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# Antibacterial-Resistant Fish-borne *Aeromonas hydrophila*: Prevalence and Virulence Characteristics

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## ABSTRACT

The goal of this investigation was to examine the occurrence and virulence features of the antibiotic-resistant fish-borne *Aeromonas hydrophila* that were isolated from fish sold in Aswan City, Egypt. A total of 125 fish samples (25 of each:the mullet, the saurus, the catfish, the Nile perch, and the Nile tilapia) were examined. The mean *Aeromonas* counts in the examined samples varied from 3.89± 0.35 logCFU/g in the Nile tilapia to 2.68± 0.13 log CFU/g in the Nile perch. 35.2% of the inspected samples had *Aeromonas* spp. and *A.hydrophila* as the majority, followed by*A. sobria* (11%), *A. caviae* (4%), and *A.veronii* (3.2%). Based on the species-specific *16S* rRNA gene, it was found that 16 isolates were confirmed as *A. hydrophila* and possessed 62.5, 25, and 37.5% of the virulence genes for *aerA, ahh1*, and *altA*, respectively. Additionally, *A. hydrophila* exhibits varying levels of resistance to the most extensively verified antibacterial substances. Therefore, it is recommended that hygienic techniques be used upon handling, preparing, processing, and storing fish to improve its quality and reduce microbiological contamination with *A.hydrophila*. Additionally, it is crucial to motivate researchers to pursue non-antibiotic control strategies for this type of pathogen and other bacterial infections in farmed fish.

## INTRODUCTION

Fish and their products provide easily digestible high-quality proteins, health-promoting polyunsaturated fatty acids, and other vital components of human nutrition, such as minerals and vitamins **(Khairy *et al.*, 2024)**. The shelf life of fresh fish is restricted by the rapid degradation of its quality during the handling and storage of fish products **(Hafez *et al.*, 2018)**. Exogenous issues, including contaminated waters, post-

capture pollution **(FAO, 2014)**, and unsanitary storing and management of fish, can accelerate the decomposition of fish by introducing pathogenic and putrefactive bacteria, thereby posing a healthiness hazard during food handling **(Morshdy *et al.*, 2022)**. Fish may potentially be a cause of foodborne *Aeromonas* species, which have been identified as emerging foodborne bacteria causing a severe hazard to public fitness **(Igbinosa *et al.*, 2012)**.

According to **Batra *et al.* (2016)**, *Aeromonas* species are related to food poisoning and many human sicknesses, including extra-intestinal and gastrointestinal infections, skin and soft tissue diseases, traumatic diseases, and respiratory-urinary tract diseases. The most frequently implicated *Aeromonas* spp. in human gastrointestinal disorders are *A. hydrophila*, *A. caviae*, and *A. sobria* **(Stratev*et al.*, 2012)**. One of the most significant zoonotic gastrointestinal pathogens is *A. hydrophila* **(Salunke*et al.*, 2015)**. *A. hydrophila* is considered pathogenic owing to the release of multiple virulent agents, comprising aerolysin, exotoxin, cytotoxic, and hemolytic activities. These factors cause mucosal adhesion and colonization, which may lead to fluid accumulation or epithelial changes, and in turn may cause human disease **(Kishk*et al.*, 2020)**.

The prevalent use of antibacterial in aquaculture to combat *A. hydrophila* contagion is thought to be the primary source of antibacterial resistance and the delivery of multidrug-resistant bacteria **(Yildirim-Aksoy& Beck, 2017)**. There may be a human health threat through the dispersal of antibiotic-resistant microbes to customers through foodstuffs or by the transmission of resistance agents **(Binh*et al.*, 2018)**. Hence, the objectives of the existing investigation were to ascertain the occurrence of *Aeromonas* species, and virulent-associated genes in the recognized *A. hydrophila*, in addition to examining the antibacterial resistance profiles of *A. hydrophila* from fish sold in Aswan City, Egypt.

## MATERIALS AND METHODS

### Samples collection

Approximately 125 fish samples, 25 of each:the mullet (*Mugil cephalus*), the saurus (*Elops saurus*), the catfish (*Clarias gariepinus*), the Nile perch (*Lates niloticus*), and the Nile tilapia (*Oreochromis niloticus*), weighing 180-220g, were obtained from several fish markets in Aswan City, Egypt, in 2023. The samples were immediately sent to the lab for bacteriological investigation in a refrigerated container after being sealed in sterile flexible bags.

### Processing of fish samples

The ventral, pectoral, and dorsal fins of every fish sample were detached using sterilized shears and pincers. Under aseptic conditions, the surface was cut off with sterile scissors and forceps, and 25g of flesh was aseptically conveyed into a sterilized tube

containing 225ml of peptone water 1%. The mixture was standardized at 14000rpm for 3min and allowed to remain for 5min, as per **APHA (1992)**.

### *Aeromonas* count

A standard 25g of fish muscle was incubated for 24h at 37°C in 225mL of sterile alkaline peptone water (APW, Micro Master - India). A tenfold sequential dilution was established by transferring one milliliter to a single tube containing 9ml of peptone water 0.1% **(Elbarbary *et al.*, 2023)**. Duplicate sterile Petri plates of *Aeromonas* isolation Medium Base (HiMedia-M884) complemented with ampicillin were incubated at 37°C for 24h with 1mL of each serial dilution. Using a digital colony counter (DC-8 OSK 100086, Kayagaki, Japan), the number of pale green, translucent colonies with a darker core on plates ranging from 30 to 300 was calculated. The output was recorded as log CFU/g **(Carnahan & Joseph, 2015)**.

### *Aeromonas* isolation and identification

One ml of the ready homogenate was mixed in a sterile check tube containing 9ml of enrichment broth known as brain heart infusion broth (BHI), which was incubated at 28°C for 24h. The enrichment broth loopful was distributed onto *Aeromonas* agar and maintained at 37°C for 24h **(Elbarbary*et al.*, 2024)**. Pale green, transparent colonies with darker cores and diameters ranging from 0.5 to 3.0mm were identified and biochemically characterized **(Macfaddin, 2000)**.

### Morphological and biochemical identification

The isolated *Aeromonas* were morphologically recognized through Gram staining and motility and also biochemically identified according to the outlines of **Macfaddin (2000)**.

### Molecular identification

According to the instructions provided by the vendor, DNA was taken out by QIAamp DNA Gene$^{JET}$Genomic DNA Purification Kit (Catalog No. #K0721, Thermo Scientific, USA). *A. hydrophila* isolates that were biochemically recognized in the existing investigation were exposed to molecular identification, and their virulence genes detection using primer sequences is shown in Table (A). The 25µl of PCR reaction has 12.5µl of *COSMO* PCR RED Master Mix (2x premix), 4.5µl PCR grade water, 1µl of each forward and reverse primer (20pmol), and 6µl template DNA. Electrophoresis in 1.5% agarose gel was used to identify the amplified yields (Applichem, Germany, GmbH). A gel recording system (Alpha Innotech, Biomedia) was used for photography, and computer software was used for analysis.

### Antimicrobial susceptibility test

Following the methodology of **Elbarbary and Abdelmotilib (2023)**, a straightforward disk diffusion method was utilized to investigate the spreading of

antibacterial resistance. The isolates of *A. hydrophila* were cultured in Tryptone Soya Broth (HiMedia) for 24h at 37°C. Subsequent the preparation of 0.5 McFarland dilutions, isolates were grown on Mueller-Hinton agar (Hi-Media) in the occurrence of various antibacterial discs (g/disk): cefoxitin (10), ciprofloxacin (5), cephalothin (30), norfloxacin (10), meropenem (30), imipenem (30), ampicillin (10), tetracycline (30), penicillin (10), amoxicillin (25), amikacin (30), chloramphenicol (30), streptomycin (10), sulfamethoxazole (25), and gentamicin (10) (Oxoid, UK). For 24 hours, cultures were maintained at 37°C. Following **CLSI (2018)**, the diameter of the halo surrounding the developing inhibition was measured to quantify the resistance pattern, and the observed zone was interpreted. The multiple antibiotic resistance (MAR) index for each strain was calculated using the formula specified by **Singh *et al.* (2010)** as follows: The MAR index was calculated as the number of resistances (Isolates classed as intermediate were considered sensitive for MAR index) divided by the total number of antibiotics tested.

**Table A.** Primer sequences for *A. hydrophila*

| Gene | Primer sequence (5'→3') | Bp | Reference |
|---|---|---|---|
| *16S* rRNA | CAC AGC CAA TAT GTC GGT GAA G<br>GTC ACC TTC TCG CTC AGG C | 625 | El-Hossary*et al.*(2023) |
| *aerA* | CAAGAACAAGTTCAAGTGGCCA<br>ACGAAGGTGTGGTTCCAGT | 309 | Wang *et al*. (2003) |
| *ahh1* | GCCGAGCGCCCAGAAGGTGAGTT<br>GAGCGGCTGGATGCGGTTGT | 130 | Strate*et al*. (2016) |
| *altA* | TCAACA CCATCACCGACGT<br>ATCGAACTTGAACAG GGCA | 425 | Venkataiah*et al.* (2013) |

**Data analysis**

One-way analysis of difference was utilized to evaluate all statistics (ANOVA). Every value was shown as means ± S.E. When $P< 0.05$, a significant variance was considered.

## RESULTS

The mean *Aeromonas* count (log CFU/g) in the inspected fish ranged from 3.89±0.35 in the Nile tilapia to 2.68±0.13 in the Nile perch (Table 1). Bacterial inspection of fish exposed that 35.2% of samples had *Aeromonas* spp. depending on the colonial properties of *Aeromonas*agar and biochemical characterization (Table 2).

The highest isolation percentage was in the mullet and the Nile tilapia (44%), and the lowest rate was in the Nile perch (24%) (Table 3). The major *Aeromonas* spp.

identified were *A. hydrophila* (19.2%), followed by *A. sobria* (11%), *A. caviae* (4%), and *A. veronii* (3.2%) (Table 3).

**Table 1.** The mean values of *Aeromonas* species count (log CFU/g)

| Fish species | Minimum | Maximum | Mean ± SE |
|---|---|---|---|
| Mullet | 1.87 | 4.52 | $3.54\pm 0.33^{a}$ |
| Saurus | 2.08 | 4.19 | $3.04\pm 0.63^{b}$ |
| Catfish | 2.27 | 4.84 | $3.22\pm 0.27^{b}$ |
| Nile perch | 1.65 | 3.76 | $2.68\pm 0.13^{c}$ |
| Nile tilapia | 2.37 | 4.49 | $3.89\pm 0.35^{a}$ |

Mean values within the same column with different superscript letters are significantly different ($P< 0.05$).

**Table 2.** Biochemical properties of identified *Aeromonas* species

| Test | *A. hydrophila* | *A. sobria* | *A. Caviae* | *A. veronii* |
|---|---|---|---|---|
| Motility | + | + | + | + |
| Indole | + | + | +/- | + |
| Esculine hydrolysis | + | - | - | - |
| Arginine dihydrolase | + | + | + | + |
| Oxidase | + | + | + | + |
| Methyle red | +/- | - | +/- | +/- |
| VogesProskuaer | +/- | +/- | - | - |
| Citrate utilization | +/- | + | +/- | + |
| L- lysine decarboxylase | + | + | - | + |
| Hydrogen sulphide | + | + | - | +/- |
| Nitrate reduction | + | +/- | - | +/- |
| Urease | - | - | - | - |
| Ornithine decarboxylase | - | - | - | - |
| Gelatin liquefaction | + | + | + | + |
| arginine decarboxylase | + | +/- | +/- | + |
| β- galactosidase | + | + | + | + |
| Rhamnose fermentation | + | - | - | - |
| Mannose fermentation | + | + | +/- | +/- |
| Sucrose fermentation | + | + | + | + |
| Glucose fermentation | + | + | + | + |
| Arabinose fermentation | +/- | - | + | +/- |
| Inositol fermentation | - | - | +/- | - |
| Sorbitol fermentation | +/- | - | - | - |

* Positive: (+) * Negative: (-) * Variable: (+/-).

**Table 3.** Prevalence of *Aeromonas* species in the examined fish (n=25 each)

| Sample | Positive samples | | *Aeromonas* species | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | *A. hydrophila* | | *A. sobria* | | *A. veronii* | | *A. caviae* | |
| | No. | % | No. | % | No. | % | No. | % | No. | % |
| Mullet | 11 | 44 | 7 | 38 | 1 | 4 | 2 | 8 | 1 | 4 |
| Saurus | 7 | 28 | 4 | 16 | 3 | 12 | 0 | 0 | 0 | 0 |
| Catfish | 9 | 36 | 3 | 12 | 4 | 16 | 0 | 0 | 2 | 8 |
| Nile perch | 6 | 24 | 2 | 8 | 2 | 8 | 2 | 8 | 0 | 0 |
| Nile tilapia | 11 | 44 | 8 | 32 | 1 | 4 | 0 | 0 | 2 | 8 |
| Total | 44 | 35.2 | 24 | 19.2 | 11 | 8.8 | 4 | 3.2 | 5 | 4 |

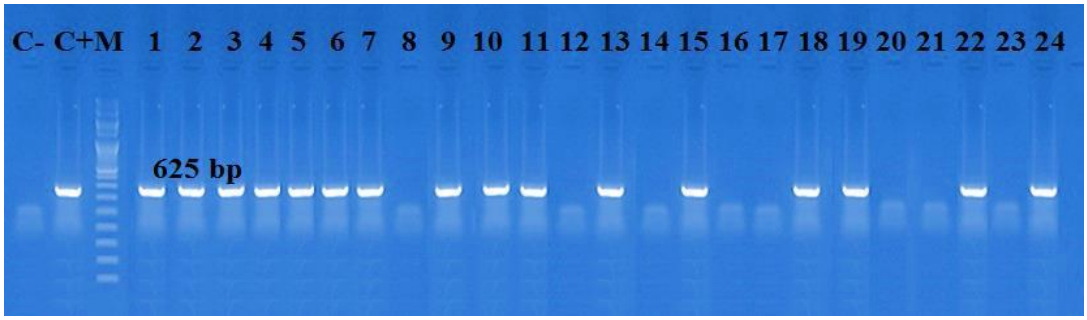


**Fig. 1.** Electrophoretic profile of amplification products of *16S* rRNAgene of *A. hydrophila* at 625bp. M: Marker (50bp), C+: Positive control, C-: Negative control.

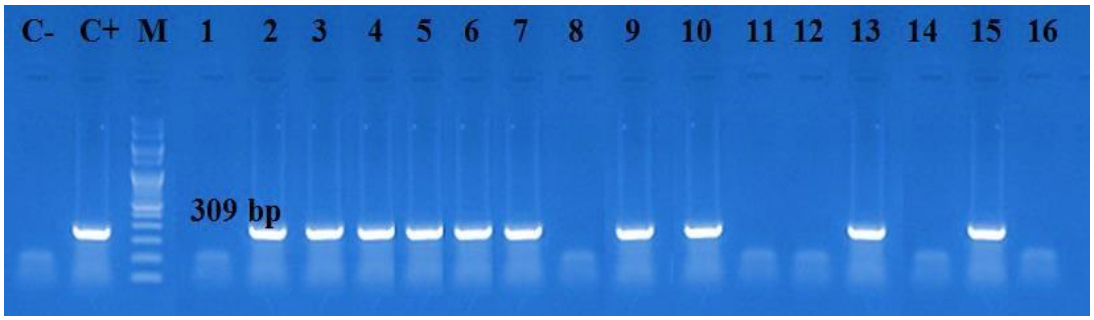


**Fig. 2.** Electrophoretic profile of amplification products of *aerA* gene of *A. hydrophila* at 309bp. M: Marker (50bp), C+: Positive control, C-: Negative control.

It was found that 16 out of 24 isolates were revealed as *A. hydrophila* depending on the species-specific *16S* rRNAgene (Fig.1) and harboring 62.5, 25, and 37.5% for *aerA*, *ahh1*and *altA* virulence gene (Figs. 2, 3, 4). On the other hand, Table (4) displays 100% resistance of the verified *Aeromonas* isolates for both tetracycline and ampicillin, 81.3 and 75% resistance to penicillin and sulfamethoxazole, respectively. Meanwhile, *Aeromonas* spp. were highly sensitive to imipenem (81.3%), cephalothin (68.8%), and

cefoxitin (68.8 %). The resistance profile for MRI of *A. hydrophila* fluctuated from 0.80 to 0.20 with an average of 0. 0.457 (Table 5).

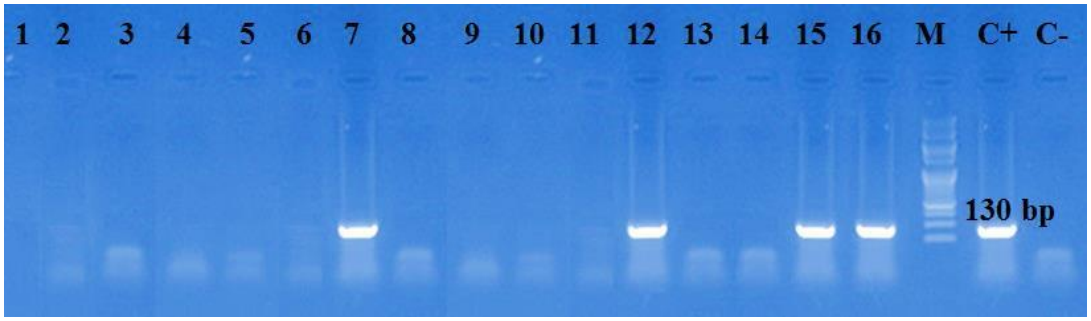


**Fig. 3.** Electrophoretic profile of amplification products of *ahh1* gene of *A. hydrophila* at 130bp. M: Marker (50bp), C+: Positive control, C-: Negative control.

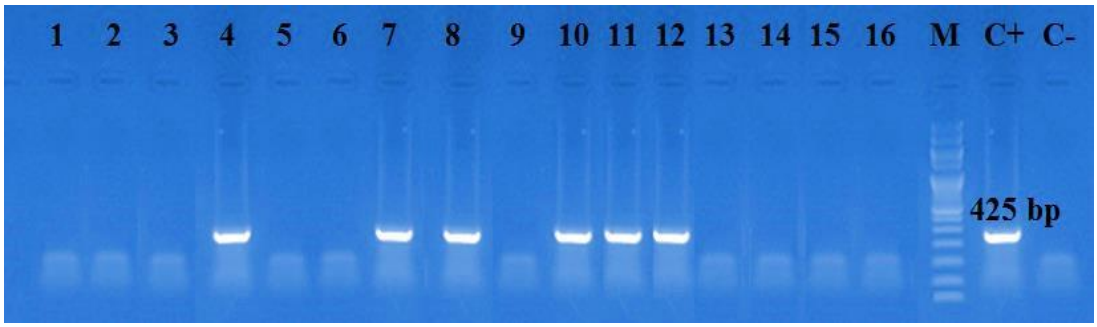


**Fig. 4.**Electrophoretic profile of amplification products of *altA* gene *A. hydrophila* at 425bp. M: Marker (50bp), C+: Positive control, C-: Negative control.

## DISCUSSION

The quality and microbiological safety of fish are of the utmost importance to consumers, retailers, and processors. Fish are predisposed to contamination by pathogens and additional microorganisms **(Khairy *et al.*, 2024)**. The outcomes of the current research suggest that the Nile tilapia had the highest *Aeromonas* counts (3.89±0.35 log CFU/g). These findings were compared to those from previous studies, providing a result that the *Aeromonas* count in the examined fish fillet ranged from 2.29 to 7.20 log CFU/g to 4.91 log CFU/g **(Herrera *et al.*, 2006)** and 4.91 log CFU/g in the mullet fillet **(Salah Eldin *et al.*, 2009)**. The variation in counts is significantly influenced by the hygienic standards of the fish being caught, the additional processing stages, and the sampling methods.

Furthermore, *A. hydrophila* has a significant public health impact on humans. Fishborne *A. hydrophila* can thrive in foods under refrigeration and has been detected along the foodstuff processing line **(Kelany*et al.*, 2024)**. The examination of the microbiological condition of fish is essential for the preservation of public health. Raw seafood may facilitate the dissemination of *Aeromonas* species, among others.

*Aeromonas* spp. were identified from all the analyzed fish samples in the current study at a rate of 35.2%, based on colonial characteristics and biochemical identification. The current results match those of **Morshdy *et al.* (2022)**, who postulated that the occurrence of *Aeromonas* spp. in the Nile tilapia and the mullets was 36 and 32%, respectively.

The present study's overall prevalence was, however, more than that of **Adah *et al.* (2021)** and **Deborah *etal.* (2023)**, who indicated 19.6 and 28.6% prevalence rates, respectively, from *Clarias gariepinus*. It was, however, lower than the prevalence specified by **Ebeed*et al.* (2017)** and **Kishk*et al.* (2020)**, who observed *Aeromonas* contamination in 68 and 64% of the examined fish samples, respectively. Additionally the results of **Abd-El-Malek (2017)** indicated that 38% of inspected fish were contaminated with *Aeromonas* spp., 20 and 1.3% of isolates obtained were categorized as *A. hydrophila* and *A. sobria*. Furthermore, **Morshdy *et al.* (2022)** found that the highest *Aeromonas* spp. recognized from the Nile tilapia were *A. hydrophila* (16%) and *A. caviae* (20%), whereas *A. caviae* (16%) was identified from the mullets.

Additionally, 18.6 and 28.8% of the examined fish samples contained *A. hydrophila*, as reported by **Deborah *et al.* (2023)** and **Elbarbary *et al.* (2024)**. Meanwhile, **Ahmed *et al.* (2018)** found *A.hydrophila* in 4.67% of the Nile tilapia and 6.25% of the *Mugilcephalus*. Nevertheless, **Kishk*et al.* (2020)** reported that the highest *Aeromonas* spp. from the Nile tilapia were *A. caviae* (40.6%), A. *hydrophila* (25%), *A sobria* (21.9%), and *A. veronii* (9.4%), whereas from *Mugil cephalus*, they were *A. sobria* (44%), *A. caviae* (28%), *A.hydrophila* (20%), and *A. veronii* (8%). Furthermore, the *Aeromonas* spp. from freshwater fish were identified by **Dhanapala *et al.* (2021)** as *A. veronii* (75.8%), A. *hydrophila* (9.3%), *A.caviae* (5%), and *A. sobria* (0.6%). The incidence of *Aeromonas* species may vary depending on factors, viz. fish species, holding facilities, sample procedures, geographical locations, and management approaches.

*Aeromonas hydrophila* is a highly pathogenic *Aeromonas* species that has been linked to numerous foodborne outbreaks worldwide, including those resulting from the consumption of uncooked fermented fish. Eventhough the majority of microorganisms can be identified by culturing them on particular media, the traditional technique of analysis was unable to exactly identify *Aeromonas* species as a result of their phenotypic assortment **(Puthucheary *et al.*, 2012)**. Conversely, molecular-based recognition is capable of more precise and dependable identification of *Aeromonas* species **(Kelany *et al.*, 2024)**. The isolation of *16S* rRNA, which is a great and quick way to identify *A. hydrophila*, can be used to corroborate the isolation of the species **(Venkataiah *et al.*, 2013; Morshdy *et al.*, 2023)**.

Hemolysin is a collection of multifunctional enzymes that contribute significantly to *A. hydrophila* pathogenicity.Hemolysins comprise *aerA*, *ahh1*, *ahyA*, and *altA*. While *ahh1* is the major spread of extracellular heat-labile hemolysin, the synergistic mixture of *aerA*, and *ahh1* is the main lethal genotype **(Morshdy *et al.*, 2023)**. *A. hydrophila* also encodes virulence factors that may manifest in several ways, such as adhesion proteins,

nucleases, catalysts, and poisons. adhesion proteins are involved in mucosal adhesion, biofilm development, cell separation, and motility **(Huang *et al.*, 2015)**. These virulence-associated variables are critical in differentiating infective from non-infective bacteria. Aerolysin is the primary virulence-associated factor linked to multiple infections, whereas *A. hydrophila* enterotoxin, which is cytotoxic, is the primary reason for gastroenteritis **(Kishk *et al.*, 2020)**. The PCR results demonstrated that *16S* rRNA was detected in 16 of 24 isolates that were confirmed as *A. hydrophila* (66.7%), indicating that *16S* rRNA can be employed as a specific aim for *A. hydrophila* discovery.

However, in the current investigation, the pathogenicity of *A. hydrophila* strains was caused by one or more virulence genes, such as *aerA*, *ahh1*, and *altA*, which had respective rates of 62.5, 25, and 37.5%. The *aerA* and *ahh1* genes in *A. hydrophila* can generate cytotoxic and hemolytic effects **(Yousr *et al.*, 2007)**. *A. hydrophila* has a detrimental effect on erythrocytes through hemolysis, which may result in diarrhea outbreaks **(Morshdy *et al.*, 2022)**. The acquired result was in agreement with **Ahmed *et al.* (2018)**, who reported that only 3 isolates (42.8 %) from the Nile tilapia fish and 5 isolates (55.6 %) from the *Mugilcephalus* were *aerA* positive, out of all the *A. hydrophila* strains found, all of which were negative for the *ahh1* gene. PCR analysis directed by **Elbarbary *et al.* (2024)** showed the occurrence of *aerA* in 60% of *A. hydrophila*. However, **Hafez *et al.* (2018)** found that 60% of *A.hydrophila* included both *aerA* and *hlyA* genes, while 20% were positive for only *aerA* and 20% for only *hlyA*.

The results are better than those of **Morshdy *et al.* (2022)**, who mentioned that total isolates possessed a high incidence of the hemolysin-encoding genes (*hlyA* and *aerA*), with proportions of 100 and 75%, respectively, and were therefore thought to be potentially pathogenic. Furthermore, **Kishk *et al.* (2020)** verified that *altA* was present in the Nile tilapia and *Mugil cephalus* at a percentage of 38.5 and 30.8%, respectively, whereas *aerA* was discovered in both species at 64.2 and 30.8%. Previous research has shown that hemolysin and aerolysin donate to the pathogenicity of *A. hydrophila* in fish and humans, enabling the bacteria to colonize, replicate, and harm the host tissues **(Elbarbary *et al.*, 2024)**. The identification of pathogenic potentials is contingent upon the determination of virulence determinants, which play multifunctional and multifactorial roles in the pathogenesis of *A.hydrophila* **(Nawaz *et al.*, 2010)**. The incidence rates of *A. hydrophila* have undergone modest fluctuations in previous research in which **Hafez *et al.* (2018)** proposed that the occurrence of *Aeromonas* species may be influenced by a variety of factors, including the different species, sample location and time, geographic region, postcapture contamination, fish species, types of water, and the manipulations and handling that occur during fish handling, storage, and transportation.

Antibiotics are routinely used on fish farms to avoid and manage bacterial contagions. Aquaculture has been associated with the progress of resilient bacteria and the diffusion of these bacteria to other animals and humans through the use of a diverse array of antimicrobial agents **(Srinivasan & Saranraj, 2017)**. Furthermore, the

establishment of the microbial drug resistance phenomena is encouraged by the use of similar antibacterial in veterinary and human medicine, among other domains **(Abdallah *et al.*, 2022)**. This has prompted the necessity of antibacterial susceptibility challenging, which is critical for the identification of the level of antibacterial resistance and the selection of the appropriate medications to treat infections in fish aquaculture, thus decreasing the human health threat **(Nhinh*et al.*, 2021)**.

**Table 4.** The interpretation of antimicrobial resistance of *A. hydrophila* isolates (n=16)

| Antimicrobial agent | Conc. (µg) | Resistance | | Intermediate | | Sensitive | |
|---|---|---|---|---|---|---|---|
| | | No. | % | No. | % | No. | % |
| Tetracycline | 30 | 16 | 100 | 0 | 0 | 0 | 0 |
| Gentamicin | 10 | 9 | 56.3 | 7 | 43.8 | 0 | 0 |
| Ciprofloxacin | 5 | 3 | 18.8 | 5 | 31.3 | 8 | 50 |
| Norfloxacin | 10 | 6 | 37.5 | 4 | 25 | 6 | 37.5 |
| Cephalothin | 30 | 5 | 31.3 | 0 | 0 | 11 | 68.8 |
| Imipenem | 30 | 2 | 12.5 | 1 | 6.3 | 13 | 81.3 |
| Streptomycin | 10 | 8 | 50 | 3 | 18.8 | 5 | 31.3 |
| Ampicillin | 10 | 16 | 100 | 0 | 0 | 0 | 0 |
| Penicillin | 10 | 13 | 81.3 | 2 | 12.5 | 1 | 6.3 |
| Amoxicillin | 25 | 6 | 37.5 | 3 | 18.8 | 7 | 43.8 |
| Cefoxitin | 10 | 3 | 18.8 | 2 | 12.5 | 11 | 68.8 |
| Amikacin | 30 | 4 | 25 | 0 | 0 | 12 | 75 |
| Meropenem | 30 | 3 | 18.8 | 3 | 18.8 | 10 | 62.5 |
| Chloramphenicol | 30 | 11 | 68.8 | 5 | 31.3 | 0 | 0 |
| Sulfamethoxazole | 25 | 12 | 75 | 4 | 25 | 0 | 0 |

Regardless of the different phenospecies, the isolate of *A. hydrophila* in this investigation showed a significant level of resistance to the altered antibacterial utilized. There was a high degree of resistance to the tetracycline group and β-lactam antibiotics (ampicillin, penicillin, and amoxicillin), which is consistent with studies of **Borella *et al.* (2020)**, **Nhinh *et al.* (2021)**,**Deborah *et al.*(2023)** and **Morshdy *et al.* (2023)**. This could be credited to the creation of numerous, inducible, chromosomally encoded beta-lactamases. Additionally, **Dhanapala *et al.* (2021)** and **Li *et al.* (2022)** described *A. hydrophila* resistance to tetracycline, sulfamethoxazole, and gentamycin. This may be related to the widespread usage of these readily available over-the-counter medications, which are administered as feeds or baths **(Adah *et al.* 2022)**. Notably, the *Aeromonas* species was vulnerable to imipenem (81.3%), cephalothin (68.8%), and cefoxitin (68.8%). The outcomes corroborate with those of **Rahman *et al.* (2021)**, **Morshdy *et al.***

**(2022)** and **Woo *et al.* (2022)**. These conclusions may be credited to the lower incidence usage of these drugs in aquaculture in comparison to the other antibacterial.

**Table 5.** Antimicrobial resistance profile of *A. hydrophila* (n=16)

| Species | Isolates no. | Antimicrobial resistance profile | No. of antibiotic | MAR index |
|---|---|---|---|---|
| *A. hydrophila* | 13 | T, G, CP, CN, AP, AX, NR, AK, MP, IP, ST, CM, | 12 | 0.80 |
| | 10 | T, CP, AM, AX, SM, AP, P, CM, ST | 9 | 0.60 |
| | 8 | T, NR, AX, AP, ST, MP, CF, P | 8 | 0.533 |
| | 7 | T, G, CP, CN, AK, AP, CM, | 7 | 0.467 |
| | 5 | T, NR, SM, CM, AM, ST | 6 | 0.40 |
| | 3 | T, AP, NR | 3 | 0.20 |
| | 2 | T, AP, MP | 3 | 0.20 |
| | | Average | | 0.457 |

MAR: Multiple Antibiotic Resistant indexes. T: tetracycline; G: gentamicin, CP: ciprofloxacin, NR: norfloxacin, CN: Cephalothin, IP: imipenem, SM: streptomycin; AP: ampicillin, P: penicillin, AX: amoxicillin, CF: Cefoxitin, AK: Amikacin, MP: Meropenem, CM: chloramphenicol, ST: sulfamethoxazole.

The various resistance patterns of *A. hydrophila* in the various fish samples under study may be caused by differences in the incidence, period, dose, and use of antibacterial drugs. It is possible for a variety of antibiotic resistance patterns to develop; however, these patterns are susceptible to change in response to selective pressure and the environment **(Borella *et al.*, 2020; Nhinh *et al.*, 2021)**.

Furthermore, the results of this study's MAR index of *A. hydrophila* of > 0.2 match those of **Salem *et al.* (2020)**, **Saleh *et al.* (2021)**, **Morshdy*et al.* (2022)** and **Morshdy*et al.* (2023)**. These findings indicate that *A. hydrophila* from these studies was subjected to antibacterial agents throughout culture, leading to the development of antibacterial resistance. As observed in this investigation, this resistance may influence the effectiveness of treatments in fish farms.

## CONCLUSION

The current research indicated that the majority of the fish samples under analysis were contaminated with antibacterial-resistant *Aeromonas*, particularly *A. hydrophila*, which poses potential foodborne bacteria and may facilitate its dissemination. *A. hydrophila* that was identified includes one or more virulence genes that could be harmful to people's health. Additionally, *A. hydrophila* exhibits varying resistance levels to the most extensively verified antibacterial agents. It is recommended that sanitary procedures

should be implemented during the handling, preparation, processing, and storage of fish to reduce microbial contamination with *A. hydrophila* and prevent the prevalence of pathogens. This will enhance the quality of the fish. Additionally, it is suggested that the overuse of antibiotics in aquaculture should be reduced and that scientists ought to be encouraged to pursue alternate non-antibacterial control approaches for various bacterial contaminations in farmed fish.

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