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# *Aeromonas hydrophila* Isolated from the River Nile *Oreochromis niloticus*: Molecular Characterization and Antimicrobial Susceptibility

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## ABSTRACT

The goal of this study was to detect *Aeromonas hydrophila* and its antimicrobial resistance in *Oreochromis niloticus* sampled from the Nile River in Dakahlia Governorate, Egypt. A total of 150 moribund live and freshly dead tilapia fish (mean weight: 80 ± 10g, mean length: 20 ± 1.5cm) were collected during the 2022–2023 seasons. Fish samples were analyzed in the laboratory of the Fish Disease Research Department, Animal Health Research Institute, Dokki, Cairo. The clinical symptoms observed in the fish included surface hemorrhage, erosions, scale loss, and some samples showed exophthalmia, cataracts, and skin discoloration. Postmortem assessments revealed internal organ deterioration, yellowish fluid in the abdominal cavity, gallbladder enlargement, a pale liver, and pigmentation on the liver's surface. *A. hydrophila* identification was based on phenotypic features and homology of 16S rRNA gene sequences. The 16S rRNA gene of the isolates was submitted to GenBank under accession number PP829284.1, showing high nucleotide identity with other Egyptian isolates. Antimicrobial sensitivity testing indicated that the *A. hydrophila* isolates were sensitive to ciprofloxacin, nalidixic acid, oxolinic acid, amikacin, ofloxacin, flumequine, and nitrofurantoin, but resistant to tetracycline, lincomycin, gentamicin, amoxicillin/clavulanic acid, erythromycin, trimethoprim/sulfamethoxazole, and oxytetracycline. Genetic characterization confirmed the presence of aerolysin genes and resistance genes blaTEM and tetA, while hemolysin, ermB, and mcr1 genes were absent. These findings highlight the need for effective monitoring and management strategies to control the *A. hydrophila* pathogen in aquaculture.

## INTRODUCTION

In recent years, Egypt's aquaculture sector has grown substantially, reaching a production value of approximately $1.2 billion as of 2020, with the Nile tilapia being the largest contributor **(FAO, 2020; Samaddar *et al.*, 2024)**. The Nile Delta region,

including Dakahlia Governorate, provides an ideal environment for tilapia farming due to abundant water and favorable climate **(Munguti *et al.*, 2022)**.

*A. hydrophila* is an opportunistic pathogen causing various infections in fish, such as septicemia, ulcerative diseases, and fin rot. Moreover, it is a Gram-negative, rod-shaped bacterium widely distributed in all aquatic environments **(Janda & Abbott, 2010; Sherif & Kassab, 2023)**. Infections caused by *A. hydrophila* in the Nile tilapia can lead to high mortality rates, which in turn affect the productivity and economic viability of aquaculture operations **(Anantasuk *et al*., 2024)**.

The pathogenicity of *A. hydrophila* is attributed to virulence factors as siderophores **(Mateos *et al.*, 1993; Anantasuk *et al*., 2024)**, adhesins **(Sen & Rodgers, 2004)**, aerolysin (aerA) **(Chopra *et al*., 2009)**, enterotoxins (alt) **(Janda & Abbott, 2010; Lu *et a*l., 2024)**, and hemolysins (hlyA) **(Beaz-Hidalgo & Figueras, 2013)**.

Antibiotic resistance is a growing concern in aquaculture, primarily due to the overuse and misuse of antibiotics. *A. hydrophila* demonstrated resistance to multiple antibiotics, complicating the treatment of infections in fish. Several studies summarized high levels of resistance to commonly used antibiotics, such as tetracycline, ampicillin, and chloramphenicol **(Ayoub *et al*., 2024)**. Additionally, mutations in target genes can reduce the efficacy of these antibiotics, rendering them ineffective **(Peng *et al*., 2024)**. The mechanisms of antibiotic resistance in *A. hydrophila* include the production of beta-lactamases, which degrade beta-lactam antibiotics, and the presence of efflux pumps that expel antibiotics from the bacterial cell **(Peng *et al*., 2024)**.

In this context, the current study aimed to isolate and identify *Aeromonas hydrophila* from natural populations of *O. niloticus* along the River Nile in Dakahlia Governorate. Additionally, the study sought to detect virulent genes and antimicrobial resistance genes of *A. hydrophila*.

## MATERIALS AND METHODS

### 1. Fish sampling

A total of 150 moribund live and freshly dead tilapia (*Oreochromis niloticus*), with average body weight of 80 ± 10g and length of 20 ± 1.5cm were collected randomly from the Nile River in Dakahlia Governorate, Egypt, during various seasons from March 2022 to February 2023. The samples were then transferred to the laboratory in Fish Diseases Research Department at the Animal Health Research Institute in Al-Dokki, Cairo, Egypt. All specimens collected have been checked for abnormal symptoms, and the pathological findings were evaluated following the guidelines described by **Schaperklaus *et al*. (1992)**.

## 2. Bacteriological assay

Fish samples were subjected to bacteriological examination under sterile conditions. Loopful samples from various organs of each fish, including kidney, liver, gills, spleen, were directly inoculated onto tryptic soy agar (TSA) and *Aeromonas* selective agar base (Titan Media, India) with an ampicillin supplement of 2.5mg per 500ml of diluted media and incubated at 37°C for 24 hours. The suspected colonies, subjected for further examination based on cultural characteristics, were identified phenotypically by the Gram- staining reaction and biochemical tests (Gram- stain microscopy, motility testing, Voges-Proskauer, citrate utilization, urease, oxidase, catalase, indole, methyl red, H2S production and sugar fermentation tests). All tested colonies were identified using Diagnostics SRO GN24 (www.diagnostics.sk), following the manner described by **Austin and Austin (2016)**.

## 3. Antibiogram of *A. hydrophila*

Antibiotic susceptibility of the retrieved bacterial isolates was determined using the disk diffusion method according to **Bauer *et al*. (1966)** on Mueller-Hinton agar. Seventeen different antibiotic discs (Oxoid, Basingstoke, UK) were selected to cover the different antibiotic groups used in the aquaculture as follows: lincomycin (MY; 10µg), ciprofloxacin (CIP; 5µg), gentamycin (CN; 10µg), colistin sulphate (CT; 25µg), flumequine (UB; 30µg), erythromycin (E; 15µg), nitrofurantoin (F; 300µg), amoxycillin (AML; 10µg), nalidixic acid (NA; 30µg), amoxicillin / clavulanic acid 20 /10 (AMC; 30µg), oxalinic acid (OA; 2µg), oflicin (OFX; 10µg), tetracycline (TE; 30µg), trimethoprim-sulphamethoxazol (SXT; 25µg), ampicillin (AMP; 10µg), amikacin (AK; 30µg), oxytetracycline (T; 30µg). The bacterial growth inhibition zones were scored, and the results were interpreted as susceptible (S), intermediate (I), or resistant (R) according to **CLSI (2024)**.

## 4. Molecular approach and sequence analysis of *A. hydrophila* and virulence genes

*A. hydrophila* isolates were confirmed by detecting the 16S rRNA gene and examining the presence of virulence genes, specifically the Aerolysin (aero) and Hemolysin (hyl) genes, using oligonucleotide primers (Metabion, Germany) through conventional PCR (Table 1). Briefly, the isolates were inoculated into Brain Heart Infusion (BHI) broth (Oxoid) and incubated for 24 hours. Genomic DNA was extracted from the bacterial culture using the PathoGene-spin™ DNA extraction kit, following the manufacturer's instructions; PCR reactions were conducted according to the protocols described in Table (1).

The PCR products, along with a 100 bp DNA ladder, were loaded onto an agarose gel and subjected to electrophoresis to separate the DNA fragments based on size. The gel

was then visualized to analyze the presence or absence of specific PCR products corresponding to the target genes.

**5. Molecular identification of antibiotic-resistant genes in *A. hydrophila* strains**

PCR amplification was performed using specific prefixes targeting *bla*$_{TEM}$, *tet*A, *erm*B, and *mcr1* genes. PCR reaction mixtures were prepared using DNA extracted from the bacterial strain and suitable primers (Table 1). This step aimed to selectively amplify areas of interest within bacterial DNA.

**6. Sequencing and phylogenetic analysis**

PCR products of *A. hydrophila* 16srRNA were purified using the QIAquick PCR Product extraction kit (Qiagen, Valencia). Bigdye Terminator V3.1 cycle sequencing kit (PerkinElmer) was used for the sequence reaction. DNA sequences were obtained by Applied Biosystems3130 genetic analyzer (HITACHI, Japan), and 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 done using neighbor joining in MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms **(Kumar *et al.*, 2018)**.

**7. Statistical analysis**

Statistical analysis of the event data by calculation of the ratio was made using the SPSS (Statistical Set for Social Science) Statistics 17.0 software program.

**Table 1.** The cyclic PCR conditions and specific primers the for *A. hydrophila*

| Gene | Primer sequence (5'-3') | Amplified product (bp) | Primary denaturation | Amplification (35 cycles) | | | Final extension | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | | Secondary denaturation | Annealing | Extension | | |
| *16srRNA* | F: GGGAGTGCCTTCGGGAATCAGA<br>R: TCACCGCAACATTCTGATTTG | | 95°C / 5 min | 94°C / 30 s | 55°C / 30 s | 72°C /30 s | 72°C / 5 min. | **Wang *et al.*, (2003)** |
| *aero* | F: CACAGCCAATATGTCGGTGAAG<br>R: GTCACCTTCTCGCTCAGGC | 326 bp | 95˚C / 5 min | 94˚C / 30 s. | 52˚C/ 30 s. | 72˚C/ 30 s | 72˚C/ 5 min | **Singh *et al.*, (2008)** |
| *hly* | F:GGCCGGTGGCCCGAAGATACGG G<br>R:GGCGGCGCCGGACGAGACGGGG | 592 bp | 95°C / 5 min | 94°C / 30s. | 55°C/ 30 s. | 72°C / 30 s | 72°C / 5 min | **Lee *et al.*, (2023)** |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| $bla_{TEM}$ | F: ATCAGCAATAAACCAGC<br>R: CCCCGAAGAACGTTTTC | 516 bp | 94°C / 5 min | 94°C / 30 s | 54°C/ 40 s. | 72°C / 45 s | 72°C / 10 min | **Colom *et al.*,(2003)** |
| *tetA* | F: GGTTCACTCGAACGACGTCA<br>R: CTGTCCGACAAGTTGCATGA | 576 bp | 94°C / 5 min | 94°C / 30 s | 50°C/ 40 s. | 72°C / 45 s | 72°C / 10 min | **Randall *et al.*, (2004)** |
| *ermB* | F: GAAAAAGTACTCAACCAAAT A<br>R: AATTTAAGTACCGTTACT | 639 bp | 94°C / 5 min | 94°C / 30 s | 45°C/ 40 s. | 72°C / 45 s | 72°C / 10 min | **Nguyen *et al.* (2009)** |
| *mcr1* | F: CGGTCAGTCCGTTTGTTC<br>R: CTTGGTCGGTCTGTAGGG | 308 bp | 94°C / 5 min | 94°C / 30 s | 60°C/ 40 s. | 72°C / 45 s | 72°C / 10 min | **Newton Foot *et al.*, 2017** |

## RESULTS

### Clinical and postmortem observations

The fish displayed various severe symptoms, including extensive surface hemorrhage, erosions, loss of scales, erosion of fins and tail. Upon clinical examination, it was evident that the fish were diseased and exhibited signs consistent with hemorrhagic septicemia. These signs included hemorrhage at the base of the fin, and some samples even showed exophthalmia and eye cataract. Additionally, there were instances of skin discoloration (Fig. 1A, B). During the postmortem examination, further abnormalities were observed. Internal organs showed signs of deterioration, an enlargement of gall bladder, pale liver and pigmentation on surface of the liver (Fig. 1C, D) were observed. These findings indicate the severity and extent of the infection or disease affecting the fish.

### Prevalence of *Aeromonas* species among of *O. niloticus*

The *A. hydrophila* isolates were confirmed for 16S rRNA gene by PCR giving a specific band at 356bp. The prevalence of *Aeromonas* species in diseased *O. niloticus* fish was screened from the River Nile in Dakahlia Governorate as 68% (102/150). Out of 102 samples, *A. hydrophila* was observed in 56% (57/102) samples, including 31.6% (18/57) of the kidneys, 28.1% (16/57) liver, 15.8% (9/57) spleen, 14% (8/57) intestine, and 10.5% (6/57) gills. The genetic characterization of virulence genes showed that all *A. hydrophila* isolates were positive for *aero* gene at 326bp, while were negative for *hly* gene (Fig. 2).

## Antibiogram of *A. hydrophila* strains

Antimicrobial susceptibility testing was performed on all recovered isolates of *A. hydrophila*. The results indicated marked resistance to several antibiotics, with the tested isolates showing the following resistance rates: amoxicillin/clavulanic acid (73.7%), trimethoprim-sulfamethoxazole “63.2%”, amoxicillin “54.4%”, lincomycin “54.4%”, ampicillin “52.6%”, erythromycin “49.1%”, gentamicin “47.4%”, oxytetracycline “47.3%”, and tetracycline “43.9%”. In contrast, the isolates exhibited sensitivity to ciprofloxacin “82.5%”, ofloxacin “52.6%”, amikacin “50.8%”, flumequine “47.4%”, nalidixic acid “45.6%”, nitrofurantoin “45.6%”, and oxolinic acid “42.1%” (Table 2). Molecular detection of antibiotic-resistant genes in *A. hydrophila* strains revealed the presence of the blaTEM and tetA genes, while the ermB and mcr1 genes were absent (Fig. 2).

## Sequence analysis of *A. hydrophila* 16S gene

PCR product of 16S rRNA for one representative strain of our collection were purified and sequenced and sent for the gene bank data base under the accession no. PP829284.1. The resulted sequence isolate showed high nucleotide identity with other *A. hydrophila* Egyptian strains isolated from tilapia (OQ687115.1, OQ625314.1, OQ625313.1, and OQ625312.1. The phylogenetic tree for the obtained sequence was constructed (Fig. 3).

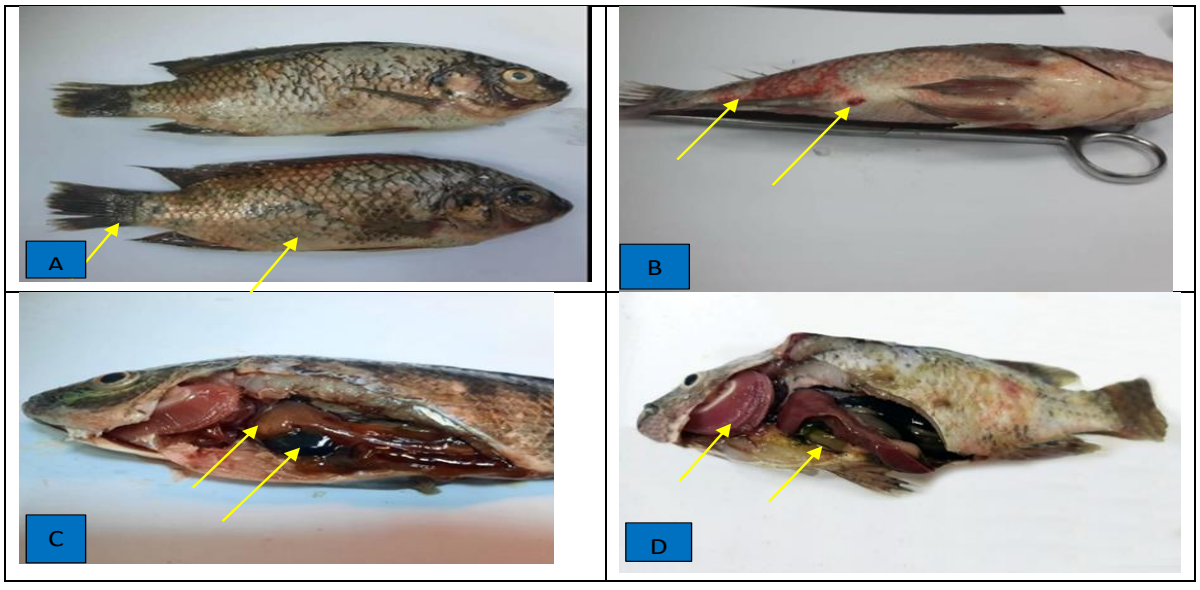


**Fig. 1.** Clinical and postmortem observations of hemorrhagic septicemia caused by *Aeromonas hydrophila*: **(A)** *O. niloticus* shows skin discoloration, desquamation of scales and erosion of tail. **(B)** Natural infected *O. niloticus* shows eye cataract, hemorrhage around anal opening and skin discoloration. **(C)** Natural infected *O. niloticus* shows enlargement of gall bladder and pigmentation on surface of the liver. **(D)** Natural infected *O. niloticus* shows enlargement of gall bladder and pale gills

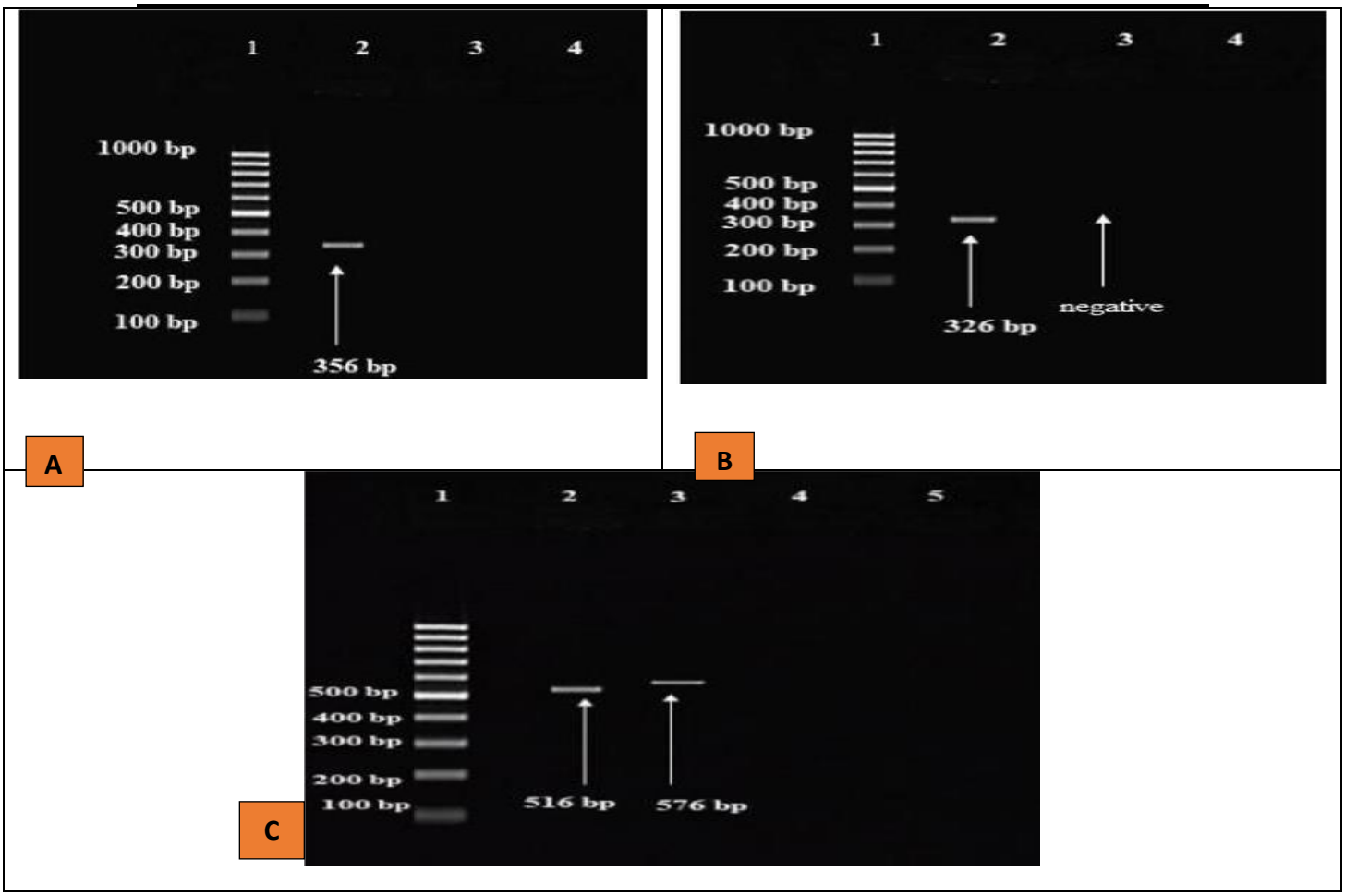


**Fig. 2.** Agarose gel electrophoresis showing amplification of *A. hydrophila*; **A)** 16srRNA gene *at* 356bp. Lane 1: 1 00bp Ladder, Lane 2: Positive. **B)** Virulence genes (*aero* at 326bp and *hly at* 592bp). Lane 1: 100bp Ladder, Lane 2: Positive for *aero* gene, Lane 3: negative for *hly* gene. **C)** Antibiotic resistance genes. Lane (1): 100bp DNA Ladder, Lanes (2): positive for *blaTEM* gene, Lanes (3): positive for *TetA* gene, Lanes (4, 5): negative for *ErmB* and *mcr1* genes, respectively

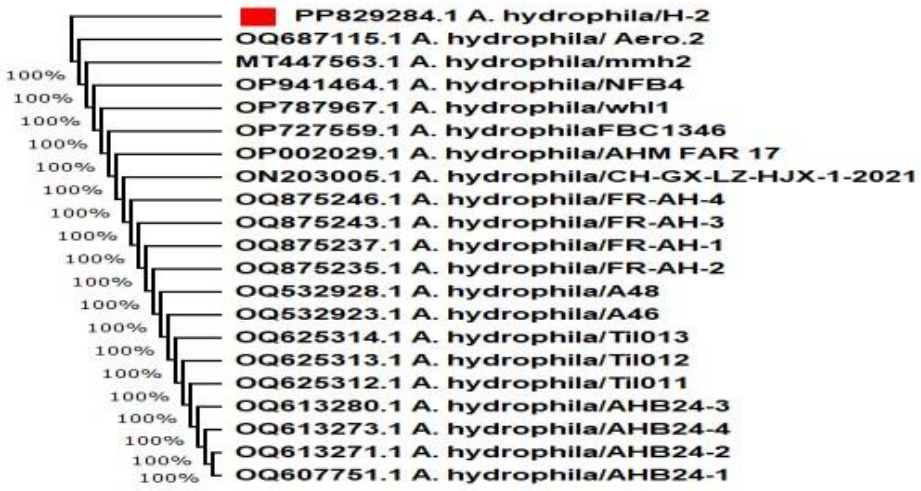


**Fig. 3.** The phylogenetic tree of *A. hydrophila* 16s gene red triangle: this research isolate

**Table 2.** Antibiotic sensitivity testing of *A. hydrophila* isolates

| Item | Antimicrobial class | No.(%) | | |
|---|---|---|---|---|
| | | Sensitive | Intermediate | Resistant |
| Amoxicillin / Clavulanic acid | Penicillin Combination with Beta-lactamase inhibitor | 6 (10.5%) | 9 (15.8%) | 42 (73.7%) |
| Trimethoprim Sulphamethoxazol | Folate Pathway Inhibitors | 7 (12.3%) | 14 (24.5%) | 36 (63.2%) |
| Amoxicillin | Penicillins | 9 (15.8%) | 17 (29.8%) | 31 (54.4%) |
| Lincomycin | Lincosamides | 11 (19.3%) | 15 (26.3%) | 31 (54.4%) |
| Ampicillin | Penicillins | 9 (15.8%) | 18 (31.6%) | 30 (52.6%) |
| Erythromycin | Macrolides | 13 (22.8%) | 16 (28.1%) | 28 (49.1%) |
| Gentamycin | Aminoglycosides | 20 (35.1%) | 10 (17.5%) | 27 (47.4%) |
| Oxytetracyclin | Tetracyclines | 14 (24.6%) | 16 (28.1%) | 27 (47.3%) |
| Tetracycline | Tetracyclines | 15 (26.3%) | 17 (29.8%) | 25 (43.9%) |
| Oxalinic acid | Quinolones | 24 (42.1%) | 20 (35.1%) | 13 (22.8%) |
| Nitrofurantoin | Nitrofurans | 26 (45.6%) | 19 (33.3%) | 12 (21.1% |

| | | | | |
|---|---|---|---|---|
| Flumequine | Fluoroquinolone | 27 (47.4%) | 20 (35.1%) | 10 (17.5%) |
| Colistin sulphat | Lipopeptides | 21 (36.8%) | 26 (45.7%) | 10 (17.5%) |
| Oflicin | Quinolones | 30 (52.6%) | 18 (31.6%) | 9 (15.8%) |
| Nalidixic acid | Quinolones | 26 (45.6%) | 23 (40.4%) | 8 (14%) |
| Ciprocin Ciprofloxacin | Fluoroquinolone | 47 (82.5%) | 3 (5.3%) | 7 (12.2%) |
| Amikacin | Aminoglycosides | 29 (50.8%) | 23 (40.4%) | 5 (8.8%) |

## DISCUSSION

In this current research, infected *O. niloticus* exhibited external signs consistent with hemorrhagic septicemia such as extensive surface hemorrhage, erosions, loss of scales, erosion of fins and tail, and some samples even showed exophthalmia and eye cataract and skin discoloration **(Austin & Adams, 1996; Thomas *et al*., 2013; El-Bahar *et al*., 2019; Hamouda *et al*., 2019; Abdelsalam *et al*., 2021; Bakiyev *et al*., 2022; Farouk *et al*., 2023)**. The postmortem examination displayed signs of deterioration; there was an enlargement in the gall bladder, in addition to a pale liver and pigmentation detected on the surface of the liver, as reported by **Austin and Austin (2016)**, **Aboyadak *et al*. (2017)** and **Hamouda *et al*. (2019)**.

In the current study, the bacteriological assay confirmed the presence of *A. hydrophila*, Gram-negative bacteria infected *O. niloticus*. The colonies observed on TSA medium displayed characteristics of being round, convex, and had a white, creamy, and opaque appearance. On RS medium, the colonies ranged from deep creamy to light yellow with smooth edges. While, MacConkey's agar medium produced pale colonies, and on TCBS medium turned yellow. In TSI tests, *A. hydrophila* exhibited acid production without $H_2S$ generation. It tested negative for urease and positive for oxidase, catalase, and Voges Proskauer, aligning with the report of **Aboyadak *et al*. (2017)**, **Ayoub *et al*. (2021)** and **Mansour and El-Shaer (2023)**.

Antibiotics have a significant role in managing diseases in both animals and humans, but their use can lead to bacterial resistance. Bacteria's capacity to withstand a broad spectrum of antibiotics can lead to the development of additional virulence traits. The global issue of Multiple Antibiotic Resistance (MAR) in *A. hydrophila* arises from the inappropriate use of antibiotics **(Ahmed *et al*., 2018)**. Quinolones are proven to be highly efficient antimicrobial agents when combating Gram-negative bacterial infections. Both ofloxacin and oxalinic acid have received approval for treating MAS and columnaris in aquatic animals **(Thaotumpitak *et al*., 2023)**. The current findings, based

on the examination of 150 wild *O. niloticus* specimens, revealed a 20% prevalence of *A. hydrophila*. It was observed that *A. hydrophila* displayed sgensitivity to ciprofloxacin, nitrofurantoin, oxalinic acid and ofloxacin whereas it exhibited high resistance to penicillin and tetracycline. These results are in close alignment with findings from previous research **(Nasser *et al*., 2022; Thaotumpitak *et al*., 2023)**.

The insight analysis of the achieved antibiogram of *A. hydrophila* isolates revealed significant variation in their responses to different antibiotics. The isolates exhibited notable sensitivity to ofloxacin, ciprofloxacin, oxalinic acid, nitrofurantoin, flumequine, amikacin, and nalidixic acid, with inhibition zones measuring 22, 26, 21, 22, 25, 18, and 20mm, respectively **(Laith *et al*., 2014)**.

Conversely, the isolates demonstrated resistance to several commonly used antibiotics, including tetracycline, lincomycin, gentamicin, amoxicillin, amoxicillin/clavulanic acid, erythromycin, trimethoprim/sulfamethoxazole, ampicillin, and oxytetracycline, with no inhibition zones observed **(Sarder *et al*., 2016)**. This resistance pattern may be owing to the mutations of the bacterial genome and virulence; especially given the widespread use of these antibiotics; however, this issue needs further investigation to be confirmed.

Genetic characterization of the *A. hydrophila* isolates confirmed the presence of the aerolysin gene (aero), a known virulence factor, while the hemolysin gene (*hly*) was absent. The presence of the aerolysin gene indicates that the isolates have the potential to exert significant pathogenic effects in infected fish. Moreover, the isolates possessed the resistance genes $bla_{\text{TEM}}$ and tetA, indicating resistance to beta-lactam and tetracycline antibiotics, respectively. The absence of the *ermB* and *mcr1* genes suggests that the isolates may still be susceptible to macrolide and colistin antibiotics, which could be considered in treatment plans **(Zhang *et al*., 2000)**.

Analysis of the 16S ribosomal RNA gene sequence of an *A. hydrophila* isolate, submitted to GenBank under accession number PP829284.1, showed high nucleotide identity with other isolates from Egypt. This genetic similarity suggests the presence of a common strain prevalent in the region, possibly due to environmental factors or aquaculture practices that facilitate the spread of this pathogen **(Yuan *et al*., 2021)**.

In this study, the 16S rRNA gene sequence was utilized to identify *A. hydrophila*, revealing a characteristic homology at 356 base pairs.] These findings align with those reported by **Hamouda *et al*. (2019)** and **Umutoni *et al*. (2020)**.

The detection of the $bla_{\text{TEM}}$ gene indicates that the bacterial strain harbors a specific beta-lactamase gene. Beta-lactamase enzymes are known to confer resistance to beta-lactam antibiotics, such as penicillin and cephalosporin. The presence of this gene suggests that the strain possesses the ability to produce beta-lactamase enzymes, which

can hydrolyze and inactivate beta-lactam antibiotics, ultimately leading to antibiotic resistance.

A positive result for the *TetA* gene indicates that the bacterial strain possesses a tetracycline resistance determinant. The *TetA* gene is linked to resistance to tetracycline antibiotics, which are commonly used to treat a wide range of bacterial infections. The presence of the *TetA* gene indicates that the strain has acquired genetic elements that enable it to resist the effects of tetracycline antibiotics; this result agrees with that reported by **El- Bahar *et al*. (2019)** and **Mansour and El-Shaer (2023)**.

The absence of the *ErmB* gene in the strain, as indicated by the negative result in lane (4), indicates that the strain does not contain this specific macrolide resistance gene. Macrolide antibiotics are a class of antibiotics that includes erythromycin, and genes such as *ErmB* can mediate resistance to these antibiotics. The absence of the *ErmB* gene suggests that the strain may be sensitive to macrolide antibiotics that target protein synthesis in bacteria.

A negative result for the *mcr1* gene in line (5) indicates that the strain does not carry this colistin resistance gene. Colistin is an antibiotic used as a last resort to treat multidrug-resistant Gram-negative bacterial infections. The absence of the *mcr1* gene indicates that the strain may remain susceptible to the effects of colistin and related antibiotics, which is important information for understanding its antibiotic susceptibility profile.

The results of this study have significant implications for aquaculture in Egypt. The high prevalence of antimicrobial-resistant *A. hydrophila* strains highlights the urgent need for strict monitoring and management strategies to prevent the spread of resistant pathogens. Identifying effective antibiotics, such as ciprofloxacin and nalidixic acid, offers valuable insights for developing appropriate treatment protocols **(Sarder *et al*., 2016)**. However, the presence of multidrug resistance highlights the need for alternative approaches, such as the use of probiotics, phage therapy, or improved biosecurity measures, to manage bacterial infections in aquaculture. Future research should focus on exploring the mechanisms of resistance in *A. hydrophila* and on identifying new therapeutic agents or strategies to combat infections. Additionally, studies on the environmental factors contributing to the spread of resistant strains and the impact of aquaculture practices on pathogen dynamics would provide a comprehensive understanding of the challenges in managing *A. hydrophila* infections.

## CONCLUSION

The research showed that the Nile tilapia isolates exhibited notable sensitivity to ciprofloxacin, nalidixic acid, oxalinic acid, amikacin, oflicin, flumequine and

nitrofurantoin, while demonstrating resistance to tetracycline, lincomycin, gentamicin, amoxicillin, amoxicillin/clavulanic acid, erythromycin, trimethoprim/sulfamethoxazole, ampicillin, and oxytetracycline. Additionally, genetic characterization confirmed the presence of the *aerolysin* gene and resistance genes *bla*TEM and *tet*A, while the hemolysin, *erm*B, and *mcr*1 genes were absent. The 16S rRNA gene sequence analysis of the isolate revealed a high nucleotide identity with other isolates from Egypt. These findings have significant implications for aquaculture in the region, highlighting the need for effective monitoring and management strategies to control *A. hydrophila* infections in the Nile tilapia. Identifying effective antibiotics, such as ciprofloxacin and nalidixic acid, provides valuable information for developing treatment protocols. However, the presence of multidrug resistance underscores the need for alternative approaches, such as the use of probiotics, phage therapy, or improved biosecurity measures, to manage bacterial infections in aquaculture. The research contributes to understanding the dynamics of *A. hydrophila* in the Nile tilapia farming, emphasizing the importance of continuous monitoring and innovative treatment methods to ensure the sustainability of aquaculture in Dakahlia Governorate and beyond.

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