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Virulent and Multiple Antimicrobial Resistance *Aeromonas hydrophila* Isolated from Diseased Nile Tilapia Fish (*Oreochromis niloticus*) in Egypt with Sequencing of Some Virulence-Associated Genes

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***Aeromonas hydrophila* is a major waterborne pathogen, which induces various diseases in freshwater fish with the capability for zoonotic potential. This study was applied to investigate the prevalence of *A. hydrophila* in diseased Nile tilapia fish, genetic characterization of the virulence encoding genes (*act*, *aerA*, *alt*, and *ast* genes), and antibiotic susceptibility. Out of the 500 diseased Nile tilapia fish samples, 70% (350/500) *Aeromonas* species were isolated. From which 53.4% (187/350) of *Aeromonas hydrophila* strains were identified. *A. hydrophila* was detected in kidneys, followed by liver, spleen, intestine, and gills. The results of virulotyping displayed the presence of *act*, and *aerA* genes in a high percentage of 40%, followed by *alt* gene (30%), but *ast* gene was not detected (0%) in *A. hydrophila* strains. Based on DNA sequence analysis of three virulence associated-genes (*act*, *aerA*, and *alt* genes), the phylogenetic tree showed the genetic relationship with related species. Finally, the antibiotic susceptibility tests revealed high resistance toward chloramphenicol (67.4%), followed by amikacin (51.9%) and gentamicin (47.1%), whereas a high sensitivity was exhibited toward meropenem (90.9%), followed by ciprofloxacin (84.2%), amoxicillin-clavulanic acid (73.3%) and trimethoprim-sulfamethoxazole (64.2%). The multidrug-resistant *A. hydrophila* strains were observed in 69.0% of strains with six resistance patterns.**

Key words : *Aeromonas hydrophila* / Virulence genes / Nile tilapia fish / Multi-antimicrobial resistance.

INTRODUCTION

Aeromonas species are facultatively anaerobic, Gram-negative, motile, short, rod-shaped bacilli, and non-spore-forming bacterium with widely host affection as fish, domestic chicken, most vertebrates, and humans (Rey et al., 2009). *Aeromonas* species were related to diseases in freshwater and marine fish species including Motile *Aeromonas* Septicemia and Epizootic Ulcerative Syndrome in addition to deleterious signs

accompanied with affected tilapia as skin darkness, scales detachment, red sores, exophthalmia, and fin erosions (Viji et al., 2011; Dias et al., 2016). The postmortem examination showed hemorrhages with enlargement of internal organs (El Deen et al., 2014). Most human affections arise to immunocompromised patients with multiple gastrointestinal tract infections, meningitis, endocarditis, peritonitis, hemolytic uremic syndrome, septicemia, and food poisoning (Guerra et al., 2007).

A. hydrophila, one of the most vigorous *Aeromonas* species, able to induce incurable problems to freshwater fish aquaculture in Egypt (Aboyadak et al., 2017) and

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has zoonotic importance with the capability to possess multiple antimicrobial resistance toward different classes of antibiotics (Le et al., 2018). Multifactor and the virulence genes work together to induce the virulence of *A. hydrophila* which contribute to infect, spread and kill the host such as cytotoxic enterotoxin gene, aerolysin gene, cytotoxic enterotoxin heat-labile gene, cytotoxic enterotoxin heat-stable gene, lipases, proteases, beta-lactamases, amylases, chitinases and nucleases with disease development (Furmanek- Blaszk, 2014; Stratev et al., 2015).

The irrational massive usages of antibiotics with the presence of antimicrobial resistance enzymes promote resistance in different species with induction of multiple antimicrobial resistances. *Aeromonas* spp could arise beta-lactamases resistance, which is mediated by three beta-lactamases including metallo beta-lactamase, cephalosporinase, and penicillinase (Janda and Abbott, 2010). In order to continuous arising of multiple antimicrobial resistances spreading *A. hydrophila* bacteria around the world with increase the affections to different hosts, so that massive efforts should be done to monitor the great distribution of pathogen, which would help to investigate disease diagnosis, prevention, and control (Kashhedikar and Chhabra, 2010). Consequently, in the current study *A. hydrophila* isolates from diseased (*Oreochromis niloticus*) Nile tilapia fish collected from freshwater fish aquaculture in Egypt, were tested for their genetic characterization of the virulence encoding genes and antibiotic susceptibility with the determination of multiple antimicrobial resistance patterns.

MATERIAL AND METHODS

Ethical Considerations

The current research work was permitted by the local guidance of the Research Ethics Committee of the Faculty of Veterinary Medicine, Mansoura University, Egypt.

Fish collection and sampling

A total of 500 clinically diseased (*Oreochromis niloticus*) Nile tilapia fish was indiscriminately assembled from three private freshwater fish aquaculture located at El-Manzala city, Dakahlia Governorate, Egypt. Over the study period, fish were collected from April to September 2019. The diseased collected fish showed hemorrhages on gills covers, ulceration on the body surface, cloudiness of both eyes with bilateral exophthalmia, and abdominal distension, congested, and enlarged liver, as well as congested spleen, and kidneys with several hemorrhages on internal contents. All fish under test were labeled with number, date, time of collection, name of the farm owner and kept in an icebox to be transferred to

the bacteriological laboratory for examination as soon.

Bacteriological examination

Samples for bacteriological examination were obtained under sterilized and hygienic conditions. The diseased fish samples from gills, liver, kidneys, spleen, and intestine were separately streaked onto *Aeromonas* selective agar base (Titan Media, India) with ampicillin supplement 2.5 mg/500 ml of diluted media and incubated at 37°C for 24 h with recording the characteristic features of the isolated bacterial colonies. They were refreshed in tryptone soya broth (Oxoid, Hampshire, UK) and incubated at 37°C for 24 h, then a loopful of the incubated broth was streaked onto tryptone soya agar (Oxoid, Hampshire, UK) and incubated at 37°C for 24 h for further examination. Single pure colonies were picked up and examined by Gram's staining and biochemically tested (oxidase, catalase, indole, esculin hydrolysis, Voges-Proskauer, and oxidation-fermentation tests). The tests were performed according to Bergy's manual of determinative bacteriology criteria which was previously described (Quinn et al., 2011). Purified biochemically identified strains were kept as pure stock isolates in tryptone soya broth containing 20% glycerol stored at -20 °C for further investigation.

Molecular determination of virulence associated-genes in *Aeromonas hydrophila* strains

PCR assays were performed to investigate four virulence genes including cytotoxic enterotoxin (*act*) gene, aerolysin (*aerA*) gene, cytotoxic enterotoxin heat-labile (*alt*) gene, and cytotoxic enterotoxin heat-stable (*ast*) gene. The conventional boiling method had been used for bacterial DNA extraction of purified suspected colonies (Zeinali et al., 2015). PCR reactions were performed in a total volume of 25 µl containing 12.5 µl of 2X PCR master mix (Promega, Madison, USA), 1µl of each primer (Metabion, Germany), 6 µl of DNA template, and 4.5 µl PCR grade water was added to bring the final volume in Applied Biosystem, 2720 Thermal Cycler (USA). The Animal Health Research Institute at Dokki, Cairo, Egypt, kindly provided a positive control, which acts as a reference strain (*A. hydrophila* ATCC 7966). The specific primers and the cyclic PCR conditions for PCR amplification of various virulence genes specific for molecular identification of *A. hydrophila* are summarized in Table (1). The amplified PCR products were arranged on a 1.5% agarose gel (Lonza, USA) stained by 1% ethidium bromide (Sigma, England) and photo-documented under UV illumination (Ltd., USA).

Sequencing of the virulence associated-genes in *Aeromonas hydrophila* strain

The purification of amplified PCR products for three

TABLE 1. The specific primers and the cyclic PCR conditions for *Aeromonas hydrophila*.

Gene	Primer sequence (5'-3')	Amplified product (bp)	Primary denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondary denaturation	Annealing	Extension		
<i>aerA</i>	F: CACAGCCAATATGTCGGTGAAG R: GTCACCTTCTCGCTCAGGC	326 bp	94°C / 5 min	94°C / 30 sec.	52°C / 40 sec	72°C / 40 sec	72°C / 10 min	Singh et al., 2008
<i>act</i>	F: AGAAGGTGACCACCACCAAGAACA R: AACTGACATCGGCCTTGAAGTC	232 bp	94°C / 5 min	94°C / 30 sec.	55°C / 30 sec.	72°C / 30 sec.	72°C / 7 min	Nawaz et al., 2010
<i>ast</i>	F: TCTCCATGCTTCCCTTCCACT R: GTGTAGGGATTGAAGAAGCCG	331 bp	94°C / 5 min	94°C / 30 sec.	55°C / 30 sec.	72°C / 30 sec.	72°C / 7 min	
<i>alt</i>	F: TGACCCAGTCCTGGCACGGC R: GGTGATCGATCACCACCAGC	442 bp	94°C / 5 min	94°C / 30 sec.	55°C / 40 sec.	72°C / 45 sec.	72°C / 10 min	

virulence genes including cytotoxic enterotoxin (*act*) gene, aerolysin (*aerA*) gene, and cytotoxic enterotoxin heat-labile (*alt*) gene of one representative *A. hydrophila* strain was applied by a QIAquick extraction kit (Qiagen, Valencia, CA) and was investigated for sequencing by BigDye Terminator cycle sequencing ready reaction kit (Applied Biosystems, Foster City, CA) with an Applied Biosystems 3130 Genetic Analyzer (Hitachi, Tokyo, Japan) in accordance to the manufacturer instructions.

Phylogenetic analysis

The comparison of sequences was accomplished with other strains in BLAST 2.0 and PSI-BLAST research programs (NCBI) in the GenBank database. A comparative analysis of nucleotide sequences was done using the CLUSTAL W multiple sequence alignment programs, version 1.83 of MegAlign module of Lasergene DNASTar software Pairwise that was prepared by Thompson et al. (1994). The Phylogenetic trees were constructed by using maximum likelihood, the neighbor-joining method, and maximum parsimony in MEGA6 program (Tamura et al., 2013).

Nucleotide accession number

The nucleotide sequences of the three virulence genes (*act*, *aerA*, and *alt* genes) of one representative *A. hydrophila* strain were recorded in GenBank under the accession number (**MW086870**, **MW086871**, and **MW086872**) respectively.

Antibiotic sensitivity testing

Antibiotic susceptibility and resistance patterns of *A. hydrophila* strains were performed toward ten antibiotic discs of various antimicrobial classes on Mueller-Hinton agar (Oxoid, Basingstoke, UK) by Kirby-Bauer disc diffusion method as recognized by the Clinical and

Laboratory Standards Institute (CLSI) (M45-A2, 2010). The antibiotic discs (Oxoid, Basingstoke, UK) under investigation were chloramphenicol (30 µg), amikacin (30 µg), gentamicin (10 µg), ceftriaxone (30 µg), tetracycline (30 µg), cefotaxime (30 µg), trimethoprim-sulfamethoxazole (25 µg), amoxicillin-clavulanic acid (30 µg), ciprofloxacin (5 µg), and meropenem (10 µg). The results were assessed according to the guidelines of CLSI (2010) criteria by measuring the inhibition diameter zone. Multiple antimicrobial-resistant (MAR) strains exhibited resistance to more than two antibiotic classes and the Multiple antibiotic resistance (MAR) index was calculated by dividing the number of antibiotics to which the isolate showed resistance on the total numbers of tested antibiotics (Krumperman, 1983).

Statistical analysis

Statistical analysis of the results by calculation of the ratio was made using the SPSS Statistics 17.0 software program.

RESULTS

Prevalence of isolated *Aeromonas hydrophila* in diseased Nile tilapia fish

The prevalence of *Aeromonas* species was screened in the present study of collected diseased Nile tilapia fish samples at El-Manzala city in Egypt as 500 samples, *Aeromonas* species were identified in 350 (70%) samples. Out of 350 samples, *Aeromonas hydrophila* strains were observed in 53.4% (187/350) samples that were 31% (58/187) of the kidneys, 26.2% (49/187) of the liver, 18.7% (35/187) of the spleen, 14.4% (27/187) of the intestine, and 9.6% (18/187) of the gills.

FIG. 1. Prevalence of virulence *act* gene (232 bp) in *A. hydrophila* strain isolated from kidney. Lane L: 100:1000 bp DNA size marker; lane1: positive control; lane2: negative control; lanes 4, 7, 8, 9: positive samples.

FIG. 2. Prevalence of virulence *aerA* gene (326 bp) in *A. hydrophila* strain isolated from kidney. Lane L: 100:1000 bp DNA size marker; lane1: positive control; lane2: negative control; lanes 3, 4, 6, 8: positive samples.

FIG. 3. Prevalence of virulence *alt* gene (442 bp) in *A. hydrophila* strain isolated from kidney. Lane L: 100:1000 bp DNA size marker; lanes1: positive control; lane2: negative control; lanes 4, 7, 8: positive samples.

Molecular detection of virulence-associated genes in *Aeromonas hydrophila* strains

A. hydrophila strains were subjected to the existence of four virulence-associated genes (*act*, *aerA*, *alt*, and *ast*) using uniplex PCR (Figure1-3). From all samples, the cytotoxic enterotoxin (*act*) and aerolysin (*aerA*) genes were investigated with a high percentage of 40% (75/187), followed by cytotoxic enterotoxin heat-labile (*alt*) gene with 30% (57/187) and cytotoxic enterotoxin heat-stable (*ast*) gene 0% (0/187).

Identification of the virulence-associated gene sequences in *Aeromonas hydrophila* strain

DNA sequence analysis of three virulence associated-genes (*act*, *aerA*, and *alt* genes) from purified PCR products of one *A. hydrophila* strain AHEA70 isolated from the kidneys of diseased Nile tilapia fish was accomplished. These virulence associated-genes of *A. hydrophila* strain *act*, *aerA*, and *alt* genes were identical to several *A. hydrophila* strains with a similarity of 91.4%, 93.1%, and 97.7%, respectively, and the sequences

FIG. 4. Phylogenetic tree showing the genetic relatedness among *A. hydrophila* strains based on nucleotide sequence analysis of the *act* gene.

FIG. 5. Phylogenetic tree showing the genetic relatedness among *A. hydrophila* strains based on nucleotide sequence analysis of the *aerA* gene.

FIG. 6. Phylogenetic tree showing the genetic relatedness among *A. hydrophila* strains based on nucleotide sequence analysis of the *alt* gene.

were recorded in GenBank under the accession number (**MW086870**, **MW086871**, and **MW086872**) for *act*, *aerA*, and *alt* genes, respectively. The phylogenetic trees depending on nucleotide sequence analysis of these virulence associated-genes were showed the relationships with related species (Figure 4-6).

Antibiotic susceptibility and MAR index value of identified *Aeromonas hydrophila* strains

The phenotypic resistance of *A. hydrophila* strains was

performed using the disc diffusion assay as shown in Table (2). The highest resistance was observed against chloramphenicol 67.4% (126/187), followed by amikacin 51.9% (97/187), and gentamicin 47.1% (88/187). The intermediate resistance was exhibited against ceftriaxone 32.6% (61/187), followed by tetracycline 28.9% (54/187), and cefotaxime 28.3% (53/187). The highest susceptibility was displayed toward meropenem 90.9% (170/187), followed by ciprofloxacin 84.2% (154/187), amoxicillin-clavulanic acid 73.3% (137/187), and trimethoprim-sulfamethoxazole 64.2% (120/187). The MAR of *A. hydrophila* strains displaying resistance to more than two classes of antibiotics were 69.0% (129/187) with revealing six resistance patterns. The MAR index of the strains was ranged from 0.2 to 0.8 (Table 2).

The presence of virulence-associated genes (*act*, *aerA*, and *alt*) in *A. hydrophila* strains isolated from fish samples displayed numerous antimicrobial resistance patterns (Table 3). A detailed analysis exhibited associations of phenotypic resistance and virulence possession pattern.

DISCUSSION

A. hydrophila has a worldwide distribution with infections to fishes, birds, and humans with causing fatal problems for the fish farming industry in Egypt and massive economic losses. The present investigation showed a high prevalence of *Aeromonas* spp (70%) in diseased Nile tilapia fish. Similarly, the previous study

TABLE 2. Antimicrobial resistance results and patterns of *Aeromonas hydrophila* isolates (n=187).

Antibiotic agent	Antimicrobial Class	Disc code	Total (n=187)			Resistance pattern	Total no of isolates	MAR index
			Sensitive	Intermediate	Resistant			a/b
			No. (%)	No. (%)	No. (%)			
Chloramphenicol	Phenicol	C	39 (20.9 %)	22 (11.8%)	126 (67.4%)	C, AK, CN, CRO, TE, CTX, SXT, AMC	2	0.8
Amikacin	Aminoglycoside	AK	75 (40.1%)	15 (8%)	97 (51.9%)	C, AK, CN, CRO, TE, CTX, SXT	8	0.7
Gentamicin	Aminoglycoside	CN	67 (35.8%)	32 (17.1%)	88 (47.1%)	C, AK, CN, CRO, TE, CTX	19	0.6
Ceftriaxone	Third Generation Cephalosporin	CRO	93 (49.7%)	33 (17.7%)	61 (32.6%)	C, AK, CN, CRO, TE	27	0.5
Tetracycline	Tetracyclines	TE	84 (44.9%)	49 (26.2%)	54 (28.9%)	C, AK, CN, CRO	33	0.4
Cefotaxime	Third Generation Cephalosporin	CTX	99 (52.9%)	35 (18.7%)	53 (28.3%)	C, AK, CN	40	0.3
Trimethoprim-Sulfamethoxazole	Sulphonamides	SXT	120 (64.2%)	20 (10.7%)	47 (25.1%)	TE, CN	19	0.2
Amoxicillin-Clavulanic acid	Penicillins And B-Lactamase	AMC	137 (73.3%)	21 (11.2%)	29 (15.5%)	AK, CTX	28	0.2
Ciprofloxacin	Fluoroquinolone	CIP	154 (82.4%)	10 (5.4%)	23 (12.3%)	CN, CRO	11	0.2
Meropenem	Carbapenem	MEM	170 (90.9%)	0 (0%)	17 (9.1%)			

MAR index = a/b (a) is the number of antibiotics to which the isolate shows resistance and (b) is the number of antibiotics to which the isolate was exposed.

revealed the high occurrence of *Aeromonas* spp in Nile tilapia fish (68%) collected from Egypt (Saleh et al., 2017), while El-Gamal et al. (2018) detected only 25.9% of *Aeromonas* spp in diseased fish. Moreover, *A. hydrophila* is the most prevalent pathogen affecting freshwater fish (Abd El-Tawab et al., 2018). The current results showed the occurrence of *A. hydrophila* strains in a percentage of 53.4% that was higher than previous studies (Abd El-Kader and Mousa-Balabel, 2017). Regarding the prevalence of *A. hydrophila* strains in organs, kidneys were the dominant affected organ (31%), followed by liver (26.2%), spleen (18.7%), intestine (14.4%), and gills (9.6%). The high occurrence of *A. hydrophila* in internal organs was very likely as the pathogen establishes its way to the blood and quickly proliferated, dissemination to the internal organs of the fish. The kidneys might be the major affected internal organs as their main functions are the excretion and removal of materials from the fish body. The presence of virulence factors; extracellular enzymes and enterotoxins; are responsible for the initiation of progressive and degenerative changes in the endothelial cells and

parenchymatous organs with systemic damage to the internal organs with subsequent death (Citarasu et al., 2011). In contrast, Algammal et al. (2020) found that the liver was the major affected organ (54.1%), followed by kidneys (26.3%) and spleen (19.6%). The high prevalence of *A. hydrophila* strains in freshwater fish could be attributed to the presence of predisposing stress factors, bad handling, and hygienic conditions with massive careless antimicrobial usage and treating the disease.

A. hydrophila possess multiple virulence factors, which combined to evade the host defense system and induction of disease and mortalities (Chacon et al., 2003). The cytotoxic enterotoxin gene induces multifactorial actions with enterotoxic, hemolytic, cytotoxic activities and promotes cell lysis (Xu et al., 1998). The aerolysin toxin induces pore formation with the destruction of target cells so that acts as a marker for *A. hydrophila* pathogenicity (Al-Maleky et al., 2011). The massive harmful effects as cytotoxic, enterotoxic, and hemolytic effects are produced by aerolysin and cytotoxic enterotoxin with cell lysis. In this study, cytotoxic

TABLE 3. Antimicrobial resistance patterns and virulence genes of *Aeromonas hydrophila* isolates.

Source	Resistance pattern	Total no of isolates	virulence genes			
			<i>act</i>	<i>aerA</i>	<i>alt</i>	<i>ast</i>
Kidneys	C, AK, CN, CRO, TE, CTX, SXT, AMC	1	+	+	+	-
Kidneys	C, AK, CN, CRO, TE, CTX, SXT, AMC	1	-	+	+	-
Kidneys	C, AK, CN, CRO, TE, CTX, SXT	8	+	-	+	-
Kidneys	C, AK, CN, CRO, TE, CTX	19	-	-	+	-
Kidneys	C, AK, CN, CRO, TE	17	+	-	-	-
Kidneys	C, AK, CN, CRO, TE	5	-	+	-	-
Liver	C, AK, CN, CRO, TE	5	+	-	+	-
Liver	C, AK, CN, CRO	33	-	+	-	-
Liver	C, AK, CN	11	+	-	-	-
Spleen	C, AK, CN	29	+	-	-	-
Spleen	TE, CN	6	-	-	+	-
Intestine	TE, CN	13	-	-	+	-
Intestine	AK, CTX	14	-	+	-	-
Gills	AK, CTX	14	-	+	-	-
Kidneys	CN, CRO	7	-	+	-	-
Gills	CN, CRO	4	+	-	+	-

C, Chloramphenicol; AK, Amikacin; CN, Gentamicin; CRO, Ceftriaxone; TE, Tetracycline; CTX, Cefotaxime; SXT, Trimethoprim-Sulfamethoxazole; AMC, Amoxicillin-Clavulanic Acid; CIP, Ciprofloxacin; MEM, Meropenem.

enterotoxin (*act*) and aerolysin (*aerA*) genes were present in 40% of examined isolates. Such genes were previously investigated (El-Bahar et al., 2019). Also, the cytotoxic enterotoxin heat-labile (*alt*) gene was demonstrated in 30% of *A. hydrophila* strains, whereas no strain had cytotoxic enterotoxin heat-stable gene (*ast*) (0%). The minority of presence of *ast* gene possession strain was commonly detected in fish and water sources (Rather et al., 2019). Younes et al. (2015) found *alt* and *ast* genes in 54.5% and 0.09% of tested strains, respectively.

DNA sequence analysis of virulence-associated genes (*act*, *aerA*, and *alt* genes) of one *A. hydrophila* strain was identical to the virulence genes related to several *A. hydrophila* strains found in different countries. The same *act* gene was previously specified in *A. hydrophila* as *A. hydrophila* JKAHM3 (KC687134) isolated from mutton in India with 91.4% similarity (Rather et al., 2014), whereas a similar *aerA* gene was earlier stated in *A. hydrophila* as *A. hydrophila* EGA (KM592977) recovered from the liver of rabbits in Egypt (Abd El-Tawab et al., 2014) with 93.1% similarity. Also, a similar *alt* gene was

previously indicated in *A. hydrophila* as *A. hydrophila* NJ-35 (CP006870) obtained from diseased Carp in China (Pang et al., 2015), *A. hydrophila* pc104A (CP007576) (Pridgeon et al., 2014) isolated from soil of a catfish pond in West Alabama, and *A. hydrophila* ML09-119 (CP005966) gotten from commercial catfish farm in West Alabama (Tekedar et al., 2013) with 97.7% similarity.

Multiple variations of antimicrobial susceptibility had been recorded between *Aeromonas* spp which depend on the different isolate sources, the antimicrobial agent usage, and the specific geographic area (Aravena-Roman et al., 2012). Thus, the performance of antimicrobial surveys against *A. hydrophila* strains is necessary to reduce deleterious effects and economic losses in aquaculture. Interestingly, erythromycin, enrofloxacin, flurofenicol and doxycycline were often used in the Egyptian aquaculture as their effectiveness against *Aeromonas*. The current study showed high resistance of *A. hydrophila* strains to chloramphenicol, followed by amikacin and gentamicin with intermediate resistance against ceftriaxone, tetracycline, and cefotaxime that

were previously stated (Kaskhedikar and Chhabra, 2010; Yi et al., 2014; Ramadan et al., 2018). The highest resistance of *A. hydrophila* to chloramphenicol recently recorded in Egypt (Algammal et al., 2020; El-Gohary et al., 2020) might be regarded as indiscriminate use of the antimicrobial agent in fish farms. In contrast, a maximum susceptibility was presented toward meropenem, ciprofloxacin, amoxicillin-clavulanic acid, and trimethoprim-sulfamethoxazole in this investigation as formerly recorded (Aravena-Roman et al., 2012; Algammal et al., 2020). As a result of the maladministration of growth promoters, therapeutic agents and possess beta-lactamases resistance in the animal and fish industry resulting in the development of multiple antimicrobial-resistant bacteria (Janda and Abbott, 2010; Stratev and Odeyemi, 2016). Moreover, the present investigation revealed 69.0% of MAR *A. hydrophila* strains with six resistance patterns. Inconsistent, Salem et al. (2020) and Elkamouny et al. (2020) detected 66.6% and 77.5% MAR isolates of *A. hydrophila* in Egypt, respectively. Furthermore, the present investigation showed that the existence of virulence determinants (*act*, *aerA*, *alt*) in different *A. hydrophila* strains exhibited various antimicrobial resistance patterns. The connection between antibiotic susceptibility and virulence possession pattern among *A. hydrophila* strains is attributed to that the genetic determinants for both antibiotic resistance and virulence genes could be harbored by the same transferable part (Soto, 2009; Cepas and Soto 2020). The bacterial virulence is supported by elevation of antimicrobial resistance (Beceiro et al., 2013).

The antimicrobial pattern of *A. hydrophila* strains under investigation revealed that the MAR index values were ranged from 0.2-0.8, which might be due to massive usage and high risk of antibiotic exposure during treatment (El-Bahar et al., 2019; Salem et al., 2020). MAR index of more than 0.2 is evidence of high-risk source contamination (Krumperman, 1983). The origin of the isolates from highly contaminated areas executes a much higher risk of spreading MAR to the aquatic environment, subsequently will affect aquaculture production (Pokhrel et al., 2018). The recommendation of decrease antibiotics usage in aquacultures besides raising aquaculture immunity would inhibit the irrational spreading of drug-resistant pathogens. For example, the usage of organic acids/ salts mixtures as yuccamax extract, propionic, and lactic acids as food additives might be effective as they have bacteriostatic and bactericidal effects (Ng and Koh, 2017). Organic acid mixtures aid to increase growth performance and disease resistance in aquaculture through maximal antibacterial and immunomodulatory effects with subsequent transmission of these resistances to humans and animal livestock (Reda et al., 2016).

CONCLUSION

This study investigated the prevalence of virulent and multi-drug resistant strains of *Aeromonas hydrophila* in freshwater fish aquaculture in Egypt. The observed MAR might have resulted from the abuse of antibiotics in fish farms. Therefore, awareness of drug-resistant pathogens, as well as a good disease management and control strategy, should be considered in fish farms. Also, more studies about acidifier usage in diets to improve growth, immunity, and economy should be applied to control the massive spreading of drug-resistant pathogens and get rid of massive economic losses.

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Conflict of interest statement

No conflict of interest.

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