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ORIGINAL ARTICLE

Investigation of Nile Tilapia Mortalities During Summer 2019 in El-Manzala Fish Farms in Dakahlia Governorate, Egypt

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Abstract

OBJECTIVE: To identify suspected causes of mass mortality in *Oreochromis niloticus* at El-Manzala fish farms in Dakahlia Governorate, Egypt, during disease outbreaks in the summer of 2019.
DESIGN: Observational study.
ANIMALS: Two hundred diseased and freshly dead *O. niloticus* (Nile tilapia) weighting 100−150 g.
PROCEDURES: Collected fish samples were subjected to clinical, postmortem and bacteriological investigations regarding histopathological alterations, pathogenicity tests, and antibiogram susceptibility of the main isolated bacterial species.
RESULTS: A total of 286 bacterial isolates were detected from all examined fish samples. Bacteriological examination revealed that all bacteria were gram-negative rods, which were identified as *Aeromonas hydrophila* (43.36 %), *Aeromonas sobria* (20.28 %), *Vibrio fluvialis* (12.24 %), *Vibrio parahemolyticus* (10.14 %), *Vibrio cholera* (7.34 %) and *Ps. putida* (4.895 %). Confirmatory identification of the most predominant isolated species (*A. hydrophila*) was carried out using conventional polymerise chain reaction (PCR). An antibiogram was performed for the isolated *A. hydrophila* strains, revealing that the isolates showed high sensitivity to flumequine (UB) and cefepime (FEP), with a percent (83.33 %) for each. The Multiple Antibiotic Resistance (MAR) indices of the 12 identified *A. hydrophila* ranged from 0.2 to 0.6 with an average (0.32).
CONCLUSION AND CLINICAL RELEVANCE: This study concluded that *A. hydrophila* is one of the main causes of summer mass mortality in *O. niloticus* in the study area.

Keywords: *A. hydrophila*, Antibiogram, Histopathology, Nile tilapia

1. Introduction

Aquaculture production has recently expanded as a valuable source of animal protein and essential nutrients with a feasible cost compared with other animal protein sources [1]. It accounts for over half of worldwide fish production [2]. Egypt ranks tenth in global fish farming production and first among African countries. The industry supplies ~77 % of the total fish production in Egypt [3]. Most fish farms are located near the Nile in northern Egypt and the Delta region [4].

Nile tilapia, *Oreochromis niloticus*, is the most predominant farmed freshwater fish species worldwide, including Egypt [5]. The intensive and semi-intensive practices of aquaculture production have caused an increase in disease outbreaks, resulting in a partial or total loss of fish production [6]. In fish farms, bacterial infections are a major cause of mass mortality among fish populations. They are attributed to stressful environmental conditions (such as high or low water temperature, high stocking rate, ammonia emissions, lack of dissolved oxygen, malnutrition, handling and poor water quality) rather than other infections [7].

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Aeromonas spp. (*Aeromonas hydrophila*, *Aeromonas veronii*, *Aeromonas sobria*, *Aeromonas caviae,* and others) are opportunistic pathogens that can cause diseases only in immunocompromised fish populations or as secondary invaders in fish suffering from other diseases [8]. *A. hydrophila* is a gram-negative motile rod that produces circular, yellow-colored colonies on R−S media and is characterized by catalase and cytochrome oxidase-positive but H2S negative, facultatively anaerobic, glucose, sucrose, arabinose, and salicin-fermenting. Nitrates were reduced to nitrites with no gas production, and the Voges−Proskauer reaction was positive, but not the methyl red test [9,10].

Motile *Aeromonas* septicemia is a hemorrhagic and ulcerative syndrome in Nile tilapia (*O. niloticus*), usually resulting in high mortality rates [11]. Clinical abnormalities of *A. hydrophila* typically appear in the acute phase, including shallow to deep necrotizing ulcers in the skin and fins, extensive irregular hemorrhages on the body surface, skin darkness, scale detachment with ascites in the abdomen, and exophthalmia [10]. Postmortem examination is associated with hemorrhage and enlargement of the internal organs [12].

The histopathological changes usually associated with *A. hydrophila* infection in Nile tilapia were observed mainly in the liver, kidney, spleen, and gills. The most common findings included necrosis and dissociation of hepatocytes in the liver, congestion with hyperplasia of melanomacrophages in the spleen, while the kidney showed desquamation of the renal tubular epithelium with severe destruction and desquamation of the gill lamellar epithelium [13,14].

In vitro, the experimental challenge of healthy *O. niloticus* with pathogenic *A. hydrophila* induced septicemic signs with high mortality rates [12,15]. Pathogenicity is attributed to the production of many extracellular proteins, which are the host's main virulence factors, such as enterotoxins, aerolysin and hemolysin, proteases, and cytolytic enterotoxins [16,17].

The Multiple Antibiotic Resistance (MAR) index is valuable for evaluating contamination sources [18]. The MAR value becomes equal to or less than 0.2 when the antibiotics usage for the animal treatment was seldom or in low doses. On the other hand, the elevated MAR index value of 0.2 indicated a high risk of exposure to antibiotics for animal treatment. Because of the misuse of antibiotics, *A. hydrophila* shows MAR, causing a worldwide problem with a public health hazard [19,20].

Therefore, the present study aimed to investigate the prevalence of bacterial pathogens associated with the high mortality rates of naturally infected Nile tilapia (*O. niloticus*) in El-Manzala fish farms throughout the summer 2019 outbreak, in addition to antibiotic sensitivity evaluation and MAR index of the most isolated bacterial species.

2. Materials and methods

2.1. Study area and fish collection

A total of 200 freshly dead and moribund *O. niloticus* samples (average weight of 100−150 g) were aseptically collected from semi-intensive earthen ponds of private fish farms in the El-Manzala region, Dakahlia Governorate, Egypt, during the summer months (June, July, and August) of 2019. Each fish sample was packed in a separate sterile labeled plastic bag, immediately transported in an ice box with cooled ice bags to the laboratory and processed immediately.

2.2. Fish clinical signs and postmortem (PM) examination

The collected fish samples were examined both clinically and microbiologically. Necropsy and histopathological examinations were performed. The Clinical examinations were performed according to Schäperclaus [21]. Necropsy was performed on different numbers of freshly dead or dying fish to recognize postmortem lesions according to the method described by Stoskopf [22].

2.3. Bacteriological examination

Bacteriological examination of samples from the skin, gills, liver, kidney, and heart was performed through culture in tryptic soya broth (TSB) (Oxoid, CM 0129) and incubated at 28 °C for 24−48 h, then streaked on different laboratory media: tryptic soya agar (TSA) (Oxoid, CM0131), *Aeromonas* Base Medium (Oxoid, CM0833), and Thiosulfate-citrate-bile salts-sucrose agar media (TCBS) (Oxoid, CM0333). The inoculated plates were incubated at 28 °C for 24−48 h [23].

Presumptive colonies were purified and identified based on microscopic and cultural characteristics and biochemical identification, according to Austin and Austin [10]. Further identification was achieved using VITEK2 compact (bioMérieux, France) biochemical marker [24].

2.4. Molecular characterization

Molecular identification of *A. hydrophila* isolates was carried out using a species-specific primer of *A.*

hydrophila [25]. Genomic DNA was extracted using the DNA extraction kit (QIAamp DNA Mini Kit (Catalogue no. 51304)). PCR amplification with *A. hydrophila*-specific primer (*A. hydrophila*-specific 16S rRNA) was used to amplify 625 bp from the general bacterial 16S rRNA (Table 1).

2.5. Histopathological examination

For histopathological examination, tissue specimens of the naturally infected fish were carefully removed from (gills, liver, spleen, and kidney). Samples were trimmed and fixed in a 10 % neutral buffered formalin solution for 24 h. Paraffin sections of 5 μ thickness were prepared, stained with hematoxylin and eosin (H and E), and then examined microscopically [26].

2.6. Antibiotic sensitivity and multiple antibiotic resistance (MAR)

The sensitivity of *A. hydrophila* isolates to antibiograms was studied using different chemotherapeutics as described by Finegold and Martin [27]. The results were interpreted following Clinical and Laboratory Standards Institute guidelines [28]. The MAR index was determined for isolates resistant to more than two antibiotics. According to the following equation: MAR index = a/b; (a) is the number of antibiotics to which the isolate was resistant, and (b) is the number of antibiotics to which the isolate was tested [29].

2.7. Challenge trial

The isolated *A. hydrophila* was stored in a glycerol solution at −80 °C. *A. hydrophila* inoculum was prepared for challenge by culturing the organism in tryptic soya agar (TSA) at 27 °C for 24 h. Then, the isolate was inoculated in tryptic soya broth (TSB) for another 24 h at 27 °C. Finally, the culture broth was centrifuged and washed with phosphate buffer saline (PBS). The bacterial density was then determined using the agar plate-spread method on TSA after serial dilution [30]. The number of bacterial CFUs was calculated (between 30 and 300 number of bacterial colonies). The following formula was used to calculate colony counts in CFU/ml for bacterial suspension:

(1) Microbial load = number of colonies × dilution factor/volume of inoculum used.

The bacterial suspension was then kept at 4 °C until it was used. The applied concentration of *A. hydrophila* was 1.5×10^6 CFU/ml.

Forty healthy *O. niloticus* with an average body weight of 40 ± 20 g were used for the experimental infection. After adopting fish in an aquarium for 2 weeks, healthy fish were assigned into two groups (20 fish/group), with 10 fish in each glass aquaria (100 × 80 × 40 cm). During the experiment, the water quality in the aquaria was maintained at optimum levels. One group was injected I/P with 0.5 ml of (1.5×10^6 CFU/mL) of an isolated strain of *A. hydrophila* (18 h culture). The second group was injected I/P with PBS as a control group and kept under examination for 7 days to record clinical signs and mortality rate in addition to re-isolation and identification of *A. hydrophila*.

3. Results

3.1. Clinical and postmortem findings of naturally infected O. niloticus

Diseased *O. niloticus* showed dullness, loss of appetite, absence of reflexes, sluggish movements, and swimming near the surface water. As shown in Fig. 1a,b, the clinical investigation revealed septicemic signs as severe hemorrhage and ulcer formation on the body at the base of pectoral fins, under the lower jaw, and on skin with depigmentation in addition to severe congestion of gills and exophthalmia. As shown in Fig. 1c,d, Postmortem findings showed congestion and hemorrhage of the internal organs.

3.2. Prevalence of bacterial isolates from naturally infected O. niloticus

Bacteriological analysis revealed the primary isolation of 286 bacterial isolates from all examined fish samples, as shown in Table 2. The highest prevalence of bacterial isolates of naturally infected Nile tilapia (*O. niloticus*) was recorded for *A.*

Table 1. Primers used for Polymerase chain reaction amplification of isolated A. hydrophila.

Target	Gene	Sequence	Amplified product (bp)	Reference
Aeromonas hydrophila	*16S rRNA*	GAAAGGTTGATGCCTAATACGTA CGTGCTGGCAACAAAGGACAG	625	[31]
Bacteria	*16S rRNA*	AGAGTTTGATCMTGGCTCAG TACGGYTACCTTGTTACGACTT	1485	[32]

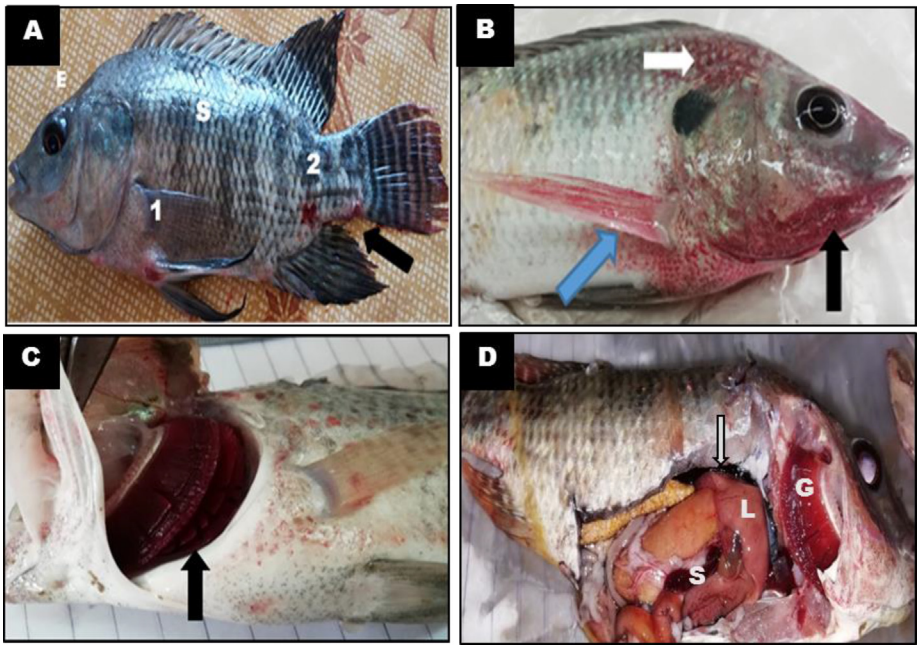

Fig. 1. (A–D): Naturally infected O. niloticus revealed A: dark coloration of skin (S), ulcer formation beside pectoral fin (1) and tail (black arrow), hemorrhage on the body (2) and exophthalmia (E). B: Infected fish showed severe hemorrhage all over the body at the base of pectoral fins (blue arrow), under the lower jaw (black arrow), and on skin (white arrow) with depigmentation. C: Severe congestion of gills (black arrow). D: The postmortem lesions showed congested gills (G), congested and enlarged liver (L), congested spleen with splenomegaly (S) and dark congested kidney (white arrow).

hydrophila (43.36 %), followed by *A. sobria* (20.28 %), *Vibrio fluvialis* (12.24 %), *Vibrio parahemolyticus* (10.14 %), *Vibrio cholera* (7.34 %), *Ps. putida* (4.895 %). However, the lowest prevalence was recorded for *K. oxytoca* at a percentage of (1.75 %).

3.3. Bacteriological identification

3.3.1. Morphological and biochemical characters

Morphological features of colonies of the obtained isolates showed 2–5 mm round, smooth, creamy, whitish, or grey opaque colonies on tryptic soya agar plates. Aeromonads appeared on *Aeromonas* base media as dark green opaque with black center colonies. *Aeromonas* and *vibrio spp.* Showed complete hemolysis on blood agar. *Vibrio* spp. are susceptible to O/129 vibriostatic agents. Microscopically, all isolates were gram-negative rod-shaped bacteria, as shown in Table 3.

Table 2. Prevalence of bacterial isolates from naturally infected O. niloticus.

Bacterial isolates	No. (%[a])
A. hydrophila	124 (43.36)
A. sobria	58 (20.28)
V. fluvialis	35 (12.24)
V. parahemolyticus	29 (10.14)
V. cholera	21 (7.34)
Ps. putida	14 (4.895)
K. oxytoca	5 (1.75)
Total	286 (100.00)

[a] Related to total No. of the bacterial isolates recovered from fish.

Isolated bacteria were further identified by the microbial identification system (VITEK 2 Compact), and all isolates were identified as *A. hydrophila* (the most prevalent isolated spp.) followed by *A. sobria*, *V. fluvialis*, *V. parahemolyticus*, *V. cholera*, *Ps. putida*. The lowest prevalence was recorded for *K. oxytoca*.

3.3.2. Molecular identification of Aeromonas hydrophila by conventional PCR

Twelve isolates were randomly selected from the most predominant isolated species (*A. hydrophila*) for confirmatory identification using traditional PCR. The 16S rRNA gene was amplified from all isolates using a specific primer for *A. hydrophila,* which was detected at 625 bp (Fig. 2).

3.4. Pathogenicity test

The tested *A. hydrophila* was highly pathogenic at a dose of 1.5×10^6 CFU/ml to the experimentally infected *O. niloticus,* which showed typical septicemic clinical signs and necropsy findings related to *A. hydrophila,* similar to those observed in naturally infected fish in our study. The control group showed

Table 3. Morphological and biochemical characters of bacterial isolates from naturally infected O. niloticus.

	A. hydrophila	*A. sobria*	*V. fluvialis*	V. parahemolyticus	V. cholera	Ps putida	*K. oxytoca*
Colony on TSA	**2–2.5 mm opaque creamy white round colonies with smooth edges**	**2–3 mm grey, creamy, shiny round colonies**	**2–3 mm translucent grayish colonies**	**2–3 mm grayish colonies**	**1–2 mm moist translucent disk-like colonies**	**3 mm shiny smooth moist convex whitish opaque round**	**2–3 mm Circular, mucoid, translucent or opaque, yellow to cream-colored colonies.**
Gram stain	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**
growth on Aeromonas agar-based medium	+	+		**-**	**-**	+	**-**
Colony on TCBS	**yellow colonies**	**Yellow colonies**	**Yellow colonies**	**Blue colonies with green center**	**Yellow colonies**	**Blue-green**	**-**
Growth in NaCl							
0 %	+	+	**-**	**-**	+	+	+
2 %	+	+	+	++	++	+	**-**
6 %	**-**	**-**	+	+	+	**-**	**-**
Motility	+	+	+	**-**	+	+	**-**
O/129 Vibriostatic agent susceptibility	**-**	**-**	+	+	+	**-**	**-**
Hemolysis on blood agar	+	+	+	+	+	**-**	**-**
Bipolar staining	**-**	**-**	+	+	+		**-**
Cytochrome oxidase	+	+	+	+	+	+	**-**
Catalase	+	+	+	+	+	+	+
Citrate utilization	**v**	**v**	+	**-**	+	+	+
Voges–Proskauer	+	+	**-**	**-**	**-**	**-**	+
Trible Sugar Iron agar	**A/A(gas)**	**A/A(no gas)**	**A/A(no gas)**	**K/A(no gas)**	**A/A(no gas)**	**K/K(no gas)**	**A/A(gas)**

0 % mortality, while the infected group showed 65 % mortality at the end of test periods with re-isolation of *A. hydrophila* from the internal organs (liver, spleen, kidney, and gills). Phenotypic and biochemical confirmations of the re-isolated bacteria were performed.

3.4.1. Antibiotic susceptibility of A. hydrophila *isolated strains from naturally infected Nile tilapia (O. niloticus)*

A. hydrophila strains were tested according to their susceptibility as resistant, intermediate, or sensitive to each antibiotic group. An antibiogram sensitivity test was performed on 12 *A. hydrophila* isolates PCR identified. *A. hydrophila* identified showed high sensitivity to flumequine (UB) and cefepime (FEP) with a percent of 83.33 % followed by tetracycline (TE) (75 %). In contrast, trimethoprim and sulphamethoxazole (SXT), ciprofloxacin (CIP) and cefoxitin (FOX) showed intermediate efficacy against *A. hydrophila*. Regarding the resistance pattern among the other tested drugs, the highest resistance was recorded against penicillin (P), with a percentage of

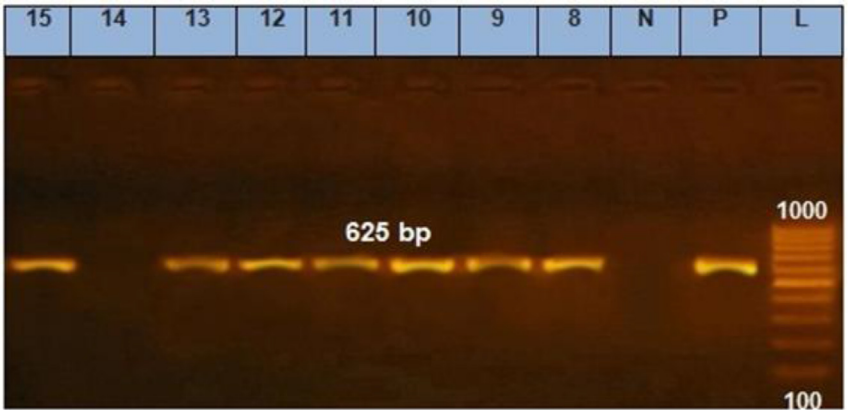

Fig. 2. 1.5 % agarose gel electrophoresis of 12 A. hydrophila isolates, representative of 124 isolates, PCR product exhibits specific A. hydrophila 16s rRNA bands at 625 bp. Lane (L): 100 bp ladder, Lane P: control positive, Lane N: control negative and Lanes 8–13 and 15: positive samples that demonstrated the expected 625 bp fragment of Aeromonas 16s rRNA gene, Lane 14: negative sample.

Table 4. Antimicrobial resistance phenotypes of A. hydrophila isolated strains from infected O. niloticus.

Antimicrobial Agents	Concentrations μg	Susceptibility Patterns		
		Resistance No (%)	Intermediate No (%)	Sensitivity No (%)
Amikacin (AK)	30	9 (75)	2 (16.67)	1 (8.33)
Flumequine (UB)	30	1 (8.33)	1 (8.33)	10 (83.33)
Cefepime (FEP)	30	1 (8.33)	1 (8.33)	10 (83.33)
Gentamycin (CN)	10	7 (58.33)	2 (16.67)	3 (25)
Tetracycline (TE)	30	0	3 (25)	9 (75)
Trimethoprim & sulphamethoxazole (SXT)	25	1 (8.33)	4 (33.33)	7 (58.33)
Ciprofloxacin (CIP)	5	2 (16.67)	5 (41.67)	5 (41.67)
Erythromycin (E)	15	9 (75)	2 (16.67)	1 (8.33)
Cefoxitine (FOX)	30	1 (8.33)	4 (33.33)	7 (58.33)
Penicillin (P)	10	11 (91.67)	0	1 (8.33)

(91.67 %) followed by amikacin (AK) and erythromycin (E), with a percentage of (75 %) for each, as well as gentamycin with a percentage of (58.33 %) as shown in Table 4.

On the other hand, our results showed that the MAR index values of the 12 identified *A. hydrophila* ranged from 0.2 to 0.6 with an average of 0.32, as shown in Table 5, which indicates multiple resistance patterns exhibited by the isolated *A. hydrophila*. Seven isolates were multi-resistant to four antibiotics, whereas four showed considerable resistance against two antibiotics. Additionally, only one strain showed multi-resistance to the six antibiotics.

3.5. Histopathological results of naturally infected O. niloticus

The histopathological findings of tissue samples collected from organs of naturally infected *O. niloticus* gills, kidney, liver, and spleen showed lesions of septicemia, as shown in Fig. 3e-h.

4. Discussion

With regards to the clinical symptoms and necropsy findings of the examined *O. niloticus*, the naturally infected fish showed loss of appetite, dullness, loss of equilibrium, sluggish swimming at the water surface, detached scales, exophthalmia in addition to septicemic signs such as hemorrhage on the body surface, ulcers on the skin, congested gills, and pale gills with excessive mucus in others. Postmortem lesions showed congestion of internal organs such as the liver, spleen, and kidney, with liver paleness in some cases. Moreover, splenomegaly and liver and gall bladder enlargement were observed, and abdominal distension and serosanguinous fluid in the abdominal cavity were obvious. These findings are consistent with [12,33,34]. These results could be due to the effect of toxic extracellular metabolites of the isolated bacteria, including aerolysin, hemolysin, and cytotoxins, which possess cytolytic, hemolytic, and enterotoxic activities that cause liver necrosis, degeneration of renal tubules, and tissue hemorrhage with serum and fibrin exudates [35].

Table 5. Multiple Antibiotic Resistance index value of A. hydrophila *isolated strains from infected O. niloticus.*

A. hydrophila strains	Resistance antibiotic	MAR
Ah1	E & p	0.2
Ah2	CN, P	0.2
Ah3	AK, FEP, CN, E	0.4
Ah4	AK, UB, CIP, E, FOX, P	0.6
Ah5	AK, SXT, E, P	0.4
Ah6	AK, P	0.2
Ah7	AK, CN, E, P	0.4
Ah8	AK, CN,E, P	0.4
Ah9	AK, CN, E, P	0.4
Ah10	AK, CN, E, P	0.4
Ah11	CN, P	0.2
Ah12	AK, CIP, E, P	0.4
Average = 0.32		

Regarding the prevalence of bacterial isolates from naturally infected *O. niloticus*, the results emphasized the significant impacts of *A. hydrophila* on the health status of Nile tilapia, especially during the summer period. The highest prevalence of bacterial isolates during the entire period of examination of naturally infected *O. niloticus* was *A. hydrophila* (43.36 %), followed by *A. sobria* (20.28 %), *V. fluvialis* (12.24 %), *V. parahemolyticus* (10.14 %), *V. cholera* (7.34 %), *Ps. putida* (4.9 %). However, the lowest prevalence was recorded for *K. oxytoca* at a percentage of (1.75 %). These results were consistent with earlier work [36–38]. Also, Hamouda et al. [39] studied the most prevalent bacterial diseases infecting *O. niloticus* at Aswan fish hatchery and found that the total incidence of bacterial infections was (73 %) among them, *A. hydrophila* was predominant with a percentage of (56 %), followed by *A. sobria* (45 %), *P. fluorescens* (33 %) and *Enterococcus faecalis* (11 %). The authors noticed that outbreaks of the detected bacterial pathogens usually occurred

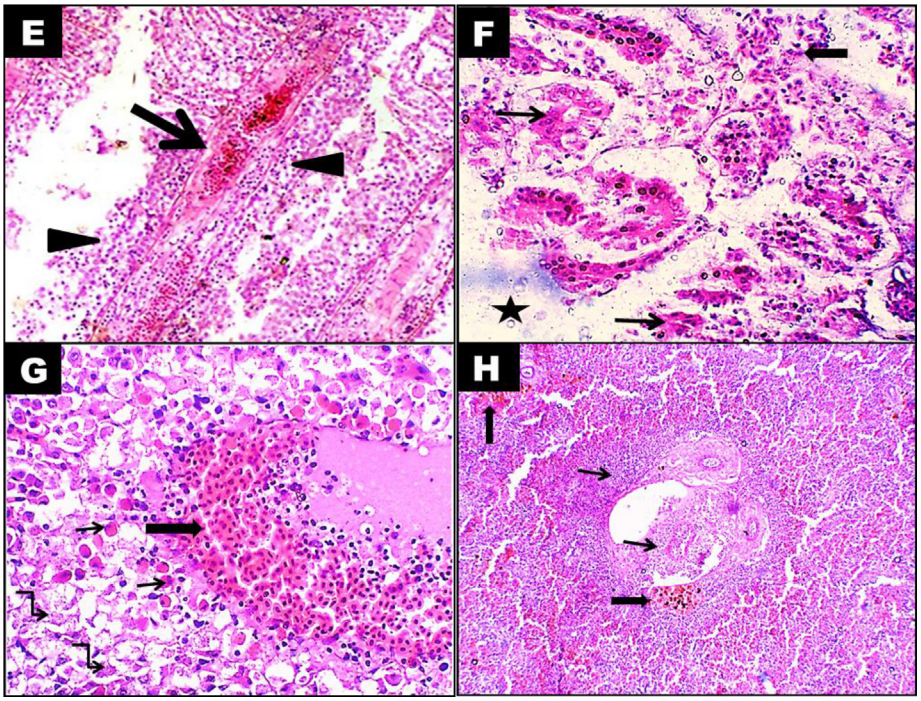

Fig. 3. (E–H): Naturally infected O. niloticus revealed (E): Gills showing diffuse necrosis and desquamation of lamellar epithelium with numerous inflammatory and leukocytic infiltrates (arrowheads) beside secondary lamellar blood vessels dilation and inter lamellar hemorrhage (black arrow), H and E, 400X. (F): Kidney showing diffuse, severe lytic damage and degeneration of renal glomeruli (thin arrow) with edema (star) and hemorrhage (thick arrow), H and E, 400X. (G): Liver showing lytic necrosis (twisted arrow) with dilated, congested blood vessel (thick arrow) and numerous perivascular large eosinophilic cells with an eccentric nucleus (thin arrows), H and E, 400X. (H): Spleen showing multifocal granuloma formation around ellipsoids (thin arrows) with hemosiderosis (thick arrow), H and E, 100X.

during induced spawning in April and May each year.

In contrast, a lower prevalence of *A. hydrophila* (13.2 %) and (10 %) in the isolated Nile tilapia was recorded by Algammal et al. [12] and Ibrahem et al. [40], respectively. These differences in the prevalence of isolated bacteria in fish can be attributed to the species, geographical range and sampling time [41]. The higher incidence of *A. hydrophila* in our study may be related to the elevated water temperature during the summer months, which acts as a stress factor for fish and induces susceptibility to infection [15]. The cultural and morphological characteristics of the bacteria isolated from the examined *O. niloticus* are consistent with Austin and Austin [10,42]. The biochemical characteristics of the retrieved isolates were consistent with Eissa et al. [15,43] and in agreement with Martin Carnahan and Joseph [44] Bergey's Manual.

Regarding the molecular identification of isolated *A. hydrophila* from naturally infected *O. niloticus*, our results confirmed the presumptive identification of *A. hydrophila* isolates by the vitek2 compact system with the consistent identification of these isolates by PCR amplification using *A. hydrophila* 16s rRNA specific primers that are essential for epidemiological investigations by outbreak management, threat analysis, and source tracing [17]. Bacterial isolates were identified by particular bands that appeared by electrophoresis at a molecular weight of 1485 bp, which is specific for bacterial identification, as described by Lagacé et al. [32]. PCR amplification with *A. hydrophila*-specific primer (*A, hydrophila*-specific 16S rRNA) identified 12 isolates as *A. hydrophila,* and a particular band appeared by electrophoresis at a molecular weight of 625 bp. These results are consistent with El-son [14] and Gordon et al. [31].

The histopathological examination of tissue samples from different organs of naturally infected *O. niloticus* revealed generalized septicemic and hemorrhagic findings. Gills showed diffuse necrosis and desquamation of the lamellar epithelium with numerous inflammatory and leukocyte infiltrates, secondary lamellar blood vessel dilation, and congestion with interlamellar hemorrhage. In addition, the kidney revealed diffuse, severe lytic damage and degeneration of the renal glomeruli epithelium with edema, replacing renal tissue with hemorrhage and hemosiderosis. The liver showed lytic and vascular necrosis, replacing hepatic tissue with dilated and congested blood vessels. The spleen showed multifocal granuloma formation

around ellipsoids with hemosiderosis. These results agree with Afifi et al. [33,34,45]. These changes could be attributed to extracellular toxins, such as enterotoxins, proteases, hemagglutinins, adhesions [46], and hemolysins [47].

In the pathogenicity test, the tested *A. hydrophila* was highly pathogenic to fish at a dose of (1.5×10^6 CFU/ml) and experimentally infected *O. niloticus* showed typical septicemic clinical signs and necropsy findings related to *A. hydrophila* causing 65 % mortality in the challenged group. These observations followed [15], who challenged healthy Nile tilapia (I/P) with isolated *A. hydrophila* recovered from naturally infected Nile tilapia during summer mortality in Kafr El-Sheikh Governorate, Egypt. The bacterial isolates exhibited virulence to Nile tilapia and produced severe disease symptoms, as observed in postmortem pictures of naturally infected fish with a mortality rate of 70 %. Further, the inoculated bacteria were re-isolated from the lesions of the challenged fish and confirmed to be *A. hydrophila,* with no mortalities recorded in the control group that was injected with 0.5 ml sterile saline I/P. Extracellular metabolites and toxins, such as hemolysin, aerolysin, protease, and cytotoxic enterotoxins, may be responsible for *Aeromonas* spp. Virulence [46].

The antibiotic susceptibility of *A. hydrophila* isolated strains from naturally examined Nile tilapia (*O. niloticus*), *A. hydrophila* strains were highly susceptible to flumequine (UB) and cefepime (FEP), with a percentage of 83.33 % for each, tetracycline (TE) by (75 %). On the other hand, trimethoprim and sulphamethoxazole (SXT), CIP, and cefoxitin (FOX) showed moderate inhibition of isolated *A. hydrophila* on Muller-Hinton agar. Meanwhile, the main resistance was observed against penicillin (P), with a percentage of 91.67 %, followed by amikacin (AK) and erythromycin (E), with a percentage of 75 % for each, and gentamycin (58.33 %). This work was supported by Wei et al. [48], who demonstrated the antibiotic susceptibility patterns of potentially pathogenic bacterial isolates of *A.* spp. that were isolated from diseased red hybrid tilapia. Most isolated bacteria were resistant to ampicillin, while all the bacterial isolates were sensitive to flumequine, nalidixic acid, and oxytetracycline. In contrast, these results disagree with Essmat [49], who tested 70 of *A. hydrophila* isolates for antibiotic sensitivity and declared that all isolates (70/70) were sensitive to gentamycin (100 %) and 63/70 isolates were susceptible to cefotaxime (90 %). In comparison, three isolates (4.2 %) were intermediate, and four (5.7 %) were resistant.

A MAR index above 0.2 is a parameter used to evaluate the spread of bacterial resistance in certain populations [29]. The MAR values of the identified *A. hydrophila* isolates against the 10 antibiotics used in our study ranged from 0.2 to 0.6, with an average of 0.32, suggesting that these strains originated from an area with a high risk of exposure to antibiotics and, therefore, threatens the spreading of MAR to aquatic environments, which has an impact on aquaculture production and the direct spread of resistance from aquatic environments to humans with the development of resistance to human health [50]. These results follow Laith and Najiah [51], who studied *A. hydrophila* antimicrobial susceptibility and found that the MAR index for *A. hydrophila* isolates ranged from 0.10 to 0.50. Similarly, Sreedharan et al. [52] reported that the MAR index value for isolated aeromonads from ornamental fish ranged from 0.2 to 0.46.

5. Conclusions

It can be suggested that *A. hydrophila* is one of the main causes of summer mortality outbreaks in Nile tilapia in Egypt, and molecular detection methods are more rapid and cost-effective than bacteriological methods for detecting causative agents. Flumequine and cefepime were the most effective antibiotics against *A. hydrophila.* The MAR index in our study revealed that bacteria originated from a habitat in which different antibiotics were used. Therefore, farmers should be aware of basic fish health control and avoid multidrug-resistant bacteria by promoting alternative non-antibiotic control strategies for bacterial infections in farmed fish.

Research ethics committee permission

The Research Animal Ethics Committee of the Faculty of Veterinary Medicine, Mansoura University, approved the study ethically.

Data availability statement

The datasets generated or analyzed during the current study are not publicly available but are available from the corresponding author upon reasonable request.

Author's contribution

The study is part of a Philosophy Doctoral Degree thesis presented to the Department of Aquatic Animal Medicine, Faculty of Veterinary Medicine, Mansoura University by A. M. where V. H. Z., M. M. F., and M. M. S. acted as thesis supervisors.

Conflicts of interest

The authors declare no conflict of interest to disclose.

References

[1] Dawood MA. Nutritional immunity of fish intestines: important insights for sustainable aquaculture. Rev Aquacult 2021;13:642−63.
[2] Verdegem M, Buschmann AH, Latt UW, Dalsgaard AJ, Lovatelli A. The contribution of aquaculture systems to global aquaculture production. J World Aquacult Soc 2023; 54:206−50.
[3] FAO. The state of world fisheries and aquaculture 2020. In: Sustainability in action; 2020.
[4] FAO. FishStat J − software for fishery and aquaculture statistical time series. Rome, Italy: Fisheries and Aquaculture Division; 2018.
[5] El-Sayed AFM, Fitzsimmons K. From Africa to the world—the journey of Nile tilapia. Rev Aquacult 2023;15:6−21.
[6] Bondad-Reantaso MG, Subasinghe RP, Arthur JR, Ogawa K, Chinabut S, Adlard R, et al. Disease and health management in Asian aquaculture. Vet Parasitol 2005;132:249−72.
[7] Haenen OL, Dong HT, Hoai TD, Crumlish M, Karunasagar I, Barkham T, et al. Bacterial diseases of tilapia, their zoonotic potential and risk of antimicrobial resistance. Rev Aquacult 2023;15:154−85.
[8] Ana F, Maria J. An update on the genus Aeromonas: taxonomy, epidemiology, and pathogenicity. Microorganisms 2020;8:129.
[9] Pund R, Theegarten D. The importance of aeromonads as a human pathogen. Bundesgesundh Gesundh Gesundheit 2008;51:569−76.
[10] Austin B, Austin DA. Aeromonadaceae representatives (motile aeromonads). Switzerland: Bacterial Fish Pathogens Springer International Publishing; 2016. p. 161−214. https://doi.org/10.1007/978-3-319-32674-0_4.
[11] Nicholson P, Mon-on N, Jaemwimol P, Tattiyapong P, Surachetpong W. Coinfection of tilapia lake virus and Aeromonas hydrophila synergistically increased mortality and worsened the disease severity in tilapia (Oreochromis spp.). Aquaculture 2020;520:734746.
[12] Algammal AM, Mohamed MF, Tawfiek BA, Hozzein WN, El Kazzaz WM, Mabrok M. Molecular typing, antibiogram and PCR-RFLP based detection of Aeromonas hydrophila complex isolated from Oreochromis niloticus. Pathogens 2020;9: 238.
[13] Hassanien AE, El-Bendary N, Sweidan AH, Mohamed AE-K, Hegazy OM. Hybrid-biomarker case-based reasoning system for water pollution assessment in Abou Hammad Sharkia, Egypt. Appl Soft Comput 2016;46:1043−55.
[14] El-son MAM. Advanced techniques used in diagnosis and control of some fish diseases. Fac Vet Med 2019. Mansoura University.
[15] Elsheshtawy A, Yehia N, Elkemary M, Soliman H. Investigation of nile tilapia summer mortality in Kafr El-Sheikh governorate, Egypt. Genet Aquat Organ 2019;3:17−25.
[16] Pattanayak S, Priyadarsini S, Paul A, Kumar PR, Sahoo P. Diversity of virulence-associated genes in pathogenic Aeromonas hydrophila isolates and their in vivo modulation at varied water temperatures. Microb Pathog 2020;147: 104424.
[17] Hu M, Wang N, Pan Z, Lu C, Liu Y. Identity and virulence properties of Aeromonas isolates from diseased fish, healthy controls and water environment in China. Lett Appl Microbiol 2012;55:224−33.
[18] Krumperman PH. Multiple antibiotic resistance indexing of Escherichia coli to identify high-risk sources of fecal contamination of foods. Appl Environ Microbiol 1983;46:165−70.
[19] Sharma I, Rabha D, Das S, Ningombam D. Hemolytic activity and antibiotic resistance of Aeromonas sp. isolated from marketed fish. Eur J Pharm Med Res 2015;2: 304−12.
[20] Ahmed HA, El Bayomi RM, Hussein MA, Khedr MH, Remela EMA, El-Ashram AM. Molecular characterization, antibiotic resistance pattern and biofilm formation of Vibrio parahaemolyticus and V. cholerae isolated from crustaceans and humans. Int J Food Microbiol 2018;274:31−7.
[21] Schäperclaus W. Fish diseases. CRC Press; 1992.
[22] Stoskopf MK. Fish medicine. 1993.
[23] Buller NB. Bacteria from fish and other aquatic animals: a practical identification manual. Cabi; 2004.
[24] Malainine S, Moussaoui W, Prévost G, Scheftel JM, Mimouni R. Rapid identification of V ibrio parahaemolyticus isolated from shellfish, sea water and sediments of the Khnifiss lagoon, Morocco, by MALDI-TOF mass spectrometry. Lett Appl Microbiol 2013;56:379−86.
[25] Trakhna F, Harf-Monteil C, Abdelnour A, Maaroufi A, Gadonna-Widehem P. Rapid Aeromonas hydrophila identification by TaqMan PCR assay: comparison with a phenotypic method. Lett Appl Microbiol 2009;49:186−90.
[26] Roberts RJ. Fish pathology. 4th Ed. UK: John Wiley & Sons; Blackwell Publishing Ltd.; 2012. https://doi.org/10.1002/9781118222942.
[27] Finegold S, Martin S. Diagnostic microbiology 6th ed. the CV Mosby Company, St. Louis Tranto. London Wiener Tierarstilich Mschr 1982;6:233−6.
[28] NCCLS. Performance standards for antimicrobial disk susceptibility tests. Approved standard. In: 8th ed. Document M2-A8 ed. NCCLS. Wayne, Pa: National Committee for Clinical Laboratory Standards; 2003.
[29] Pokhrel H, Baishya S, Phukan B, Pillai D, Rather MA. Occurrence and distribution of multiple antibiotic resistance bacteria of public health significance in backwaters and aquaculture farm. Int J Curr Microbiol Appl Sci 2018;7: 975−87.
[30] Güven S, Demirel Zorba N. Genel mikrobiyoloji ve laboratuvar kõlavuzu. Ankara: NOBEL yayõnlarõ; 2013.
[31] Gordon L, Giraud E, Ganière JP, Armand F, Bouju-Albert A, De La Cotte N, et al. Antimicrobial resistance survey in a river receiving effluents from freshwater fish farms. J Appl Microbiol 2007;102:1167−76.
[32] Lagacé L, Pitre M, Jacques M, Roy D. Identification of the bacterial community of maple sap by using amplified ribosomal DNA (rDNA) restriction analysis and rDNA sequencing. Appl Environ Microbiol 2004;70:2052−60.
[33] Afifi S, Al-Thobiati S, Hazaa M. Bacteriological and histopathological studies on Aeromonas hydrophila infection of Nile tilapia (Oreochromis niloticus) from fish farms in Saudi Arabia. Assiut Vet Med J 2000;42:195−205.
[34] El Deen AN, Dorgham-Sohad M, Hassan-Azza H, Hakim A. Studies on Aeromonas hydrophila in cultured Oreochromis niloticus at Kafr El Sheikh Governorate, Egypt with reference to histopathological alterations in some vital organs. World J Fish Mar Sci 2014;6:233−40.
[35] Miyazaki T, Kaige N. A histopathological study on motile aeromonad disease of crucian carp. Fish Pathol 1986;21: 181−5.
[36] El-Barbary MI, Hal AM. Isolation and molecular characterization of some bacterial pathogens in El-Serw fish farm, Egypt. Egyptian J Aquat Biol Fish 2016;20:115−27.
[37] Okasha L, Ammar A, El-Hady M, Samir A, Samy A, Abdelmegeid M, et al. Identification of common fish bacterial pathogens in Kafr El Sheikh governorate, Egypt using PCR. Int J Biol Pharm Allied Sci 2016;5:522−37.
[38] Wamala SP, Mugimba KK, Mutoloki S, Evensen Ø, Mdegela R, Byarugaba DK, et al. Occurrence and antibiotic susceptibility of fish bacteria isolated from Oreochromis niloticus (Nile tilapia) and Clarias gariepinus (African catfish) in Uganda. Fish Aquat Sci 2018;21:1−10.
[39] Hamouda A, Moustafa E, Zayed M. Overview on the most prevailing bacterial diseases infecting Oreochromis

niloticus at Aswan fish hatchery, Egypt. Adv Anim Vet Sci 2019; 7:950−61.
[40] Ibrahem MD, Mostafa M, Arab R, Rezk M. Prevalence of Aeromonas hydrophila infection in wild and cultured tilapia nilotica (O. niloticus) in Egypt. 2008.
[41] Vivekanandhan G, Hatha A, Lakshmanaperumalsamy P. Prevalence of Aeromonas hydrophila in fish and prawns from the seafood market of Coimbatore, South India. Food Microbiol 2005;22:133−7.
[42] Quinn P, Markey BK, Carter M, Donnelly W, Leonard F. Veterinary microbiology and microbial disease. Oxford, UK: Blackwell Science; 2002.
[43] Eissa I, El-Lamei M, Sherif M, Desuky E, Zaki M, Bakry M. Aeromonas veronii biovar sobria a causative agent of mass mortalities in cultured Nile tilapia in El-Sharkia governorate, Egypt. Life Sci J 2015;12:90−7.
[44] Martin Carnahan A, Joseph S. Genus Aeromonas stainer 1943. In: Brenner DJ, Krieg NR, Bergey's Staley JT, editors. Manual of systemic bacteriology. 2nd Ed. New York, NY: Springer; 2005. p. 557−80.
[45] Gamal A, Moustafa M, Hussein S. Aeromonas hydrophila infection in male monosex Oreochromis niloticus fish reared in floating cages. In: Hurgada, Egypt: 6th Vet Med Zag Conference; 2002.
[46] Sha J, Kozlova E, Chopra A. Role of various enterotoxins in Aeromonas hydrophila-induced gastroenteritis: generation of enterotoxin gene-deficient mutants and evaluation of their enterotoxic activity. Infect Immun 2002;70:1924−35.
[47] Hirono I, Aoki T. Nucleotide sequence and expression of an extracellular hemolysin gene of Aeromonas hydrophila. Microb Pathog 1991;11:189−97.
[48] Wei LS, Mustakim M, Azlina IN, Zulhisyam A, An'amt M, Wee W, et al. Antibiotic and heavy metal resistance of Aeromonas spp. isolated from diseased red hybrid tilapia (Oreochromis sp.). Ann Res Rev Biol 2015;6(4):264−9.
[49] Essmat AM. Studies on current bacterial pathogens of fish mortality and low productivity. Fac Vet Med 2009. Zagazig Univ.
[50] Pokhrel H, Baishya S, Phukan B, Pillai D, Rather MA. Occurrence and distribution of multiple antibiotic resistance bacteria of public health significance in backwaters and aquaculture farm. Int J Curr Microbiol App Sci 2018;7: 975−87.
[51] Laith A, Najiah M. Aeromonas hydrophila: antimicrobial susceptibility and histopathology of isolates from diseased catfish, Clarias gariepinus (Burchell). J Aquac Res Dev 2014; 5(2):215.
[52] Sreedharan K, Philip R, Singh ISB. Virulence potential and antibiotic susceptibility pattern of motile aeromonads associated with freshwater ornamental fish culture systems: a possible threat to public health. Braz J Microbiol 2012;43: 754−65.