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ISOLATION AND CHARACTERIZATION OF *AEROMONAS VERONII* FROM NATURALLY INFECTED NILE TILAPIA

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ABSTRACT

Aeromonas species are common pathogens that induce significant economic losses in aquaculture. This study investigated *Aeromonas* infection in cultured Nile tilapia (*Oreochromis niloticus*) in Qena Province, Egypt, with fifty fish were sampled. Bacteriological examinations as well as a molecular-based approach with 16S ribosomal DNA sequences were used. Moreover, a challenge experiment was conducted to assess the pathogenicity of the isolated *Aeromonas*. Clinical findings of the naturally infected fish were characterized by generalized septicemic signs of hemorrhagic ulcerations, ascites, and exophthalmia. Based on morphological, biochemical, and molecular characterization, 30 isolates (60% prevalence) of *Aeromonas* were identified in internal organs. The *16S rDNA* gene sequencing and phylogenetic analysis confirmed the identity of the isolated *Aeromonas* as *A. veronii*. Four sequenced *A. veronii* displayed 99.92% and 99.84% identity with the *16S rDNA* gene of *A. veronii* species available in the GenBank database, accession numbers MT302825 and KC633849, respectively. The phylogenetic position of the nucleotide sequences identified here were clustered with other fish-infecting *A. veronii*. Results of the waterborne challenge experiment revealed that the *A. veronii* (accession number PV495274) produced mortality rate of 75.6 % with the same clinical picture as the naturally infected fish. Findings herein concluded that *A. veronii* is a predominant pathogen in tilapia aquaculture in Qena Province and the biochemical and genomic characterization providing critical data for disease management. The study highlights the need for targeted surveillance and control measures against Aeromonas infections in this economically vital sector.

Keywords: *Oreochromis niloticus*; motile aeromonas septicemia; fish mortality; bacterial isolation; *16S rDNA* gene sequencing; phylogenetic analysis.

INTRODUCTION

Egypt dominates African aquaculture production, with Nile tilapia (*O. niloticus*) representing its primary cultivated species. Globally, Egypt ranks among the leading tilapia producers, surpassed only by China and Indonesia while exceeding production in Thailand, Bangladesh, and the Philippines with an annual yield of 1.11 million metric tons in 2020, tilapia emerged as Egypt's predominant farmed fish species and the world's second most cultivated fish following

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various carp species (FAO, 2022). Notably, the domestic market consumes approximately 97.5% of Egypt's tilapia output, with exports totaling merely 28,110 metric tons in 2020 (GAFRD, 2020). Industry value chain assessments identify tilapia as the cornerstone of Egyptian aquaculture, complemented by mullet as the secondary species in private aquaculture operations (Eltholth *et al.,* 2015).

Aeromonas, classified within the Gammaproteobacteria class (Aeromonadales order, Aeromonadaceae family), comprises facultatively anaerobic, motile, non-spore-forming, Gram-negative rods (Dias *et al*., 2012; Piotrowska and Popowska, 2014). Traditional taxonomy divided *Aeromonas* into two phenotypic groups: (1) motile mesophiles (e.g., *A. hydrophila* and *A. veronii*) with optimal growth at 35-37 °C and zoonotic potential, and (2) non-motile psychrophiles (e.g., *A. salmonicida*) thriving at 22-28 °C with piscine specificity (Saavedra *et al.,* 2006). Contemporary genomic analyses have substantially revised *Aeromonas* taxonomy, expanding the genus to 36 validated species through both reclassification and novel discoveries (Fernández-Bravo and Figueras, 2020). Our previous study concluded that the cause of motile aeromonas septicemia and mortality in summer season in Qena Province was *A. veronii* (Bakry and Emeish, 2022).

The classification of *Aeromonas* has been further refined using 16S ribosomal RNA sequencing, DNA-DNA hybridization, and whole-genome sequencing. These methods have revealed significant phenotypic and genotypic heterogeneity within the genus, complicating species identification (Janda and Abbott, 2010; Chenia, 2016).

Motile aeromonas septicemia (MAS) is one of the most significant diseases affecting Nile tilapia, characterized by high mortality rates and severe clinical signs. Infected fish often exhibit external ulcerations, abdominal swelling, hemorrhagic septicemia, and darkening of the skin (Dong *et al.,* 2017; Haenen *et al.,* 2023).

In Egypt, unexplained mortalities in tilapia farms during summer months have been linked to *Aeromonas* infections. Affected fish show red sores on the skin, loss of activity, and hemorrhagic fins, with severe cases exhibiting abdominal dropsy and detached scales (El Asely *et al.,* 2020; Abdelsalam *et al.,* 2021). These outbreaks result in significant financial losses due to fish mortality and reduced marketability of survivors.

Thus, the aim of this study was to isolate, phenotypically characterize, and molecularly identify *Aeromonas* pathogens associated with septicemia in farmed Nile tilapia (*O. niloticus*) from Qena Province, Egypt, using *16S rDNA* gene analysis. Moreover, challenge experiment was conducted to assess the pathogenicity of the isolated *Aeromonas* species.

MATERIALS AND METHODS

1. Ethical statement: This research adhered to established ethical standards for animal studies and was approved by the Ethical Research Committee of the Faculty of Science at South Valley University in Qena, Egypt (Approval No. 010/12/24).

2. Fish sampling and clinical examination: Fifty Nile tilapia with body weight of 25-30 g showing septicemic signs were purchased from private fish farm in Qena Province, Egypt. Fish were transported to the Aquatic Diagnostic Laboratory, Faculty of Veterinary Medicine, and South Valley University in fiberglass tanks. Nile tilapia underwent clinical examination to document visible clinical signs and postmortem lesions, following the protocols described by Noga (2010).

3. Bacterial isolation: After surface sterilization and fish dissection, sterile inocula were collected from the spleen, and kidneys. These inocula were directly cultured onto tryptic soy agar (TSA; Oxoid, England)

and incubated at 28°C for 48 hours. Dominant colonies were purified on fresh TSA. Suspected *Aeromonas* isolates were sub-cultured on Aeromonas Isolation Medium Base (HiMedia, India) with added Aeromonas selective supplement (FD039) and incubated at 26 °C for 24 hours. After incubation, individual colonies were re-streaked into fresh selective media and re-incubated under identical conditions, where dark green, opaque with dark centers colonies were presumptively considered to be *Aeromonas*. Isolates were preserved in Brain Heart Infusion (BHI; Oxoid, England) broth supplemented with 20% glycerol and stored at −80 °C for future analysis.

4. Conventional identification of the suspected isolates:

Bacterial isolates were identified using standard phenotypic and biochemical methods, as described by Austin and Austin (2016). The bacterial isolates were phenotypically characterized through comprehensive biochemical profiling and morphological analysis. Initial assessment included examination of colony morphology and Gram staining characteristics. Metabolic characters were evaluated through cytochrome oxidase activity (using oxidase test strips) and catalase reaction with 3% hydrogen peroxide. Motility was determined via semisolid agar inoculation, while carbohydrate metabolism was assessed using triple sugar iron agar for fermentation patterns. Additional biochemical tests included citrate utilization (Simmons' citrate agar), esculin hydrolysis (bile esculin agar), and nitrate reduction capacity. Furthermore, indole production (Kovac's method), Voges-Proskauer, and methyl red tests. The isolates were further characterized for hydrogen sulfide production, capability to grow in 5 and 10% NaCl, and susceptibility to the vibriostatic agent O/129 (150 µg/mL; Oxoid)

5. Molecular identification of suspected isolates

5.1. DNA extraction: Genomic DNA was extracted from isolates using the GeneJET Genomic DNA Purification Kit (Thermo Scientific, EU), following the manufacturer's protocol, and stored at -20 °C until analysis. The purity and the concentration of the extracted DNA were measured using a NanoDrop Lite spectrophotometer (Thermo Scientific).

5.2. PCR amplification: The extracted DNA was subjected to polymerase chain reactions (PCR) assay to amplify the 1502 base-pair (bp) hypervariable segment of the *Aeromonas 16S rDNA* gene using genus-specific primers *16S rDNA* F 5' AGAGTTTGATCATGGCTCAG 3' and 16S-rDNA R 5' GGTTACCTTGTTACGACTT 3' according to Borrell *et al*. (1997). The PCR reactions were carried out in a total volume of 50 µl which consisted of 25 µl Cosmo PCR Red Master Mix (Willowfort, UK), 2 µl of each primer, 4 µl template DNA and 17 µl H_2O (RANase /DNase free).

The PCR amplification was performed in the CFX96 Real-Time PCR Detection System starting with an initial denaturation step at 93 °C for 3min and followed by 35 cycles consisted of denaturation at 94 °C for 1min, annealing at 56 °C for 1min, an extension step at 72 °C for 2 min, and a final extension step at 72 °C for 10 min. Negative controls to which no template DNA was added, were also included in every set of reactions.

5.3. Analysis of the PCR products: After electrophoresis in 1.5% agarose gel and ethidium bromide staining, PCR products were visualized using UV transillumination. 100 bp DNA plus ladder (Qiagen, GmbH) was used to determine the fragment sizes.

5.4. Sequencing of *16S rDNA* gene: For identification of the isolates at the species level, amplified DNA fragments of four suspected *Aeromonas* were gel-purified using the Zymoclean Gel DNA Recovery Kit (Zymo Research, USA) following the manufacturer's protocol. Macrogen Inc. (Seoul, South Korea) commercially sequenced purified amplicons. *16S rDNA* gene sequences were trimmed and edited

using the biological sequence alignment editor, BioEdit software. The nucleotide sequences obtained were analyzed using Basic Local Alignment Search Tool (BLASTn; http://www.ncbi.nlm.nih.gov/BLAST/) to establish sequence similarities (Altschul *et al.,* 1997) against the GenBank database. Sequences of the four *A. veronii* isolates were deposited in the GenBank database and assigned the accession numbers PV495274, PV495275, PV495276, and PV495277.

5.5. Phylogenetic analysis: Sequences with higher identities, query cover of 100%, E-value of zero and isolated from different fish species were downloaded from the GenBank for phylogenetic analysis of *A. veronii* (Table 1). The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model (Tamura and Nei, 1993). Sequences were aligned using the Muscle alignment tool and evolutionary analyses were conducted in MEGA11 software as per Tamura *et al.* (2021). The alignment length used for phylogenesis was 1274 bp. Bootstrap confidence values were calculated with 1000 repetitions and the analysis involved 14 nucleotide sequences.

Table 1: *Aeromonas* species used for phylogenetic tree construction

Accession number	Organism	Host origin	Infected organ	Locality
PV495274	*Aeromonas veronii*	*Oreochromis niloticus*	Internal organs	Egypt, Qena
PV495275	*Aeromonas veronii*	*Oreochromis niloticus*	Internal organs	Egypt, Qena
PV495276	*Aeromonas veronii*	*Oreochromis niloticus*	Internal organs	Egypt, Qena
PV495277	*Aeromonas veronii*	*Oreochromis niloticus*	Internal organs	Egypt, Qena
MT302825.1	*Aeromonas veronii*	*Clarias gariepinus*	Gut	-
KC633849.1	*Aeromonas veronii*	Goldfish	-	-
OQ217596.1	*Aeromonas* sp.	Aquaculture water (Sea bass)	-	South China
KT364222.1	*Aeromonas sobria*	*Clarias fuscus*	Heart	-
KC884671.1	*Aeromonas veronii*	Diseased fish	-	-
ON383477.1	*Aeromonas veronii*	Tilapia	Pune Hatchery Fry	-
MT661567.1	*Aeromonas* sp.	Fresh aquaculture pond (*Hyriopsis cumingii*)	-	-
KM585590.1	*Aeromonas* sp.	Zebrafish	Intestine	-
MG063196.1	*Aeromonas veronii*	Ornamental fish	-	China
ON606317.1	*Aeromonas veronii*	Wetland (*Lebeo catla*)	-	-

6. Pathogenicity of the *A. veronii* isolated to Nile tilapia

6.1. Fish husbandry conditions: Apparently healthy juveniles Nile tilapia (average weight, 20 ± 0.5 g) was obtained from stocks maintained at the aquatic laboratory of the Faculty of Veterinary Medicine, South Valley University and acclimated for 15 days in 260 L tanks. No clinical signs or mortalities were observed during the acclimation period. Prior to the challenge experiment, 10 randomly selected fish were subjected to

bacteriological screening to verify that all fish were negative for *Aeromonas* infection.

Throughout the experiment, tanks were supplied with dechlorinated freshwater, and the water quality parameters were maintained as follows: temperature was 28±1 °C, dissolved oxygen was 7.3 mg/L, and pH was 7.0. The photo period was set to 12 h light / dark intervals. Fish were fed once a day to apparent satiation with a pelleted commercial tilapia feed (Skretting).

6.2. Experimental design and waterborne challenge:

Nile tilapia (n= 90), apparently healthy and devoid of any signs of infection, were distributed in 120-L fiberglass tanks where they were divided into two groups (n=45 / group) with three replicates per group (n=15 / replicate), the first is bacterial challenge group for clinical signs and mortality records, and second one is the mock control group.

Before the actual challenge experiment, *A. veronii* (accession number PV495274) was passed three times in healthy Nile tilapia through IP injection of 0.1 mL of culture (~1 x 10^9 CFU/mL).

The most appropriate challenge dose used in the experiment was established based on the mortality rate recorded in a waterborne pre-challenge experiment (data not shown). Briefly, bacteria were grown in 1 liter of TSB with shaking (200 rpm) at 28°C until they reached mid-log phase (18 hr). The CFU of bacterial counts was determined using a standard plate count method following 10-fold serial dilutions as previously described (Miles *et al.* 1938).

For the actual challenge, fish in the challenge group were immersed in *A. veronii* suspension (1 × 10^7 CFU/mL) for 1hr, with continuous aeration. Mock control fish underwent the same procedure but were exposed only to sterile TSB medium. After exposure, all fish monitored twice daily for signs of illness or death over 14 days.

Freshly dead fish were bacteriologically examined to re-isolate and confirm the infection by *Aeromonas* from the internal organs. Identification of re-isolated bacteria was conducted by the molecular approaches as described above.

RESULTS

1. Clinical signs and post-mortem findings in suspected infected Nile tilapia

Suspected fish exhibited generalized signs of septicemia. Clinical examination revealed hemorrhagic lesions with associated scale detachment and erythema, particularly in the opercular region and fin bases. Severe hemorrhagic ulcerations were observed in the dorsal musculature, accompanied by abdominal distension (ascites), hemorrhagic protruded anal opening, exophthalmia and corneal clouding. Necropsy findings indicated generalized visceral congestion, hemorrhagic enteritis, hepatomegaly with gall bladder distension, and hemoperitoneum **(Fig. 1)**.

2. Morphological and biochemical identification of isolated bacteria

Bacterial cultures isolated from infected specimens developed circular, convex, glossy colonies with a cream-colored appearance on Tryptic Soy Agar (TSA). Gram staining and light microscopy confirmed the presence of Gram-negative, rod-shaped bacilli. Conventional biochemical testing revealed consistent profiles across all isolates, demonstrating positive reactions for oxidase, catalase, motility, indole production, glucose fermentation with gas production, esculin hydrolysis, nitrate reduction, and the methyl red test. Negative results were obtained for the Voges-Proskauer test, H_2S production, and citrate utilization, and couldn't grow on media containing 5 and 10% NaCl. All isolates exhibited resistance to the vibriostatic agent O/129 (150 µg), a key feature differentiating *Aeromonas* from *Vibrio* sp. (Table 2). Growth on Aeromonas Isolation Medium Base with added

Aeromonas selective supplement (FD039) produced colonies of dark green opaque with dark centers that were characteristic of the genus *Aeromonas*. These morphological and biochemical characteristics supported the taxonomic classification of all isolates within the *Aeromonas* genus according to the criteria established in Bergey's Manual of Systematic Bacteriology (Martin-Carnahan *et al.,* 2005). Among the 50 Nile tilapia examined, biochemical analysis identified *Aeromonas* sp. in 30 cases, yielding a prevalence rate of 60%.

3. Polymerase chain reaction

Molecular analysis confirmed the presence of *Aeromonas* through successful PCR amplification of a 1502 bp *16S rDNA* gene segment in 30 isolates (60% prevalence rate), as shown in **Fig. 2.**

Table 2: Conventional biochemical characteristics of the suspected *Aeromonas* isolates.

Test	Reaction
Gram stain	-
Motility	+
Oxidase	+
Catalase	+
Indole	+
Glucose with gas	+
H_2s production	-
Voges-Proskauer	-
Nitrate reduction	+
Esculin hydrolysis	+
Methyl red	+
Citrate utilization	-
Growth on 5 and 10% Nacl	-
Resistance to vibriostatic agent O/129	Resistant

Note: + means positive; - means negative

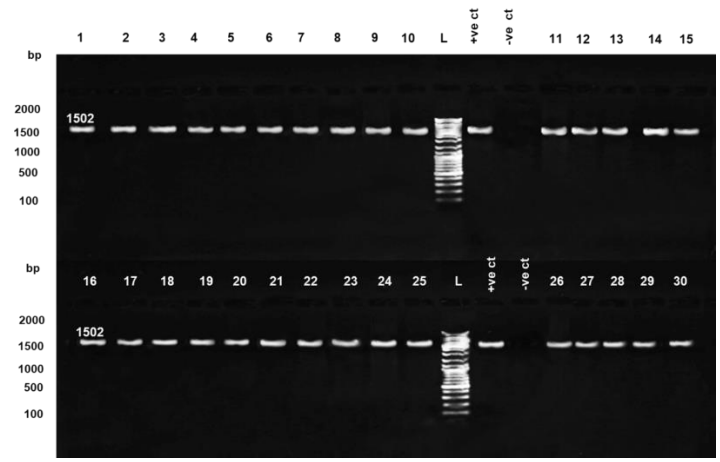

Figure 2: Amplifying a 1502 bp fragment of *16S rDNA* gene of clinical isolates of *Aeromonas* using Aeromonas-specific primers. L: 100 bp ladder, Lane 1-30: Clinical isolates of the present study, +ve ct: Positive control, -ve ct: No-template control.

4. Sequence analysis and phylogenesis of the *16S rDNA* gene

Sequencing of the *16S rDNA* gene of four isolates of *Aeromonas* sp. resulted in 1272, 1276, 1278 and 1282 bp valid sequences which revealed higher similarity with different *Aeromonas* sp. sequences available in GenBank. For example, the greatest sequence identity of 99.92% was with *A. veronii* (accession number MT302825) isolated from the gut of *C. gariepinus*, and 99.84% with *A. veronii* (accession number KC633849) isolated from goldfish. The *16S rDNA* sequences obtained from four isolates were deposited in the GenBank database using Bankit submission tool and given the accession numbers PV495274, PV495275, PV495276, and PV495277 of the 1272, 1276, 1278 and 1282 bp valid sequences, respectively.

Based on *16S rDNA* sequence data, construction of the phylogenetic tree using maximum likelihood algorithms demonstrated splitting of the tree into 2 major clades which mainly contain *A. veronii* which are isolated from various fishes (**Fig. 3**). One clade is further divided into 2 subclades, one comprising organisms infecting various fish species and the other subclade clustered *A. veronii* species of the present study. The other clade only clustered *A. veronii* (accession number MT302825) which showed the highest sequence identity with the sequences of the present study.

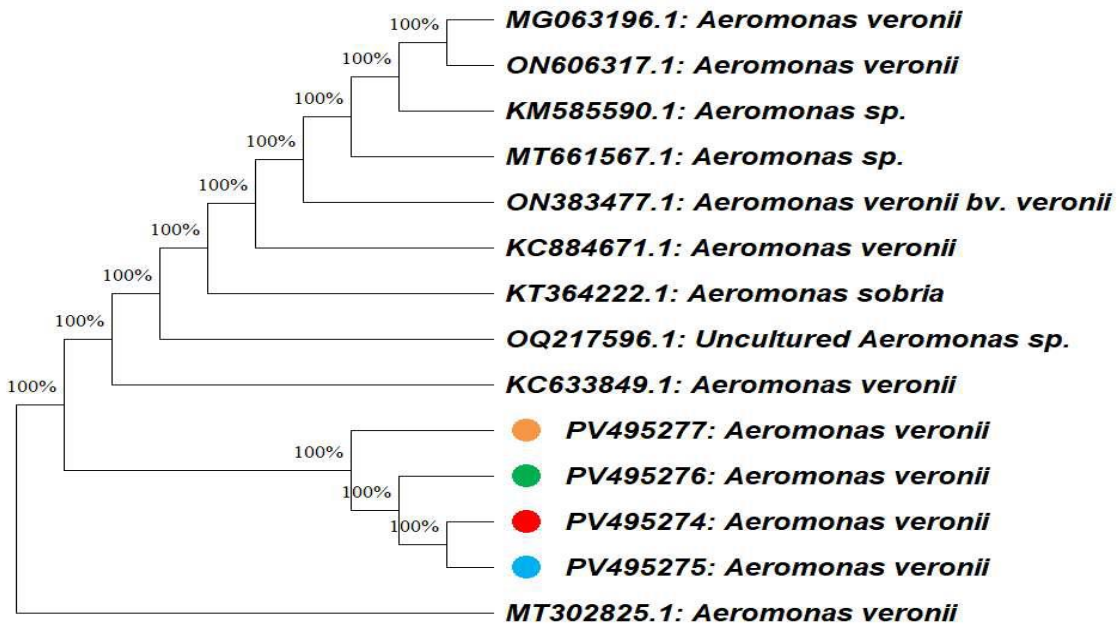

Figure 3: Un-rooted maximum likelihood phylogenetic tree showing the relationship between *Aeromonas veronii* described in this study and the closest *Aeromonas* sequences from GenBank database based on *16S-rDNA* gene sequences. Numbers at branching points indicate bootstrapping confidence levels from maximum likelihood. GenBank accession numbers are listed beside the species name. The colored circles represent the species of the present study.

5. Clinical manifestations and mortality patterns in *A. veronii*-challenged Nile tilapia

Nile tilapia experimentally infected with *A. veronii* (accession number PV495274) began exhibiting clinical signs consistent with motile aeromonad septicemia (MAS) within 2 days post-challenge (dpc), with initial mortality observed at 12 hours post-challenge. Affected fish displayed external symptoms including petechial hemorrhages around the mouth, focal scale loss leading to ulcerative skin lesions, fin erosion, and exophthalmia, often accompanied by corneal opacity or ulceration. Internally, pathological findings included hemorrhagic septicemia, gall bladder distension, petechial hemorrhage and friable degeneration of visceral organs **(Fig. 4)**.

Mortality dynamics followed a distinct pattern, peaking at 3 dpc and culminating in a 75.6% cumulative mortality rate **(Fig. 5)** at 6 dpc. No further deaths were recorded beyond 6 dpc, and surviving fish, including those initially showing clinical signs, fully recovered by 7 dpc and remained healthy until the trial's end. Bacteriological analysis confirmed *A. veronii* re-isolation from moribund or freshly deceased specimens, with colonies verified through morphological and molecular methods as described above. In contrast, mock control groups remained asymptomatic throughout the study. The challenge trial was terminated at 14 dpc following 8 days without mortality, ensuring robust observational endpoints.

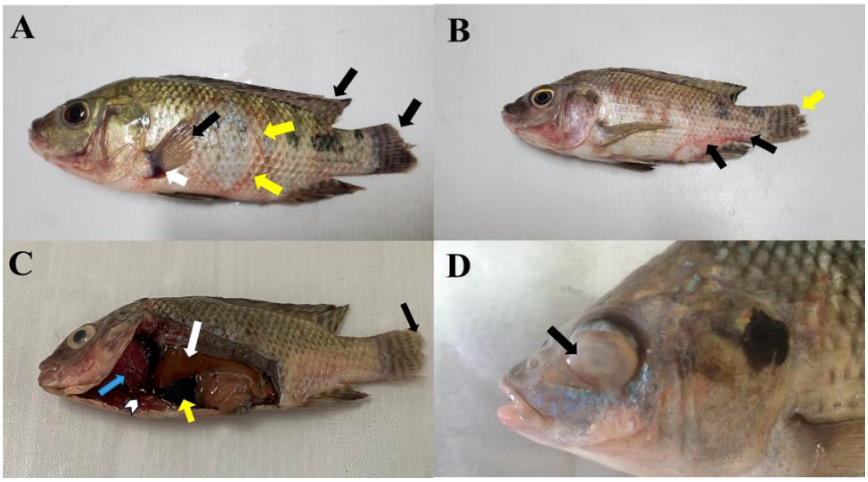

Figure 4: Gross pathology in experimentally infected Nile tilapia following 1 hour immersion challenge with $1x10^7$ cfu/mL of *Aeromonas veronii* (accession number PV495274): (A) Severe dermal lesions characterized by multifocal scale loss, ulcerative dermatitis (yellow arrows), hemorrhages at the base of the pectoral fin (white arrow) and sloughing of dorsal, pectoral, and caudal fins (black arrows). (B) Ventral hemorrhagic dermatitis with pronounced peri-anal and caudal peduncle involvement (black arrows), and progressive caudal fin erosion (yellow arrow). (C) Sloughing of the caudal fin (black arrow). Branchial hyperemia with filamentous congestion, indicating acute gill pathology (blue arrow), hepatomegaly with petechial hemorrhages (white arrow) and marked gallbladder distension (yellow arrow) and hemorrhages in the body cavity (arrowhead) suggesting systemic infection. (D) Bilateral exophthalmia with corneal opacity consistent with systemic septicemia (black arrow).

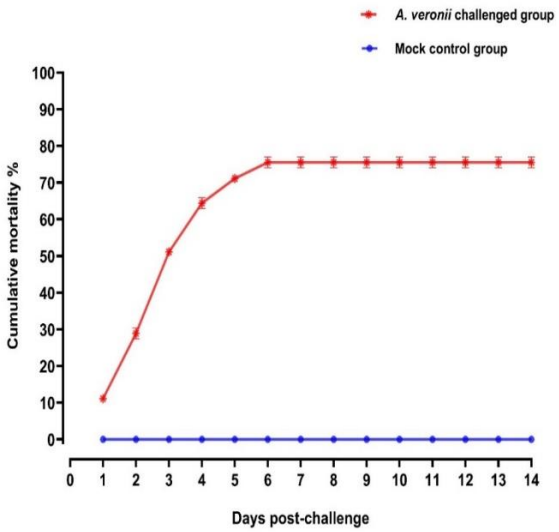

Figure 5: Percent mortality curves of Nile tilapia after 1hour immersion challenge with $1x10^7$ cfu/mL with *Aeromonas veronii* (accession number PV495274) and for mock control fish. Mortality was recorded for 14 days post-challenge. The percents represent the cumulative mortality of 3 tanks/treatment. Data is presented as the mean ± standard error of the mean (SEM).

DISCUSSION

Aeromonas is a significant pathogen in aquaculture, causing septicemia in fish and shellfish that often leads to mass mortality and substantial economic losses (Noga, 2010). Among the 36 documented species (Fernández-Bravo and Figueras, 2020), *A. hydrophila*, *A. veronii*, *A. caviae*, and *A. jandaei* are the primary species associated with MAS in fish (Janda and Abbott, 1998; Joseph and Carnahan, 2000). Previous study on *Clarias gariepinus* in Qena Province concluded that *A. hydrophila*, and *A. veronii* are the main causative agents of MAS (Emeish *et al.,* 2018).

The clinical signs and post-mortem lesions of naturally infected fish revealed typical signs of septicemia, which aligns with the previously reported clinical manifestations. Acute infections often result in rapid fatal septicemia with minimal gross signs, while affected fish may exhibit skin reddening, exophthalmia, abdominal distension, and

fluid accumulation in scale pockets (Yardımcı and Aydın, 2011).

Accurate identification of Aeromonas isolates is fundamental for determining the dominant pathogenic species in Nile tilapia. Precise pathogen characterization supports epidemiological investigations, outbreak tracing, and the development of targeted control and treatment strategies. Molecular techniques provide the most reliable identification of fish pathogens, even during early infection stages, due to their rapid and precise diagnostic capabilities (Buller, 2004). In this study, 30 isolates were presumptively identified as Aeromonas sp. through conventional morphological and biochemical analyses, though species-level classification remained inconclusive. The bacterial genus was confirmed through 16S rDNA gene amplification applying Aeromonas-specific primers, following the protocol established by Borrell et al. (1997)

The present investigation identified 30 isolates as *A. veronii*, a globally distributed pathogen of significant concern in freshwater aquaculture systems. This bacterium has been extensively documented as a highly virulent agent in numerous piscine species (Sun *et al*., 2016; Zhang *et al*., 2018; Bakry & Emeish, 2022). This pathogen is associated with severe clinical manifestations, including hemorrhagic septicemia, necrotic ulcerations, generalized cutaneous pallor, abdominal distension (dropsy), and progressive necrosis of fin and tail tissues (Hassan *et al*., 2017; Hoai *et al*., 2019). Consistent with prior research, *A. hydrophila* and *A. veronii* are the most frequently detected *Aeromonas* sp. in fish and aquatic environments (Ottaviani *et al.,* 2011; Emeish *et al.,* 2018). While *A. veronii* predominates in both fish and water samples, *A. hydrophila* exhibits higher prevalence in diseased fish compared to healthy individuals (Hu *et al.,* 2012). In contrast, *A. sobria* was the predominant species in diseased fish from Spain (Beaz-Hidalgo *et al.,* 2010).

In this study, *16S rDNA* amplification detected *Aeromonas* sp. in ٦٠% of samples, with sequencing confirming the presence of *A. veronii*. These findings are consistent with the 56% prevalence reported by Lee *et al.* (2002) but lower than the 78% observed by Hussain *et al.* (2014). Conversely, our detection rate was higher than the 42% reported by Bakry and Emeish (2022). Discrepancies in prevalence rates may reflect variations in sampling periods, fish species, and geographic location. Prior research indicates seasonal fluctuations in *Aeromonas* distribution, with pathogenic species (*A. hydrophila*, *A. veronii*, *A. caviae*) predominating in warmer months (Lee *et al.,* 2002).

The predominance of *A. veronii* in this study likely stems from its ubiquitous, opportunistic, and psychrotrophic characteristics in aquatic ecosystems, as well as its commensal presence in fish intestines. Furthermore, the outbreak occurred in summer, when elevated temperatures, reduced dissolved oxygen, and associated water parameter fluctuations likely induced physiological stress, weakening immune defenses and increasing infection susceptibility. Wassif (2018) isolated *A. veronii,* a pathogen affecting both fish and humans, from Nile tilapia and *Ictalurus punctatus* (channel catfish) during mass mortality outbreaks in Egyptian fish farms (El Sharkia and El Ismailia governorates).

According to the phylogenetic analysis based on the *16S rDNA* gene sequences of the *Aeromonas* strains, we observed that our isolates clearly belong to *A. veronii* and are grouped in a separate clade within the *A. veronii* clusters and show a relatively short phylogenetic distance from the other *Aeromonas* species with high confidence levels of 100% bootstrapping.

Concerning the results of the experimental challenge with *A. veronii* (accession number PV495274), several pathological changes were observed externally and internally on infected fish which supported the findings on naturally infected fish and *A. veronii* could be re-isolated from kidney and spleen.

Consistent with earlier reports (Hu *et al.,* 2012; El Asely *et al.,* 2020), *A. veronii* was the most frequently isolated species from the kidney, spleen, and liver likely due to their high vascularity and hematopoietic function, rendering them particularly vulnerable (Kusdarwati *et al.,* 2017). Toxemia arises from the absorption of Aeromonas-produced toxic metabolites, leading to capillary hemorrhages. These pathological effects likely stem from virulence factors, including motility, outer membrane proteins, toxins, proteases, secretion systems, quorum sensing, and iron acquisition mechanisms (Sun *et al.,* 2016). Hemolytic and cytotoxic activities, linked to virulence gene expression, play a pivotal role in disease development (Zhang *et al.,* 2018).

The mortality patterns observed in our study experienced peak deaths at 3 dpc and reached 75.6% total mortality by day 6. Bakry and Emeish (2022) documented similar outcomes, reporting 80% mortality in infected tilapia with mortalities peaking slightly later at 4 days post-infection. This difference in timing could reflect variations in experimental conditions, such as the specific bacterial strain used, the dosage administered, or water temperature during the trials. Eissa *et al*. (2016) recorded 70% mortality when injecting tilapia directly with 120 million bacterial cells per milliliter, while El Asely *et al*. (2020) observed mortality rates ranging from 60-100% depending on the bacterial isolate and dosage. Together, these studies paint a consistent picture: *A. veronii* poses a severe threat to Nile tilapia, with mortality severity depending on three key factors - how the bacteria enter the fish (through water vs injection), the specific bacterial strain's virulence, and the infection dose.

When comparing different *Aeromonas* species, *A. hydrophila* appears even more dangerous under controlled conditions. While 100% mortality rate occurred within just 48 hours when high concentrations of *A. hydrophila* were injected into muscle tissue (Ibrahem *et al.,* 2008). Overall, our results fall within the range of reported mortality rates for *Aeromonas* infections in tilapia, reinforcing the pathogen's significant threat to aquaculture. The observed differences in mortality timing and severity across studies likely stem from variations in experimental protocols, bacterial virulence factors, and host susceptibility.

CONCLUSION

This study identifies *A. veronii* as the primary pathogen in Nile tilapia, with a ٦٠% prevalence confirmed via *16S rDNA* gene sequencing. Its virulence and summer-associated outbreaks—linked to elevated temperatures and poor water quality—highlight its threat to aquaculture. Discrepancies with prior studies likely reflect geographic or seasonal variations. Proactive monitoring and strain-specific management are recommended to mitigate losses.

Abbreviations: CFU, Colony-forming unit; MAS, Motile aeromonas septicemia; PCR, Polymerase chain reaction; dpc, Days post-challenge; *A. hydrophila*, *Aeromonas hydrophila*; *A. veronii*, *Aeromonas veronii; A. caviae, Aeromonas caviae; A. jandaei, Aeromonas jandaei; A. salmonicida, Aeromonas salmonicida* ; TSA, Tryptic Soy Agar; TSB, Tryptic Soy Broth; BHI, Brain Heart Infusion; bp, base-pair; *16S rDNA*, 16S Ribosomal RNA; IP, Intraperitoneal injection; SE, standard error.

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عزل وتوصيف بكتيريا الايروموناس فيروني من أسماك البلطي النيلي المصابة طبيعياً

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أنواع الايروموناس هي مسببات أمراض شائعة تسبب خسائر اقتصادية كبيرة في تربية الأحياء المائية. ركزت هذه الدراسة في عدوى الايروموناس في أسماك البلطي النيلي المستزرعة في محافظة قنا، مصر، حيث تم أخذ عينات من خمسين سمكة. تم تقييم الفحوصات البكتريولوجية بالإضافة إلى النهج الجزيئي باستخدام تسلسل الحمض النووي الريبوسومي ١٦S. علاوة على ذلك، أجريت تجربة التحدي لتقييم مسببات الأمراض للايروموناس المعزولة. تميزت النتائج المعملية للأسماك المصابة طبيعياً بعلامات التسمم الدموى والتقرحات النزفية والاستسقاء وجحوظ العين. بناءً على التوصيف المورفولوجي والكيميائي الحيوي والجزيئي، تم تحديد 30 عينة (انتشار 60٪) من الايروموناس في الأعضاء الداخلية. أكد تسلسل جين الريبوسومي ١٦S والتحليل التطوري أن هوية الايروموناس المعزولة هي الايروموناس فيروني. أظهرت أربعة تسلسلات من الايروموناس فيروني تطابقًا بنسبة ٩٩,٩٢٪ و ٩٩,٨٤٪ مع جين النووي الريبوسومي 16S لأنواع الايروموناس فيروني المتوفرة في قاعدة بيانات GenBank، والمسجلة بارقام MT302825 و KC633849، على التوالي. تم تجميع الموقع التطوري لتسلسلات النيوكليوتيدات المحددة هنا مع غيرها من أنواع الايروموناس فيروني التي تصيب الأسماك. كشفت نتائج تجربة التحدي المنقولة بالمياه أن عزل الايروموناس فيروني أظهر نفس الصورة المعملية للأسماك المصابة طبيعياً بمعدل نفوق بلغ ٧٥,٦٪. خلصت النتائج إلى أن الايروموناس فيروني هو مرض سائد في تربية أسماك البلطي في محافظة قنا، حيث يوفر التوصيف الكيميائي الحيوي والجينومي بيانات مهمة لإدارة المرض. وتسلط الدراسة الضوء على الحاجة إلى اتخاذ تدابير مراقبة وسيطرة مستهدفة ضد عدوى الأيروموناس في هذا القطاع الحيوي اقتصاديًا.