



## Phenotypic Characteristics of *Aeromonas hydrophila* Isolated from the Wild African Catfish (*Clarias gariepinus*) in the River Nile, Egypt

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### ARTICLE INFO

#### Article History:

Received: Aug. 30, 2023

Accepted: Sept. 17, 2023

Online: Oct. 29, 2023

#### Keywords:

*Aeromonas hydrophila*,  
*Clarias gariepinus*,  
Aerolysin gene,  
Antibiotic sensitivity,  
Pathogenicity,  
Plasmid curing

### ABSTRACT

The River Nile in Egypt is the most important natural freshwater fishery that can secure thousands of tons of fish. *Aeromonas hydrophila* is a common pathogen that can induce significant deaths in African catfish (*Clarias gariepinus*). During the summer of 2022, 100 moribund and freshly dead African catfish were collected from the Nile River, Al-Manial, Cairo, Egypt for a bacteriological examination. The clinical findings of surface hemorrhage and abdominal distention hemorrhage at the anal opening resulted in skin discoloration and loss of antenna. The postmortem examinations included the deterioration of internal organs, hepatomegaly, and splenomegaly. The isolated *A. hydrophila* strains were identified based on phenotypic characterization by diagnostics SRO. GN24 features and the homology of 16S rRNA gene sequence analysis. Antibigram has revealed that isolates were sensitive to gentamicin, ciprofloxacin, nitrofurantoin, nalidixic acid, oxolinic acid, and ofloxacin, while they were highly resistant to amoxicillin, lincomycin, spiramycin, colistin, tetracycline, and cephalothin. In addition, PCR data confirmed the presence of the Aerolysin gene in the identified *A. hydrophila* isolates, while negative to the Hemolysin gene. Gene sequencing and phylogenetic analysis based on the 16S rRNA sequence confirmed that *A. hydrophila* H/A (accession No. OL771444) of the present study displayed 100% identity with the 16S rRNA gene of *A. hydrophila*. The presence of resistance genes was investigated on either the bacterial DNA or the plasmid using a plasmid curing method, which was achieved by elevated temperatures. In conclusion, *A. hydrophila* is the main cause of mortality in *C. gariepinus*, and the wise use of antibiotics in fish treatment has become compulsory to avoid antibiotic residues and the great losses due to multidrug resistance (MDR).

### INTRODUCTION

In Egypt, fish production plays an important role for maintaining food security and economic development in the agricultural sector, and the total production of natural fisheries is 418,683 tons. According to the latest 2020 statistics, fish production from the Nile River and its branches constituted 3.96% of the total natural fisheries production, amounting to 79,533 tons, indicating an increase of about 2 tons compared to the

estimates recorded in 2019. African catfish comes in the second rank after the Nile tilapia in terms of fish production from the Nile River, according to the Fish Statistical Yearbook for 2020, with an estimated quantity of 15,014 tons (GAFRD, 2020).

African catfish (*Clarias gariepinus*) suffers from widespread outbreaks due to several enteric pathogens, including *Aeromonas hydrophila*, *Edwardsiella tarda*, *E. ictalurid* and *Pseudomonas aeruginosa*, which are related to enteric septicemia of catfish (Chacón *et al.*, 2003; Enany *et al.*, 2022). *A. hydrophila*, *E. tarda* and *Streptococcus agalactiae* are pathogens mainly found in freshwater fish (Sherif *et al.*, 2021; Abdelsalam *et al.*, 2022; Elgendy *et al.*, 2022; Sherif & AbuLeila, 2022; Sherif & Kassab, 2023). These fish are commonly associated with extensive mortality through both acute and chronic infections in fishponds (Li *et al.*, 2012). The most common cause of bacterial septicemic disease in the Nile tilapia in Egypt is *A. hydrophila*, and many surveys have isolated *A. hydrophila* from healthy and diseased fish, revealing that some strains are virulent while others are not, particularly during trial infection. Antibiotic resistance is considered as a potential problem for fish health managers, who are responsible for choosing suitable and effective antibiotics that can be used for the treatment of disease in farms (El-Bahar *et al.*, 2019). *Aeromonas salmonicida* is one of the oldest bacterial pathogen in various fish species in freshwater, but the most common in tilapia and catfish is *A. hydrophila*, *E. ictaluri* & *E. tarda* (Irshath *et al.*, 2023).

In the aquatic environments, *A. hydrophila*, is a Gram-negative, facultative anaerobic rod-shaped, non-spore forming, motile bacterium, found in various aquatic environments (Ahmed *et al.*, 2018) and is considered as a potential threat to fish stocks and could be regarded as a serious threat to humans (Sellegounder *et al.*, 2018; EL-Makhzangy *et al.*, 2022). *Aeromonas* spp. are frequently isolated from freshwater, meat products and seafood, so they are considered as an initial cause of food contamination and may work as mediators in transmitting infections to humans (Shuang *et al.*, 2020). Most diseases in fish are caused by *Aeromonas hydrophila* and other *Aeromonads*. *A. hydrophila* is reported as a main bacterial pathogen for freshwater fishes like *C. gariepinus*. Dermal ulceration, fin hemorrhages, fin rots, red sores, hemorrhages and necrosis of the visceral organs are considered the common pathological symptoms of *Aeromonas* septicemia (Kumar *et al.*, 2016). The activity of multiple virulence factors of pathogenic bacteria that may act independently or in combination can trigger a disease in vulnerable hosts by producing many toxins that are harmful to host cells (El-Bahar *et al.*, 2019). It should be mentioned that disease may not be initiated by a single species of pathogen but may be initiated by the contribution of one or more bacterial taxon with viruses and/or parasites. Furthermore, the presence of stressors is capable of adversely affecting the host, leading to the development of diseases. Stressors may include handling which leads to abrasions in addition to the development of wounds due to the probable attack of finfish among one another. These stressors may produce entry sites for pathogens. Poor water flow allows the build-up of immunity stressor. In addition, poor

aeration that affects oxygen levels, and suboptimum temperatures contribute to the development of the disease (Austin, 2023). A wide range of chemical and biological agents have been used to treat and control pond sediments and water quality, resulting in environmental pollution, deterioration of water standards, and the emergence of many aquatic pathogens. Microbial diseases are a major restriction causing annual losses in fish, jobs and economy. Several studies stated that the transmission of multidrug-resistant bacterial pathogens from various origins can affect public health. Hence, the emergence of multi-drug resistant (MDR) bacterial pathogens is considered a public health threat. The molecular determination of most inherited antibiotic resistance genes should be regularly assessed to avoid antibiotic resistance strains that can affect public health (Algammal *et al.*, 2022).

16S rRNA gene sequencing has been used as a significant means for phylogenetic analysis and classification of bacteria because it contains species-specific variable areas that allow species detection. The 16S rRNA gene includes zones highly preserved in all organisms that are standard for polymerase chain reaction (PCR) primer design and sequencing (Cai *et al.*, 2003; Silva *et al.*, 2017; Sherif & AbuLeila, 2022; Mansour & El-Shaer, 2023; Sherif & Kassab, 2023).

The pathogenicity of *Aeromonas* sp. depends on many extracellular virulence proteins including exotoxins, such as haemolysins, cytotoxic and cytotoxic enterotoxin and aerolysin. These toxins have enterotoxic, cytotoxic, cytolytic and proteolytic activities. Aerolysin gene (*aerA*) is recorded to be the commonly virulence gene produced by most strains of *Aeromonas*. Thus, the detection of such gene is considered to be a convenient approach to examine pathogenic *Aeromonas* strain (Emeish *et al.*, 2018).

A plasmid is an extra chromosomal DNA located in the cell's cytoplasm. It consists of double-stranded circular DNA that can independently replicate. Plasmids often move between bacterial cells, potentially spreading throughout a population. These plasmids frequently carry genes responsible for antibiotic resistance or toxin production (El Salam *et al.*, 2016).

This study aimed to detect the virulence genes and assess the antimicrobial activity of different antibiotics against the isolated *Aeromonas hydrophila* from wild African catfish (*C. gariepinus*) in the Nile River, Al- Manial, Cairo, Egypt.

## MATERIALS AND METHODS

### 2. Fish sampling

A total number of 100 moribund and freshly dead African catfish (*Clarias gariepinus*) were randomly collected from the Nile River, Al- Manial, Cairo, Egypt, with average weight of 120- 200g. during the period of July 2022 to October 2022. Samples were transferred to the Fish Diseases Research Dept. at Animal Health Research Institute, Al-Doki, Cairo, Egypt.

### **2.1. Clinical and postmortem examination**

Fish were clinically examined for the presence of any abnormal external and internal pathological findings according to **Schäperclaus *et al.* (1992)**.

### **2.2. Bacteriological examination and isolation of *A. hydrophila***

Fish samples were exposed to bacteriological examination under sterile conditions according to the methods described by **Austin and Austin (2016)**.

In a microbiological safety cabinet, bacteria were isolated with sterile loop from different organs of each fish (liver, kidney, spleen, gills, and external lesions if present), were inoculated in TSB and incubated at 28°C for 24hrs. A loopful from each tube was streaked on TSA and MacConkey agar plates and blood agar, then they were incubated aerobically at 28°C for 24hrs. Suspected colonies were streaked on Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) agar and Rimler-Shotts (RS) medium for selection, followed by incubation for 18hrs at 28°C. All isolates were identified based on culture characters, microscopic examination using gram staining, motility test, and biochemical analysis (oxidase, catalase, indole, methyl red, Voges Proskauer, citrate utilization, urease, H<sub>2</sub>S production, sugar fermentation tests), according to **Austin and Austin (2016)**. All tested colonies were detected by Diagnostics SRO. GN24 ([www.diagnostics.sk](http://www.diagnostics.sk)) for further biochemical identification.

### **2.3. Antimicrobial susceptibility of *A. hydrophila* isolates**

The disc diffusion method was used. Twelve different discs of antibiotics were chosen to cover different antibiotic groups that are used in fish aquaculture as follows: gentamicin (CN) (10 µg), ciprofloxacin (CIP) (5 µg), lincomycin (MY) (10 µg), colistin (CT) (25 µg), spiramycin (SP) (100 µg), nitrofurantoin (F) (300 µg), amoxicillin (AML) (10 µg), nalidixic acid (NA) (30 µg), oxolinic acid (OA) (2 µg), ofloxacin (OFX) (10 µg), tetracycline (TE) (30 µg), and cephalothin (KF) (30 µg). Antibiotic sensitivity was tested on Mueller-Hinton agar, and inhibition zone diameters were interpreted as sensitive (S), intermediate (I), and resistant (R), according to **CLSI (2016)**.

### **2.4. Phylogenetic analysis of *Aeromonas hydrophila***

The *Aeromonas hydrophila* isolate was confirmed by 16SrRNA.

### **2.5. DNA extraction**

The isolates were inoculated into brain heart infusion broth (BHIB) and incubated for 24hrs; DNA was extracted using PathoGene-spin™ DNA Extraction Kit according to the manufacturer's instructions.

#### **2.5.1. Genotypic confirmation by 16srRNA *Aeromonas hydrophila***

PCR was carried out using oligonucleotide primer for the general 16SrRNA *A. hydrophila* gene (F: GGGAGTGCCTTCGGGAATCAGA and R: TCACCGCAACA

TTCTGATTTG) (Wang *et al.*, 2003). The PCR protocol was performed as follows: an initial denaturation at 95°C for 5min, followed by 35 cycles at 94°C for 2min; 35 cycles of 94°C for 30s, 55°C for 30s; 72°C for 30s and 72°C for 5min. The primer used in the amplification study was obtained from the study of Wang *et al.* (2003).

### 2.5.2. Genotypic detection of virulence genes

PCR was used to detect the presence of virulence genes, Aerolysin (*aero*) gene, and hemolysin (*hly*) in the identified isolates of *A. hydrophila* isolated from fish. The *aero* amplified primers were “F:CACAGCCAATATGTCGGTGAAG , R:GTCACCTTCTCGCTCAGGC”, and its cycling was an initial denaturation at 95°C for 5min, followed by 35 cycles at 94°C for 2min; 35 cycles of 94°C for 30s, 52°C for 30s, and 72°C for 30s; 72°C for 5min with a product size of 326bp (Singh *et al.*, 2008). While, *hly* amplified primers were “F:GGCCGGTGGCCCGAAGATACGGG, R:GGCGGCGCCGGACGAGACGGGG”, and its cycling was an initial denaturation at 95°C for 5min, followed by 35 cycles at 94°C for 2min; 35 cycles of 94°C for 30s, 55°C for 30s, and 72°C for 30s; 72°C for 5min with a product size of 592bp (Rahayu & Daruti, 2018).

### 2.5.3. Sequencing

PCR products of *Aeromonas hydrophila* 16SrRNA were purified using QIAquick PCR Product extraction kit. (Qiagen, Valencia). Bigdye Terminator V3.1 cycle sequencing kit (Perkin-Elmer) was used for the sequence reaction, and then it was purified using Centrisep spin column. DNA sequences were obtained by Applied Biosystems3130 genetic analyzer (HITACHI, Japan), a BLAST® analysis (Basic Local Alignment Search Tool) (Altschul *et al.*, 1990) was initially performed to establish sequence identity to GenBank accessions. The phylogenetic tree was created by the MegAlign Phylogenetic analysis was done using neighbor joining in MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms (Kumar *et al.*, 2018).

## 2.6. Plasmid curing

Overnight growing bacteria was achieved on TSA culture medium followed by inoculation in 10ml of broth medium. Then, Bacteria were incubated at the optimal growth temperature in shaking incubator for 18-24hrs. 50µl of the broth culture were transferred into 4 tubes with broth medium (3 as "Heat-treated" and 1 as "Control"). The "Heat-treated" tubes were placed in a water bath set to 42-44°C for plasmid curing. The tubes were treated for 20 minutes, 40 minutes and 60 minutes. After that MHA plates were inoculated with "Control" and "Heat-treated" bacterial strains, the antibiotic discs

that were resistant to the untreated strain were placed. All plates were incubated at 28°C-30°C for 18-24hrs, according to **May *et al.* (1964)**.

### **2.7. Biosafety measures**

This study applied biosafety measures according to pathogen safety data sheets: Infectious substances *Aeromonas hydrophila*, Pathogen Regulation Directorate (**Public Health Agency of Canada, 2019**).

## **RESULTS**

### **3. Clinical and postmortem observations**

Fish showed extensive surface hemorrhage, erosions, abscesses, abdominal distention and necrotic ulcers. The clinical examination revealed that, fish were diseased and exhibited signs of hemorrhagic septicemia, which is represented by the presence of surface lesions and hemorrhage at the anal opening. Some samples showed an extensive abscess with associated muscle liquefaction, skin discoloration, loss of antenna, as shown in Figs. (1, 2). The postmortem examination revealed deterioration of internal organs, congestion and enlargement of liver and spleen, as rendered in Fig. (3).

#### **3.1. Phenotypic characteristics and prevalence of *A. hydrophila* in fish**

Phenotypic analysis of *Aeromonas* spp. colonies on trypticase soy agar (TSA) medium appeared round, convex, white, creamy and opaque, while on Rimler-Shotts (RS) medium, they appeared deep creamy or light-yellow colonies with an entire margin after 24hrs of incubation. On MacConkey's agar medium, pale colonies appeared. Thiosulfate-Citrate-Bile Salts-Sucrose Agar (TCBS) medium was changed to yellow color. For Triple Sugar Iron (TSI), *A. hydrophila* produced acid without H<sub>2</sub>S production, urease-negative by gram staining, Gram-negative rod-shaped or cocco-bacilli bacteria. *Aeromonas hydrophila* is oxidase-positive, catalase-positive, Voges Proskauer-positive, diagnostics SRO. GN24 biochemical test was confirmed for *A. hydrophila*, as shown in Fig. (4).

#### **3.2. Antibiogram of *A. hydrophila* isolate**

The antimicrobial sensitivity testing was performed on all the recovered isolates of *A. hydrophila*. The tested isolates showed a notable sensitivity to gentamicin, ciprofloxacin, nitrofurantoin, nalidixic acid, oxolinic acid and ofloxacin, while they were highly resistant to amoxicillin, lincomycin, colistin, spiramycin, tetracycline, and cephalothin, as shown in Figs. (5, 6, 7). Statistically, the tested *A. hydrophila* isolates revealed a considerable difference in their susceptibility to different tested antimicrobial agents, as shown in Table (1).

### **3.3. Genetic characterization of *Aeromonas hydrophila***

The *Aeromonas hydrophila* isolate was confirmed by PCR giving a specific band at 356bp, as shown in Fig. (8). The *Aeromonas hydrophila* isolate was positive for aerolysin (*aero*) gene, while it was negative for hemolysin gene (*hly*), as shown in Fig. (9).

### **3.4. Sequence analysis of *Aeromonas hydrophila* 16S gene**

It was determined from the sequence of *Aeromonas hydrophila* 16S gene, as shown in Fig. (10). The *Aeromonas hydrophila* isolate was submitted to the GenBank database under accession no. OL771444. This isolate OL771444 showed high nucleotide identity with other isolates MW836109 and MW831507, which were isolated from the Nile tilapia in Egypt, MT384379.1 and MW494427.1 which were isolated from the Nile tilapia, MN894081.1, MN894076.1 and MN894078.1 that were isolated from fingerling of rainbow trout, *Oncorhynchus mykiss*, MW555599.1, OK634406.1 and MW556467.1 from *Acipenser baerii* and OP221548.1, ON202987.1 and OK354113.1 from *Lithobates catesbeianus* and *Procambarus clarkia* and infected Fish, respectively.

### **3.5. Susceptibility test after plasmid curing**

The sensitivity test for tetracycline (TE), cephalothin (KF), colistin (CT), amoxicillin (AML), spiramycin (SP), lincomycin (MY) (which show resistant result (Table 1) in the initial sensitivity test) were performed for control and heat-treated bacteria. The results illustrated in Table (2) and Fig. (11) show no difference in the inhibition zones of antibiotics between the control and the heat-treated bacteria, even at different periods of exposure to elevated temperature. These results may indicate the presence of resistant genes on the bacterial chromosome.



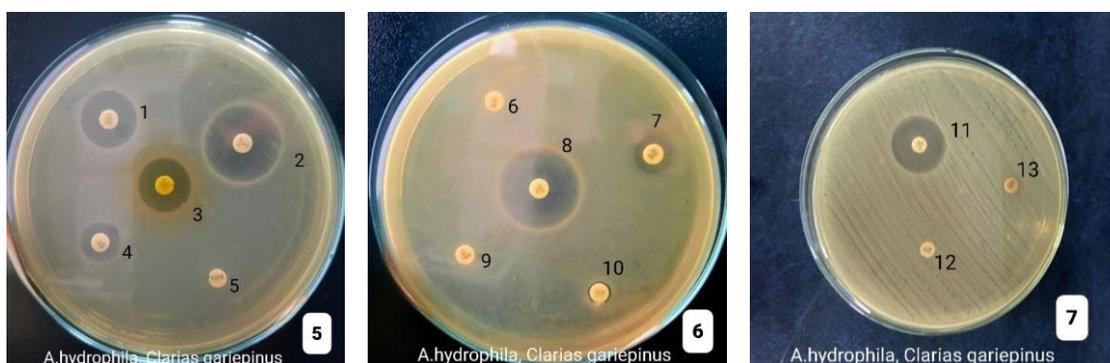
**Fig. 1.** Naturally infected *C. gariepinus* showing surface hemorrhage, abdominal distention with hemorrhage at the anal opening.

**Fig. 2.** Naturally infected *C. gariepinus* showing erosions, skin discoloration, and loss of antenna.

**Fig. 3.** Naturally infected *C. gariepinus* showing congestion and enlargement of the liver and spleen and deterioration of internal organs.



**Fig. 4.** Diagnostics SRO. GN24 biochemical test confirmed for *A. hydrophila*

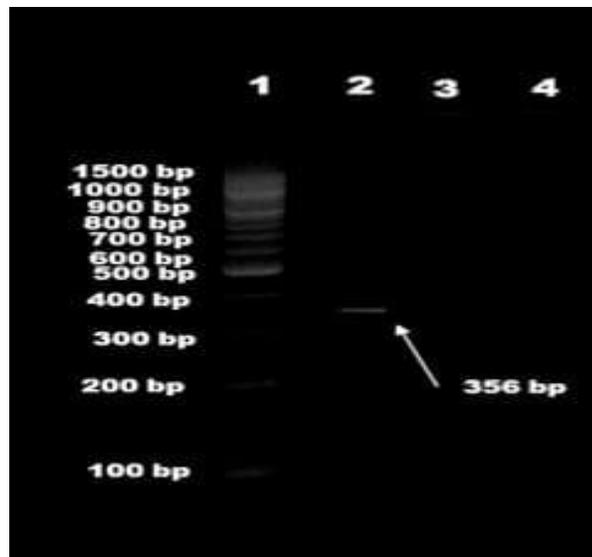


**Fig. 5.** Antimicrobial sensitivity test for *A. hydrophila* (1=NA; 2=CIP; 3 =F; 4=CN; 5=KF), **Fig. 6.** (6=KF; 7=SP; 8= OFX; 9=AML;10= CT) and **Fig. 7.** (11=OA; 12= MY; 13=TE).

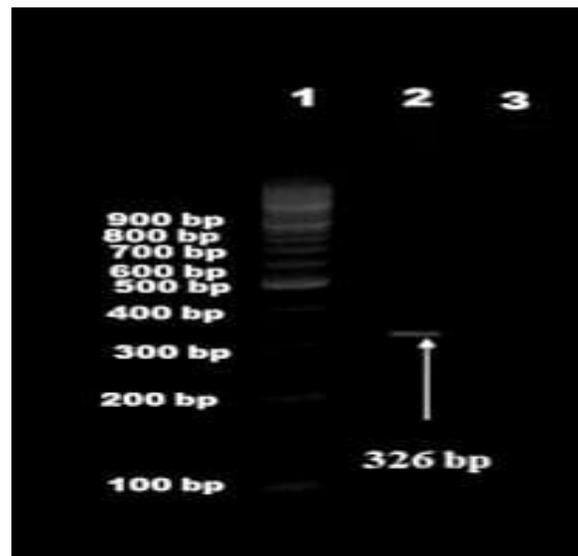
**Table 1.** Results of susceptibility test for *A. hydrophila*

Antibiotic agent	Susceptibility results
Gentamicin (CN) 10 µg	S
Ciprofloxacin (CIP) 5 µg	S
Lincomycin (MY) 10 µg	R
Colistin (CT) 10 µg	R
Nitrofurantoin (F) 300 µg	S
Amoxicillin (AML) 10 µg	R
Nalidixic acid (NA) 30 µg	S
Oxolinic acid (OA) 2 µg	S
Tetracycline (TE) 30 µg	R
Spiramycin (SP) 100 µg	R
Ofloxacin (OFX) 10 µg	S
Cephalothin (KF) 30 µg	R

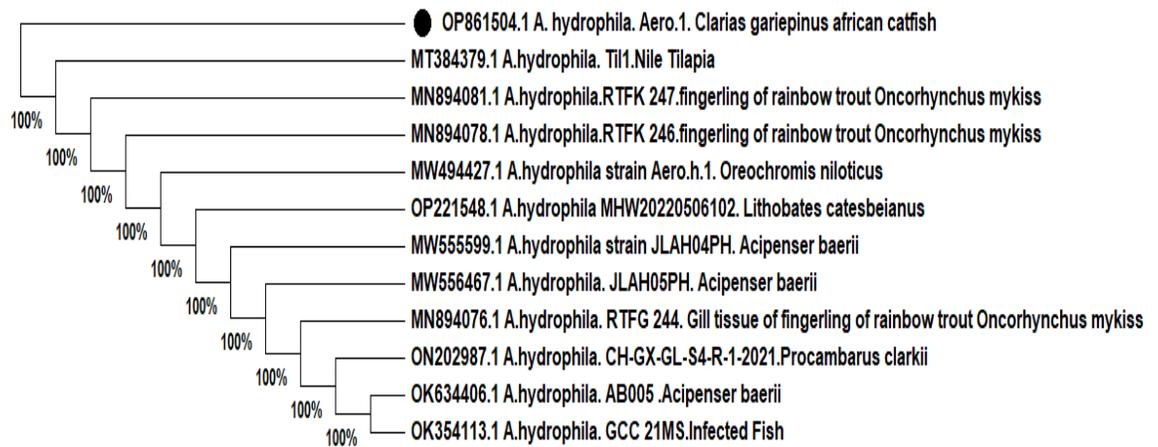
R = Resistant, I = Intermediate, S = Sensitive.



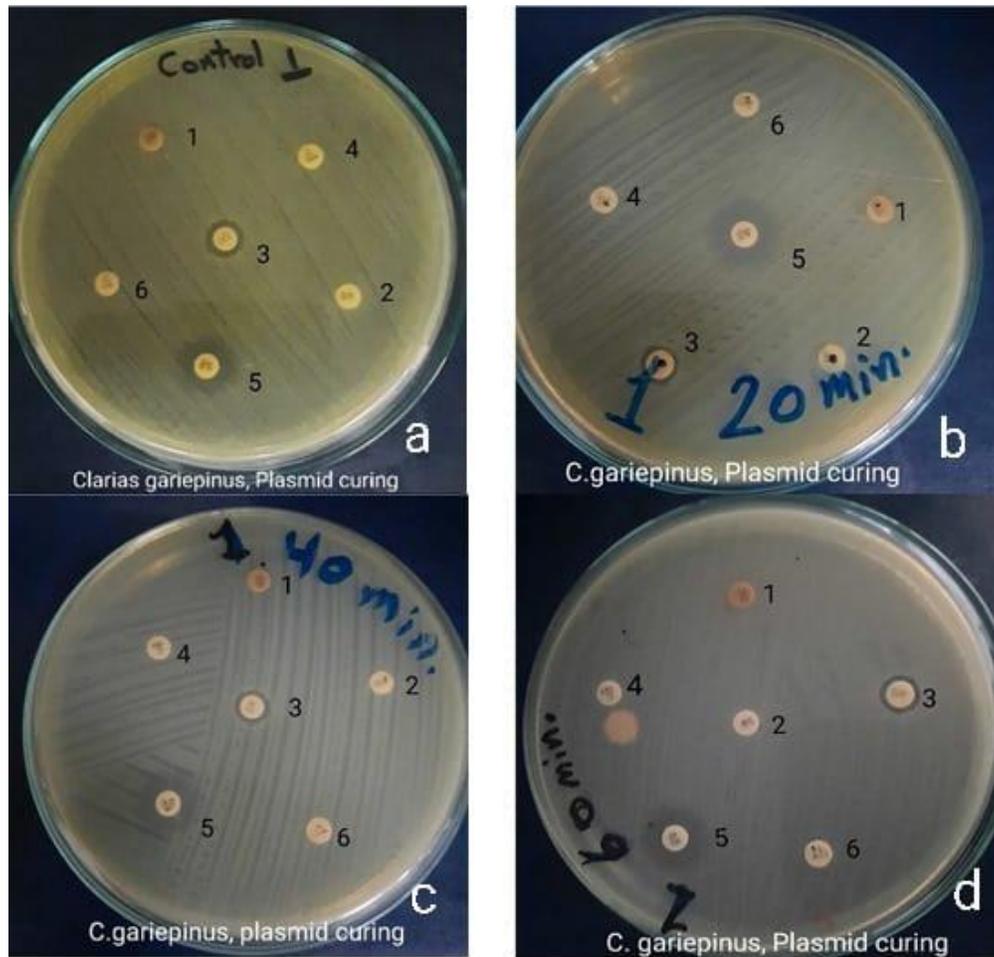
**Fig. 8.** Agarose gel electrophoresis showing amplification of 16SrRNA *Aeromonas hydrophila* using specific primer. Lane 1: 1 kb Ladder, Lane 2: Sample fragment at 356 bp for 16SrRNA *Aeromonas hydrophila*.



**Fig. 9.** Agarose gel electrophoresis showing amplification of *aero* gene and *hly* genes of *Aeromonas hydrophila* using specific primer. Lane 1: 1 kb Ladder, Lane 2: sample fragment at 326 bp for *aero* gene, Lane 3: sample negative for *hly* gene.



**Fig. 10.** Show the phylogenetic tree of *Aeromonas* spp. 16S gene black circle: this research isolate.



**Fig. 11.** The sensitivity test of "Control (a)" and "Heat-treated" *A. hydrophila* at (b) 20 min., (c) 40 min., (d) 60 min. (1=TE, 2=KF, 3=CT, 4=AML, 5=SP, 6=MY).

**Table 2.** Results of susceptibility test for plasmid cured *A. hydrophila* isolated from *C. gariepinus*

No. on plates	Antibiotic agent	Symbol	Conc. µg	Antibiotic group	Susceptible zoon (mm)			Susceptibility results
					20 Min.	40 Min.	60 Min.	
1	Tetracycline	TE	30	Tetracyclines	-ve	-ve	-ve	R
2	Cephalothin	KF	30	1 <sup>st</sup> gen. Cephalosporin	-ve	-ve	-ve	R
3	Colistin	CT	10	Lipopeptides	8mm	9mm	9mm	R
4	Amoxycillin	AML	10	Penicillins	-ve	-ve	-ve	R
5	Spiramycin	SP	100	Macrolides	11mm	12mm	12mm	R
6	Lincomycin	MY	10	Lincosamides	-ve	-ve	-ve	R

\* R = Resistant.

## DISCUSSION

The most dominant fish species in Egypt are the Nile tilapia and African catfish. Therefore, bacterial infections pose a potential threat to such a significant source of protein. In the present study, the infected catfish showed surface hemorrhage, abdominal distention, congestion and enlargement of the liver and spleen, damage to internal organs, erosions, abdominal distention, and necrotic ulcers, as reported in previous studies (Austin & Adams, 1996; El Deen *et al.*, 2014; Hamouda *et al.*, 2019; El-Bahar *et al.*, 2019; Pauzi *et al.*, 2020; Bakiyev *et al.*, 2022; Sherif & AbuLeila, 2022; Sherif & Kassab, 2023; Sherif *et al.*, 2023). The morphological shape of colonies, gram staining and the biochemical profile of the isolated bacteria are nearly identical to those reported by different authors (Hamouda *et al.*, 2019; Pauzi *et al.*, 2020; Ahangarzadeh *et al.*, 2022; Bakiyev *et al.*, 2022).

Motile aeromonads are prevalent in the aquatic environment and affect a wide range of fish species. Disease epidemics and mortality in fish have been associated to numerous *Aeromonas* spp. (Abdel-Latif & Khafaga, 2020; Sherif *et al.*, 2021). Aeromonads have been suggested as primary or secondary pathogens in various fish species, with catfish being among the susceptible species (Abdelsalam *et al.*, 2021).

Due to the misuse of antimicrobials for prophylactic or treatment purposes and inconsideration of the withdrawal period of antimicrobials, the presence of antibiotic residue was detected in the fish tissues (Morshdy *et al.*, 2022). High levels of antibiotic residues with long-term consumption of such products may cause allergic reactions in persons and antibiotic resistance in humans (Liu *et al.*, 2013).

The scope of this study was to show the high diversity of the bacterial strains that could occur due to multiple antibiotic resistance and become a potential etiological agent for mass outbreaks in *C. gariepinus*. Even though it's natural abundance in the freshwater environment, *A. hydrophila* is responsible for motile *Aeromonas* septicemia epidemics among different species of aquatic animals. The disease causes a critical problem for fish industry in Egypt as well as in other countries (Hussein *et al.*, 2021; Sherif & Kassab, 2023). According to the various clinical signs, motile aeromonad infections have been mentioned with various terminologies, identified as motile aeromonad septicemia, motile aeromonad infection, hemorrhagic septicemia, tail and fin rot, and epizootic ulcerative syndrome (Austin & Adams, 1996). The result of this study indicates that the clinical examination agrees with those of Mamun *et al.* (2022) and Hussein *et al.* (2021). Moreover, the postmortem outcomes are highly compatible to those of Austin *et al.* (2012) who observed liquefaction of the internal organs and musculature, in addition to local hemorrhages, especially in the gills, ulcers and abscesses.

In this study, bacteriological examination of *C. gariepinus* detect Gram-negative bacteria, identified as *Aeromonas hydrophila* colonies on TSA medium that appeared round, convex, white, creamy and opaque. On RS medium, deep creamy or light-yellow colonies were monitored with entire margin. Pale colonies were detected on MacConkey's agar medium. TCBS medium were changed to yellow color. For TSI, *A. hydrophila* produced acid but without H<sub>2</sub>S production. Urease –negative, oxidase-positive, catalase-positive, Voges Proskauer-positive; these results agree with those of earlier studies (Hala *et al.*, 2021; Mansour & El-Shaer, 2023).

The main approach of treating bacterial infections is the administration of antibiotics. However, the excessive use of antibiotics leads to antibiotic resistance which can reduce the effect of antibiotics in the treatment of *A. hydrophila* infections, and this can lead to a potential threat to human health (Dong *et al.*, 2018; Sherif *et al.*, 2022b). Lately, antibiotic resistance genes found in *Aeromonas* species had resistance to four main groups of antibiotics as the quinolones, aminoglycosides,  $\beta$ -lactams and tetracyclines (Piotrowska & Popowska, 2014). The existence of  $\beta$ -lactamases genes causes the resistance of *Aeromonas* species to  $\beta$ -lactam antibiotic. The present data from the examination of 100 wild *C. gariepinus* with prevalence of 40% of MAS within this study showed that *A. hydrophila* was sensitive to gentamicin and ciprofloxacin, while they were highly resistant to Penicillins and Tetracycline. These results nearly coincide with other studies (Nasser *et al.*, 2022; Austin *et al.*, 2012). This study confirmed that *A.*

*hydrophila* is resistant to  $\beta$ -lactam antimicrobials, and their effectiveness has considerably declined because of the production of  $\beta$ -lactamases and other resistant genes. However, they are still widely regarded as the best treatments for bacterial infections in fish, animals and humans.

Using 16SrRNA oligonucleotide to characterize microorganisms is the most effective and reliable approach because of their high information elements, conservative feature and universal prevalence (Lane *et al.*, 1985). In this study, the sequence of 16SrRNA gene was used to detect *A. hydrophila* with a typical homology at 356 pb; this result corresponds to the findings of some authors (Hamouda *et al.*, 2019; Abdel-Latif & Khafaga, 2020; Umutoni *et al.*, 2020).

The pathogenicity of Aeromonads may be attributed to several virulence factors, such as aerolysin, lipases, hemolysin, proteases and DNases. These toxins have an important function in the development of diseases in fish and humans. Extracellular hemolysin and aerolysin, both are the major groups of hemolysins produced by *A. hydrophila*, to immunological investigations (Ahmed *et al.*, 2018). *A. hydrophila* has multiple virulence factors that combine to overcome the host immune system and stimulate disease and death (Chacón *et al.*, 2003). Multi-virulence factors contribute to the pathogenicity of *A. hydrophila*, and do not arise from a single determinant, but it results from interactive impacts of several virulence genes. Aerolysin, cytotoxic enterotoxin, and extracellular serine protease are the highly significant genes for distinguishing potentially pathogenic *A. hydrophila* strains (Bakiyev *et al.*, 2022). The cytotoxic enterotoxin gene involves a number of factorial actions with enterotoxin, hemolytic, cytotoxic activities and promotes cell lysis (Xu *et al.*, 1998). The results reported in the studies of Saleh *et al.* (2021) and Mansour and El-Shaer (2023) indicate the presence of extracellular enzymes and enterotoxins. These are considered virulence factors responsible for detrimental changes in endothelial cells, leading to systemic damage to internal organs and subsequent death. Additionally, the prevalence of the *aerA* gene at 326 bp aligns with the findings of this study. However, some studies in fish reported evidence of increased pathogenicity of *A. hydrophila* despite the absence of certain virulence factors. This study showed the presence of *aerA* gene and the absence of *hly* gene. These results partially coincide with those of El-Bahar *et al.* (2019), Tartor *et al.* (2021) and Mansour and El-Shaer (2023), whose results were positive for *aerA* gene and *hly* gene.

Bacterial plasmids are recognized for carrying genes that are resistant to antibiotics and metals, as well as catabolic pathways. Removing plasmids from bacterial strains is a method to eradicate these plasmids and ascertain the mediation of antibiotic resistance. Numerous techniques, involving both chemical and physical agents, have been formulated to eradicate plasmids. Resistance is typically categorized as "chromosomal" if

it remains unaffected by plasmid removal, and as "plasmid-mediated" if it is affected with it (Letchumanan *et al.*, 2015).

Rendering to existing research, bacterial plasmid elimination often relies on physical measures, such as raising the growth temperature. The mechanism behind this elevated temperature approach involves causing full or partial removals of the plasmid DNA from the strain. A curing technique involves subjecting the bacteria to an incubation temperature that is 5- 7°C higher than their ideal growth temperature (Gerhardt *et al.*, 1981). Although May *et al.* (1964) reported that *Staphylococcus aureus* experienced a decline in its resistance to both penicillin and tetracycline when grown at an increased temperature ranging from 43 to 44°C. Fairbrother *et al.* (1954) reported that, the growth of certain strains of *Staphylococci* at a higher temperature (44°C) accelerated the process of penicillin resistance reduction. Positive results of plasmid-cured *Escherichia coli* were also reported by Momoh and Ayodele-Asowata (2023). These findings conflict with the results of the present study, which showed negative results for plasmid curing. This discrepancy may be due to the presence of resistant genes on the chromosomal DNA. In contrast to those encoded by plasmid DNA, these genes remained unaffected following exposure to elevated temperatures (Radi & Rahman, 2010).

## CONCLUSION

African catfish (*C. gariepinus*) is considered as a cheap source of protein and *A. hydrophila* is a potential threat, which can cause high loss in catfish stock.

In this study, we aimed to determine the contribution of different virulence genes for the development of disease and investigate the progression of pathophysiological symptoms associated with the disease. We observed severe bacterial hemorrhage signs and symptoms, and the identification of *A. hydrophila* as the causative agent was confirmed through 16SrRNA sequencing to confirm the bacterial strain. Additionally, we examined the existence of resistant genes either on the bacterial DNA or on the plasmid by plasmid curing via elevated temperature.

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