentamicin and ampicillin (Tables 3 and 4).

Table 3: Virulence genes and antimicrobial resistance profile of selected 5 isolates of *A. hydrophila* of frozen fish

***A. hydrophila* source**	**Virulence genes**	**Antimicrobial resistance**	**MAR**
Herrings (No=1)	16S rRNA, *aer*A and *ahh*1	S, Cl, E, SXT, CF, C, T, CN, AK, K, CP, N, G,AM	1.000
Fillet (No=1)	16S rRNA and *aer*A	S, Cl, E, SXT, CF, C, T, CN, AK, K, CP, N	0.857
Herrings (No=1)	16S rRNA, *aer*A and *ahh*1	S, Cl, E, SXT, CF, C, T, CN, AK	0.643
Fillet (No=1)	16S rRNA, *aer*A and *ahh*1	S, Cl, E, SXT, CF	0.357
Mackerel (No=1)	16SrRNA and ahh1	S, Cl, E	0.214

n: Number of examined samples; MAR: Multiple antibiotic resistances. S: Streptomycin; Cl: Cloxacillin; E: Erythromycin; CF: Cefotaxime; C: Chloramphenicol: Oxytetracycline; AK: Amikacin; K: Kanamycin; CP: Ciprofloxacin; G: Gentamicin; AM: Ampicillin; SXT: Sulphamethoxazol; CN: Cephalothin; N: Neomycin; *aerA*: aerolysin gene and *ahhl*: haemolysin gene.

Table 4: Percentages of antimicrobial susceptibility of *A. hydrophila* (No=10)

Antimicrobial agent	Sensitive		Intermediate		Resistant	
	No	%	No	%	No	%
S	0	0	0	0	10	100
Cl	0	0	0	0	10	100
E	0	0	0	0	10	100
SXT	0	0	2	20	8	80
CF	2	20	0	0	8	80
C	4	40	0	0	6	60
T	2	20	2	20	6	60
CN	4	40	0	0	6	60
AK	4	40	0	0	6	60
K	0	0	6	60	4	40
CP	2	20	4	40	4	40
N	4	40	2	20	4	40
G	6	60	2	20	2	20
AM	8	80	0	0	2	20

No: Number of examined samples. %: Percentage of positive samples. S: Streptomycin; Cl: Cloxacillin; E: Erythromycin; CF: Cefotaxime; C: Chloramphenicol: Oxytetracycline; AK: Amikacin; K: Kanamycin; CP: Ciprofloxacin; G: Gentamicin; AM: Ampicillin; SXT: Sulphamethoxazol; CN: Cephalothin; and N: Neomycin

Discussion

Fish flesh is an excellent substrate for growth of large number of bacteria with compositional attributes, which affect the bacterial growth and the related biochemical activities (22). Various sources are responsible for microbial contamination of fish such as water, soil and fish handlers. Bad handling of fish during fishing, transporting, freezing and shipping plays an important role in cross-contamination of fish from the surrounding environment and can act as a stress factor which results in bacterial migration from the gut to fish muscles. Some of these bacteria are associated with many diseases in humans, making the aquaculture products a potential risk factor for customer's health (23). *Aeromonas* spp. is considered as one of the emerging food-borne pathogens (24).

The present study examined the occurrence of Aeromonas spp. in three different types of frozen fish (mackerel, herrings and fish fillets) consumed in Egypt. High contamination (45.3%) of the examined fish by Aeromonas spp. was detected. Herrings were the most contaminated (80%), followed by mackerel (32%) and fish fillets (24%). This result indicated that the examined frozen fishes were subjected to contamination during storage, transportation and marketing. Leaving fish at room temperature in fish markets is a favorable condition for growth of Aeromonas spp. In addition, contaminated equipment and unhygienic standards of fish handlers during deboning process of fish fillets can increase the bacterial load of such products.

These findings were in agreement with Aeromonas-related studies conducted in other countries. For instances, in Malaysia, *Aeromonas* spp. was isolated from 69% of fish samples (25); while in Turkey, it was isolated from 82.8% of the examined seawater fish (26). Generally, the studies on prevalence of *Aeromonas* spp. in fish focused on 3 species, *A. hydrophila, A. sobria* and *A. caviae* (27). In the current study, identification of the isolated *Aeromonas* spp. from the examined fish samples revealed four different species; namely *A. hydrophila*, *A. sobria*, *A. caviae* and A. veronii. The most predominant species was A. veronii (50%) followed by *A. sobria* (20.6%) then *A. hydrophila* and *A. caviae* (14.7% each).

This result indicated the wide spreading of motile *Aeromonas* spp. in the aquatic environment; consequently fish can be easily contaminated by these microorganisms after exposure to stress as a result of rough handling during fishing and overcrowding in fish boxes. In addition, cross contamination after fish freezing and during transportation to fish

markets can increase the contamination by pathogenic motile Aeromonas spp.

In agreement with the current report, a study conducted in Serbia found that *A. hydrophila* and *A. sobria* were isolated from 66.7% and 33.3% of the examined fish, respectively (27), while in Malaysia, *A. caviae* was the predominant species isolated from fish samples (47.1%) followed by *A. hydrophila, A. sobria* and *A.veronii* (25). In India, *A. sobria* was the most common species isolated from apparently healthy fish (9,28). In China, *A. veronii* was the dominant *Aeromonas* spp. (29). Variation in the prevalence of *Aeromonas* spp. in mackerel, fish fillets and herrings could be attributed to the differences in the contamination levels during handling, transportation and storage.

Aeromonas spp. is recognized as an emerging pathogen that has the ability to cause various diseases in humans including food poisoning, gastroenteritis, septicemia, skin affections, soft-tissues and muscles infection (30). *A. hydrophila, A. sobria,* and *A. caviae* are the main causes of Aeromonas associated human diseases (31). Such microorganisms had been linked to an outbreak of Aeromonas infection due to ingestion of raw fermented fish (32). Identification of *A. hydrophila* using conventional methods remains difficult. Therefore, the multiplex PCR was used in the current study. In *A. hydrophila*, as with all pathogenic microorganisms, disease occurs because of complex molecular interactions between bacteria, host and environment (31). Virulence in *A. hydrophila* is a multifactorial due to the production of several virulence factors, such as cytotoxins, adhesins, hemolysins, proteases and lipases, as well as the ability to form biofilms by using a specific metabolic pathway and a mediate virulence factor expression (6). Enterotoxins, cytotoxins and hemolysins, are more frequently detected in isolates obtained from patients with gastrointestinal symptoms; there was a relationship between hemolysin production and human illness caused by motile *Aeromonas* spp. (33).

In the present study, selected five isolates of *A. hydrophila* were specific for 16S rRNA gene; while four isolates were producers to hemolytic toxins such as aerolysin (*aer*A) and haemolysin (*ahhl*). This result agreed with published reports in Canada, Turkey and India, where, ahh1 and aerolysin were detected (18). Hemolytic toxins were also detected in 82% of *A. hydrophila* (25); and hemolysin gene was detected in 78% of *A. hydrophila* isolates (34). Additionally, *ahh1* and *aer*A were expressed in 60.52% and 13.15%, respectively of *Aeromonas* spp. (35).

The 16S rRNA gene is an excellent and rapid way to assess the identity of *A. hydrophila*. It is complicating factor because the bacteria contain up to 15 copies of this ribosomal operon (36). It has been used for molecular identification of species by restriction fragment length polymorphism (37) or direct gene sequencing (38). Hemolysin is a group of multifunctional enzymes, which play important roles in the pathogenicity of *A. hydrophila*. Hemolysins include *aer*A, *ahh1*, *ahy*A, and *asa1*; *ahh1* is the most widely distributed extracellular heat-labile hemolysin, the synergistic combination of *aer*A and *ahh1*is the most cytotoxic genotype (18).

Other virulence factors encoded by *A. hydrophila* include adherence proteins, catalysts, nucleases, and toxins that may be expressed differently. Adherence proteins are responsible for mucosal adherence, biofilms formation, cell division and motility (39). These virulence-associated factors are very important in distinguishing between pathogenic and non-pathogenic strains. *A. hydrophila* enterotoxin, which is cytotoxic in nature, is the main cause of gastroenteritis, while aerolysin is the principle virulence-associated factor implicated in various intestinal disorders (34).

Antibiotics are widely used in fish farms for prevention and control of bacterial diseases. Using a wide variety of antimicrobial agents, aquaculture had been implicated in the development of resistant bacteria and a source of transmission of these resistant pathogens to other animals and humans (40). In addition, application of the same antibiotics in different fields including veterinary and human medicine facilitates the appearance of the microbial drug-resistance phenomenon.

In the present investigation, *A. hydrophila* showed variable degrees of resistance to the fourteen antimicrobial tested agents. The isolates were 100% resistant to streptomycin, cloxacillin and erythromycin; 80% of the isolates were resistant to sulphamethoxazol and cefotaxime; 60% were resistant to oxytetracycline, cephalothin and amikacin; 40% were resistant to kanamycin, ciprofloxacin and neomycin and 20% were resistant to gentamicin and ampicillin. In agreement with the current study similar resistance profiles were reported in Bangladesh, India and Egypt (34,41,42). However, higher resistance percentages were recorded in Turkey to gentamicin, ampicillin and cephalothin; while, low resistance rates were recorded to streptomycin (18.1%), erythromycin (54.1%), sulphamethoxazol and cefotaxime (63%) (25). In another report, resistance to sulphamethoxazol and cefotaxime was 67% (43). These differences in the antimicrobial resistance profiles may be related to the differences in fish species and the type of the used antimicrobials.

Conclusion

The results achieved in the present study revealed high contamination of mackerel, herrings and fish fillets by *Aeromonas* spp. The isolates of *A. hydrophila* harbored virulence-associated genes in addition to their resistance to different types of antibiotics. This might be due to the contamination of the aquatic environment by such pathogens as well as bad handling of fish. Such microorganisms may lead to several public health implications, especially, if such contaminated fish consumed. Therefore, hygienic measures should be adopted to control the microbial contamination either in the aquatic environment or in fish markets.

Conflict of interest

The authors declare that there is no conflict of interest

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