Research Square

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First Report and Molecular Identification of Staphylococcus warneri Infection in Aquaponic-Reared Nile Tilapia (Oreochromis niloticus) and Grass Carp (Ctenopharyngodon idella) in Egypt

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Abstract

Background

Aquaponic systems are becoming a common way to Nile tilapia production, but information on bacterial diseases that can emerge under these conditions is still limited. This study investigated the occurrence and pathogenic potential of *S. warneri* in Nile tilapia and grass carp reared in an aquaponic system in Egypt.

Results

Clinical signs were observed in 11 of 180 examined Nile tilapia (6.1%), whereas all 27 examined grass carp appeared clinically normal. Bacteriological examination recovered *S. warneri* only from diseased Nile tilapia; no isolates were obtained from apparently healthy tilapia or all examined grass carp. The isolates were identified as *S. warneri* based on biochemical characteristics and the VITEK 2 system. Molecular analysis confirmed the identification as S. warneri, through the representative strain ASUV30 that showed 99.93% sequence similarity sequences and 100% bootstrap support to reference *S. warneri* in the GenBank. The sequence was deposited in GenBank under an accession number PX531340. To our knowledge, this represents the first molecular report of *S. warneri* associated with aquaponic-reared Nile tilapia in Egypt. Experimental challenge showed that the isolate was able to develop clinical signs and a cumulative mortality rate of 40% in the infected fish.

Conclusions

The findings indicate that *S. warneri* can act as an opportunistic pathogen in aquaponic reared Nile tilapia. Its association with clinical disease and mortality highlights the importance of regular bacteriological monitoring and appropriate biosecurity measures in aquaponic systems.

Introduction

Aquaponic combine aquaculture and hydroponic in a closed, recirculating system in which fish waste is biologically converted into nutrients for plant growth, while plants simultaneously act as natural biofilters that improve water quality. This beneficial relationship reduces water consumption, minimizes environmental contamination, and eliminates the need for chemical fertilizers, allowing sustainable, year-round production of both fish and vegetables (1, 2). The continuous recirculation of water in aquaponics also creates a complex and dynamic microbial environment that can favor the growth and persistence of opportunistic bacterial pathogens. These conditions may compromise fish health, reduce system productivity, and raise public health concerns through the possible emergence of zoonotic microorganisms (3).

In Egypt, aquaponic systems are gaining increasing attention as a sustainable approach to address key challenges such as water scarcity, limited arable land, and growing environmental pressures. The integration of fish and plant production improves resource use efficiency while maintaining economic

feasibility. Despite this growing interest, studies investigating microbial dynamics, pathogen emergence, and associated health risks in Egyptian aquaponic systems remain limited (4, 5). Most available researches have focused primarily on system performance and productivity, with little attention given to microbial ecology and disease monitoring. Therefore, a better understanding of bacterial communities within aquaponic environments is essential for effective fish health management, ensuring food safety, and supporting the long term sustainability of these systems (6, 7).

Accordingly, a deeper understanding of bacterial communities in aquaponic environments is essential to improve fish health management, ensure food safety, and support the long term sustainability of these systems (6, 7). In particular, the detection and identifying of opportunistic and potentially zoonotic bacteria are of great importance, as these microorganisms can persist in biofilms, withstand environmental stress, and spread efficiently within recirculating aquaculture systems.

Staphylococcus warneri (*S. warneri*) among emerging bacterial concerns has attracted increasing attention as a potential opportunistic pathogen (8). Although it is classified as a coagulase negative staphylococcus and is typically part of the normal microbiota of the skin and mucous membranes, it can become pathogenic under certain conditions such as environmental stress, immunosuppression, high stocking density, or poor water quality. In human medicine, *S. warneri* has been associated with several clinical conditions, including septic arthritis, endocarditis, bacteremia, and meningitis, particularly in immunocompromised individuals or patients with indwelling medical devices (9). Its ability to form biofilms and exhibit antimicrobial resistance further enhances its persistence and clinical relevance, especially in aquatic environments.

In aquaculture, *S. warneri* has been isolated from a variety of fish species including rainbow trout, Siberian sturgeon (*Acipenser baerii*), catfish (*Clarias* spp.), bronze gudgeon (*Coreius guichenoti*), and seven khramulya (*Capoeta capoeta*) and is increasingly recognized as a contributor to disease outbreaks (10, 11). Infected fish often develop systemic infections characterized by ascites, exophthalmia, hemorrhages, skin ulcerations, and septicemia, frequently leading to high morbidity and mortality (12). The occurrence and pathogenic role of *S. warneri* in aquaponic systems particularly in key cultured species such as Nile tilapia (*Oreochromis niloticus*) and grass carp (*Ctenopharyngodon idella*) remains scarce.

Furthermore, the potential zoonotic implications of *S. warneri* in aquaponic environments require careful investigation. The close interaction between fish, plants, water, and human handlers in these systems may facilitate the transmission of opportunistic pathogens, especially in the absence of strict biosecurity measures (3). This highlights the importance of adopting a One Health approach that recognizes the interconnected health of aquatic animals, humans, and the environment.

This study investigated an emerging and previously unreported *Staphylococcus. warneri* infection in aquaponically reared Nile tilapia and grass carp in Egypt. Particular emphasis was placed on clinical signs, bacterial isolation, biochemical and molecular identification, experimental pathogenicity testing. These findings offer new insight into an emerging bacterial threat facing aquaponic systems.

Material and Methods

1- Aquaponic system

The investigated aquaponic system consisted of 4 rectangular cement ponds (3 × 5 x 1.25 m each) lined with a waterproof inner coating. Each pond had water inlet and outlet pipes, a continuous aeration system, and a drainage outlet for sludge removal. The effective water volume of each pond was about 15 m^3.

2- Fish for survey study:

Two hundred and seven (207) aquaponic reared fishes, one hundred and eighty (180) Nile tilapia with an average body weight 90 ± 20g, twenty seven (27) grass carp with an average body weight 60 ± 10g. A total of 30 Nile tilapia and 4:5 grass carp were examined at each sampling time point across six sampling times a long 12 month from 31/10/2023 to 1/11/2024 periods, one sampling time each two months. Fishes were collected from an aquaponic system at faculty of Science AL-Azhar University, Assiut, Egypt. Fish were stocked in the aquaponic at a density of 50 fish/m^3, comprising 40 Nile tilapia and 10 grass carp.

3- Fish for pathogenicity

A total of fifty (50) apparently healthy Nile tilapia with an average body weight 70 ±5g were obtained from a private tilapia farm at Wadi Samhoud region, New Valley province and transported to the wet lab., fish diseases and management department, for acclimation and using for the experimental infection.

4- The Gene JET genomic DNA purification kit (Thermo-Scientific, Berlin Germany)

4.1. Emerald Amp GT PCR master mix (Takara) Code No. RR310A

4.2. Oligonucleotide primers

27F (5’-AGAGTTTGATCCTGGCTCAG-3’)

1492R (5’- GGTTACCTTGTTACGACTT-3’)

5- Clinical and PM Examination of Fish

All fishes were clinically examined according to Noga, (2010) (13). The postmortem examination was performed on all freshly dead and moribund fish according to Austin and Austin, (2012) (14).

6- Bacteriological isolation.

Bacterial isolates were collected from liver, spleen and kidney of the examined fishes and inoculated in Tryptic soya broth media (Oxoid Basingstoke, United Kingdom). After 12- 24hr.’ incubation time the

inoculum was sub cultured on Tryptic soya agar media (Oxoid Basingstoke, United Kingdom) and incubated at 25 ±1°C for up to 48hs (15).

7- Bacteriological identification

7.1. **biochemical identification**

The biochemical identification tests were performed as described in Pincus, 2006 (16).

7.2. **VITEK 2 system**

The isolates identification was confirmed using the VITEK 2 system (Model VITEK® 2 60, Biomerieux, France). The biochemical reaction profile generated for each isolate was compared against the extensive VITEK 2 database to determine the closest matching organism and its associated probability of identification (17).

7.3. Molecular Identification

7.3.1. DNA extraction from the isolates

The 16S rRNA gene sequences of the isolates were sequenced in Solgent Co. Ltd, South Korea using Sanger dideoxy sequencing technology. DNA was extracted from the isolates using the Gene JET genomic DNA purification kit (Thermo Scientific, Berlin, Germany) according to the manufacturer recommendations, its concentration was measured by Nanodrop™ spectrometer (NanoDrop, Wilmington, DE, USA) then it was preserved at -20°C till be used.

7.3.2. PCR and 16S rRNA gene sequencing: -

DNA was amplified using Emerald Amp® GT PCR master mix (Takara, Japan) and two sets of universal primers amplify 16S rRNA gene; 27F (5´- AGAGTTTGATCMTGGCTCAG) and 1492R (5´-TACCTTG TTACGACTT 3´) (18, 19). PCR was conducted in 50µl reaction mixture according to master mix manufacturer's instructions where contain 25µl of MyTaq red mix **(**Takara, Japan**)**, 2µl of each primer, 4µl of template DNA and 19µl of H2O (RANase/DNase free). PCR amplification was performed in the thermal cycler (Applied Biosystems, USA) under the conditions previously described by (20); starting with an initial denaturation step at 95°C for 5 min. Then, this was followed by 35 cycles consisted of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, an extension step at 72°C for 1.5 min, and a final extension step at 72°C for 10 min. The amplification products were analyzed by gel electrophoresis of 1.5% agarose in Tris-acetate EDTA buffer, stained with ethidium bromide (50µl/L) and visualized on a UV transilluminator2000 (Bio-Rad). A 100 bp DNA ladder was used to determine the size of the PCR products. Zymoclean Gel DNA Recovery Kit (Zymo Research, USA) used to purify PCR products from gel for sequencing. Amplicons were purified and sequenced by 3500 Genetic Analyzer (Applied Biosystems, U.S.A.) using Applied Biosystems kit (Applied Biosystems, USA) and analyzed by the Basic Local Alignment Search Tool (BLAST) (http://www.ncbi.nlm.nih.gov/blast).

7.4. Phylogenetic analysis

The phylogeny of *S. warneri* was inferred using maximum-composite-likelihood distances and the neighbor - joining method implemented in MEGA 12.0 (21). A neighbor-joining tree was reconstructed based on 16S rRNA gene sequences of related taxa to assess evolutionary relationships (22).

8- Pathogenicity test

A total of fifty (50) acclimated Nile tilapia (*Oreochromis niloticus*) was randomly allocated into five groups (10 fish/group). The experimental design consisted of the following treatments:

1. Negative control group: Fish received no injection.
2. Positive control group: Fish were intraperitoneally injected with 0.1mL of sterile physiological saline.
3. Experimental infection groups: *S. warneri* challenge treatment, consisting of three replicate groups (10 fish/replicate). Fish in each of the three replicate groups were intraperitoneally injected with 0.1mL of *S. warneri* complex suspension adjusted to 0.5 McFarland standard (1.5×10^8 CFU/mL). Fish were stocked at a stocking density of 20g/L and fed a commercial pelleted ration containing 25% crude protein at a rate of 3% of body weight, twice daily.

Results

1. Clinical and post mortem findings

Clinical examination revealed that 11 Nile tilapia (6.1%) exhibited visible clinical signs, whereas 169 fish (93.9%) appeared clinically normal. While all grass carp were clinically normal (Table 1).

Nile tilapia showed a range of external clinical signs including skin depigmentation, erosion or rot of the tail, dorsal, and anal fins, scale loss. Some individuals also displayed skin blisters, marked hemorrhages around the dorsal fin, and few cases showed skeletal deformities characterized by lordosis. Post-mortem examination of diseased Nile tilapia revealed congestion and hemorrhages along the abdominal wall and enlarged livers that were either congested or pale. Eye opacity and the presence of bloody fluid within the abdominal cavity (Figures 1 - 3).

Table (1) the number of examined, clinically diseased and apparently health fishes.

Fish species	Number of examined fish	Clinically diseased fish		Apparently healthy fish	
	Species	No.	%	No.	%
Nile tilapia (*Oreochromis niloticus*)	180	11	6.1	169	93.9
Grass carp *(Ctenopharyngodon idella)*	27	0	0	0	0

2. Bacterial identification

2.1. Morphological identification

Gram staining revealed the all isolates were Gram positive bacteria

2.2. Biochemical identification

2.2.1. Traditional biochemical tests

The biochemical characterization of the bacterial isolates showed that all isolates were negative for Cytochrome oxidase, Indole and Citrate utilization, positive for Catalase and Glucose fermentation and Voges-Proskauer, while they were variable for Methyl Red and Urease.

2.2.2. VITEK 2 system

The results of VITEK-2 system confirmed the initial morphological and biochemical identification patterns and classified the 11 examined isolates to *S. warneri* (Table 2)

Table (2) results of the study isolates identification by the VITEK 2 system.

Card type	Bacterial strain	Clinically diseased fish	Probability %	Confidence Level
GP	*Staphylococcus warneri*	9	99	Excellent
		2	94	Very good

2.2.3. Molecular Identification and Phylogenetic Analysis of *Staphylococcus warneri*

Phylogenetic analysis was performed by comparing the 16S rRNA gene sequence of isolate ASUV30 with the validated *Staphylococcus* reference sequences available in GenBank. The highest sequence identity was observed between ASUV30 and *S. warneri* strain AW25 (NR-025922.1), with 99.93% identity. Lower sequence identities were observed when ASUV30 was compared with other *Staphylococcus* species.

The 16S rRNA phylogenetic tree placed this study isolate ASUV30 within the *Staphylococcus* clade and grouped it most closely with *S. warneri* strain AW25 (NR-025922.1). This relationship was supported by a

bootstrap value of 100%. The remaining *Staphylococcus* reference strains formed separate branches from ASUV30 (Figure 13).

3. Pathogenicity test

The experimentally infected Nile tilapia consistently exhibited both behavioral and physical abnormalities. Infected fish commonly congregated in the corners of the aquarium near the air stone and displayed reduced swimming activity and lethargy, noticeable scale loss, skin ulcers, fin rot and loss of skin pigmentation (Figures 5 & 6)

Cumulative mortality reflected the degree of pathogenicity. By the end of the experimental period, cumulative mortality reached 40%, indicating that *Staphylococcus warneri* exhibits high pathogenic potential in Nile tilapia

Discussion

Nile tilapia is widely used in aquaponic systems due to its rapid growth, efficient feed utilization, and tolerance to variable water quality. In these integrated systems, fish waste is converted into plant available nutrients, enabling simultaneous fish and vegetable production under relatively stable environmental conditions (23).

In the present study, clinical examination of 207 fish revealed that only 11 Nile tilapia (6.1%) exhibited visible clinical signs, while all grass carp remained clinically healthy. This marked interspecific difference suggests species-dependent susceptibility under aquaponic culture conditions. The low overall morbidity indicates a subclinical or opportunistic infection rather than an acute outbreak. These findings suggest that Nile tilapia may be more susceptible to *S. warneri* infection, consistent with previous reports describing increased vulnerability of fish under intensive or recirculating systems (24).

Clinically affected Nile tilapia showed external lesions including fin erosion, scale loss, skin depigmentation, cutaneous hemorrhages, and occasional skin blisters. Sporadic skeletal deformities (lordosis) were also observed. These lesions are consistent with chronic to subacute septicemic bacterial infections typically associated with opportunistic pathogens in fish exposed to environmental or physiological stress (25). Post-mortem examination revealed systemic pathological changes, including abdominal congestion, multifocal hemorrhages, hepatomegaly (pale or congested liver), ocular opacity, and hemorrhagic ascites. These lesions are indicative of generalized bacteremia and multi-organ dysfunction, commonly reported in warm-water fish under stress-induced immunosuppression (26, 27). In contrast, the absence of similar findings in grass carp suggests species-specific resistance or lower susceptibility to infection.

Bacteriological examination consistently recovered Gram positive cocci from all clinically affected Nile tilapia. Phenotypic characterization showed uniform biochemical profiles, which were confirmed using

the VITEK 2 system, identifying all isolates as *S. warneri*, supporting the reliability of both conventional and automated identification methods (28).

Molecular analysis of representative isolate ASUV30 confirmed its identity as *S. warneri*, showing 99.93% 16S rRNA sequence similarity and 100% bootstrap support with *S. warneri* strain AW25 (NR-025922.1). The sequence was deposited in GenBank under accession number SUB15776313 (Staphylococcus PX531340), this is the first report and molecular confirmation of *S. warneri* from aquaponic-reared Nile tilapia in Egypt. These findings meet the accepted criteria for species-level identification based on 16S rRNA gene sequencing (29, 30).

Experimental infection demonstrated that *S. warneri* has moderate pathogenic potential in Nile tilapia. Infected fish exhibited lethargy, reduced swimming activity, aggregation near aeration sources, and pronounced pigmentation loss, all indicative of systemic infection and physiological stress. Cumulative mortality reached 40%, confirming that the bacterium can induce significant disease under experimental exposure, particularly when host resilience is compromised (31). These findings support the classification of *S. warneri* as an opportunistic pathogen in aquaponic Nile tilapia. Its exclusive recovery from diseased Nile tilapia, combined with its absence in all grass carp, suggests that infection outcome is strongly influenced by host susceptibility and species-specific immune responses (32). Aquaponic environments may further enhance this risk by promoting bacterial persistence through organic enrichment, biofilm formation, and continuous host exposure (28).

Although *S. warneri* is classically considered part of the environmental and commensal microbiota, its increasing detection in diseased aquatic animals suggests a shift in opportunistic pathogen dynamics under intensive production systems. While it is also recognized as an opportunistic pathogen in humans, its detection in aquaculture settings does not necessarily indicate zoonotic transmission (32, 33). Rather, it highlights aquaculture and aquaponic systems as shared microbial ecosystems where environmental, animal, and public health interfaces converge (33). Accordingly, strict biosecurity measures, routine microbial surveillance, and improved management practices are essential to minimize opportunistic infections and ensure safe and sustainable aquaponic production (34).

Conclusion; Aquaponic-reared Nile tilapia was more susceptible to opportunistic bacterial infections than grass carp, with *S. warneri* identified as an emerging pathogen. Molecular, clinical, and experimental evidence confirmed its ability to cause systemic infections and stress-related mortality. This first molecular report of *S. warneri* in Egyptian aquaponic reared Nile tilapia highlights the need for strict biosecurity, routine microbial monitoring, and improved management to ensure fish health and sustainable production.

Declarations

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Ethics approval and consent to participate

The experiments were approved by the Veterinary Medical Ethics Research Committee, Faculty of Veterinary Medicine, Sohag University, Egypt (Code no. Soh. un. vet/ 000121M3). This study follows the ARRIVE guidelines (https://arriveguidelines.org).

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Data availability

All data supporting the findings of this study are available with co-authors. Microsatellite primer sequences are provided in text page 6, along with original reference describing the microsatellites used in this study.

Competing of interest

I declare that the authors have no competing interests as defined by BMC, or other interests that might be perceived to influence the results and/or discussion reported in this paper

Consent for publication

This article is not applicable.

Authors' contributions

M.A.A.A., M.H.M., and A.G.M. contributed to the conception of the study, experimental design, and methodology development, and participated in data analysis, interpretation, and manuscript revision. R.M.Y.A. and O.H.S.Z. were responsible for sample collection, data recording, and drafting the initial manuscript. All authors critically revised the manuscript, approved the final version, and agree to be accountable for all aspects of the work.

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Figures

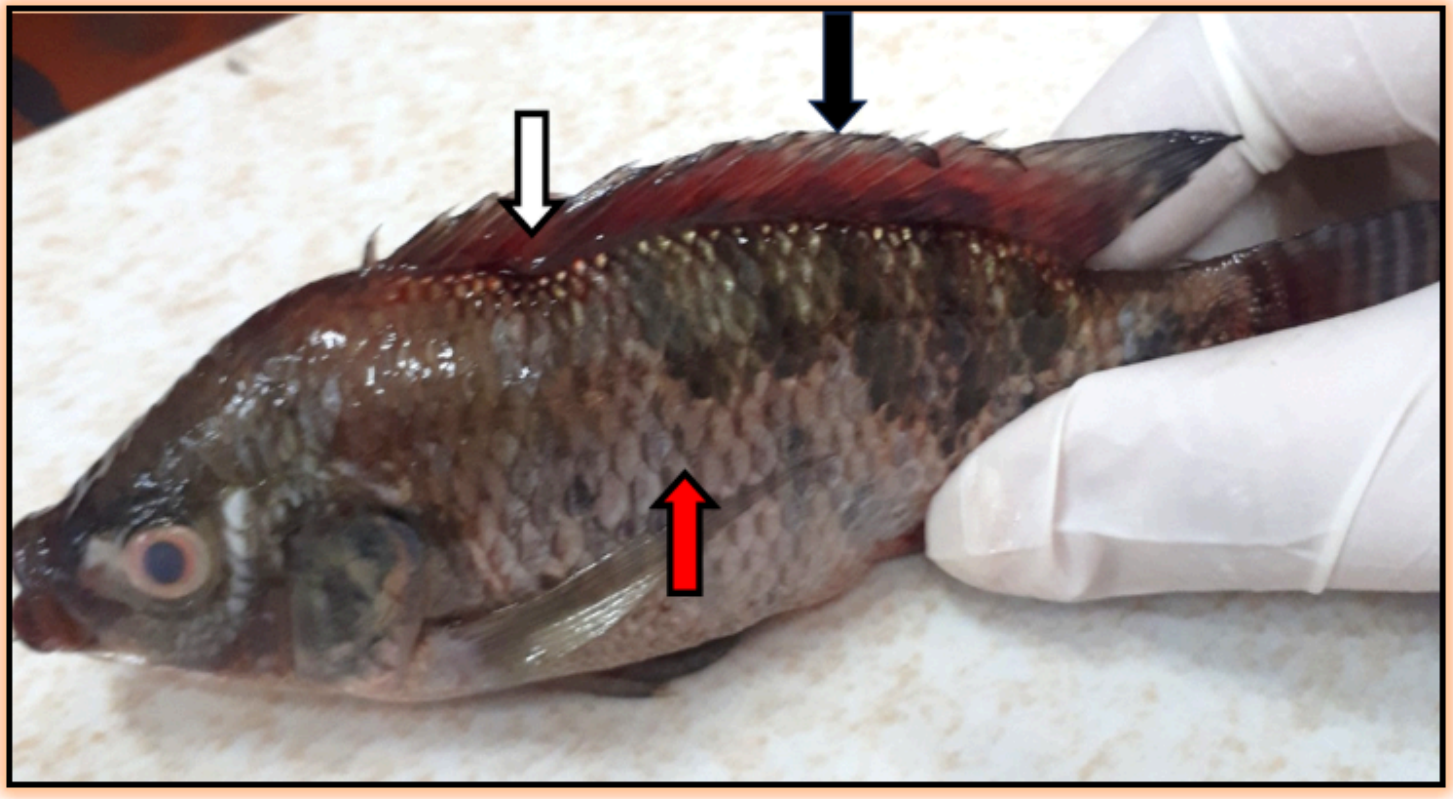

Figure 1

Nile tilapia showed hemorrhages at the dorsal fin (black arrow), scale-loss (red arrow) and skeletal deformity (Lordosis)

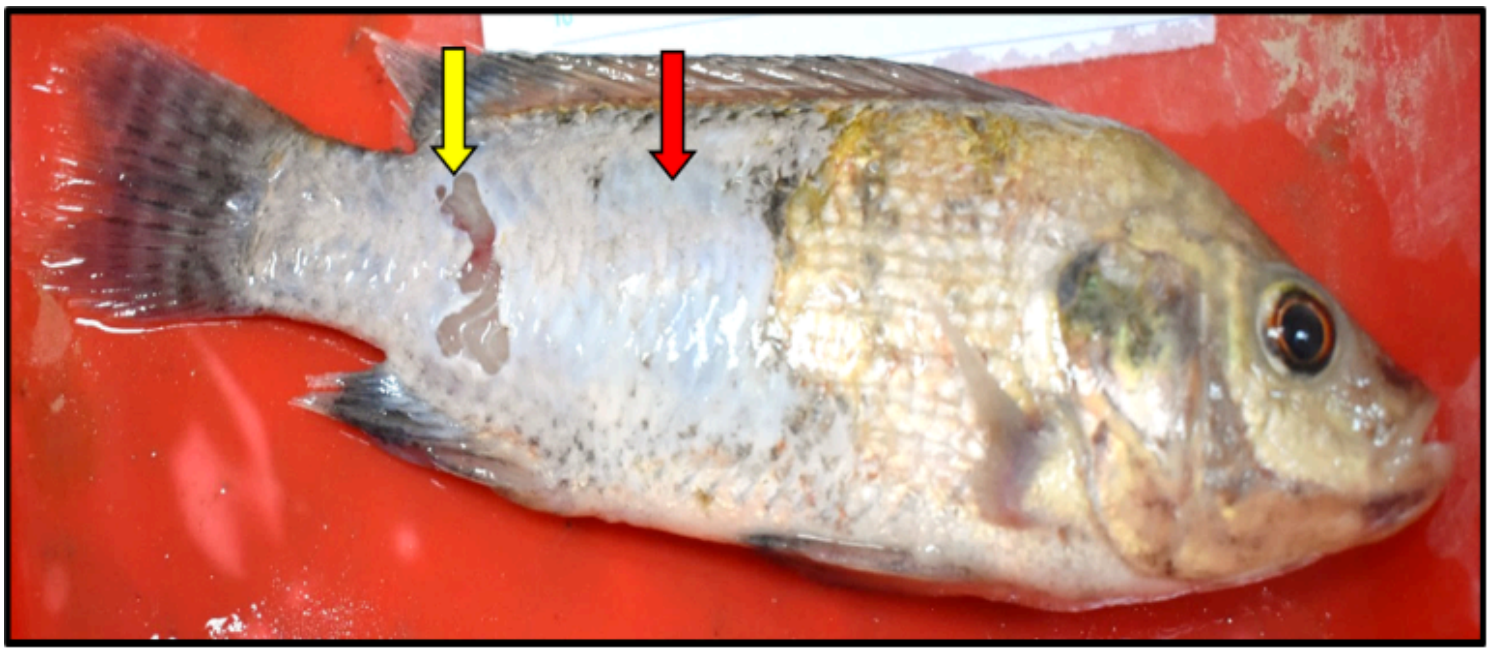

Figure 2

Nile tilapia with skin depigmentation, scale-loss and skin blister (red arrow) and skin ulcer (yellow arrow)

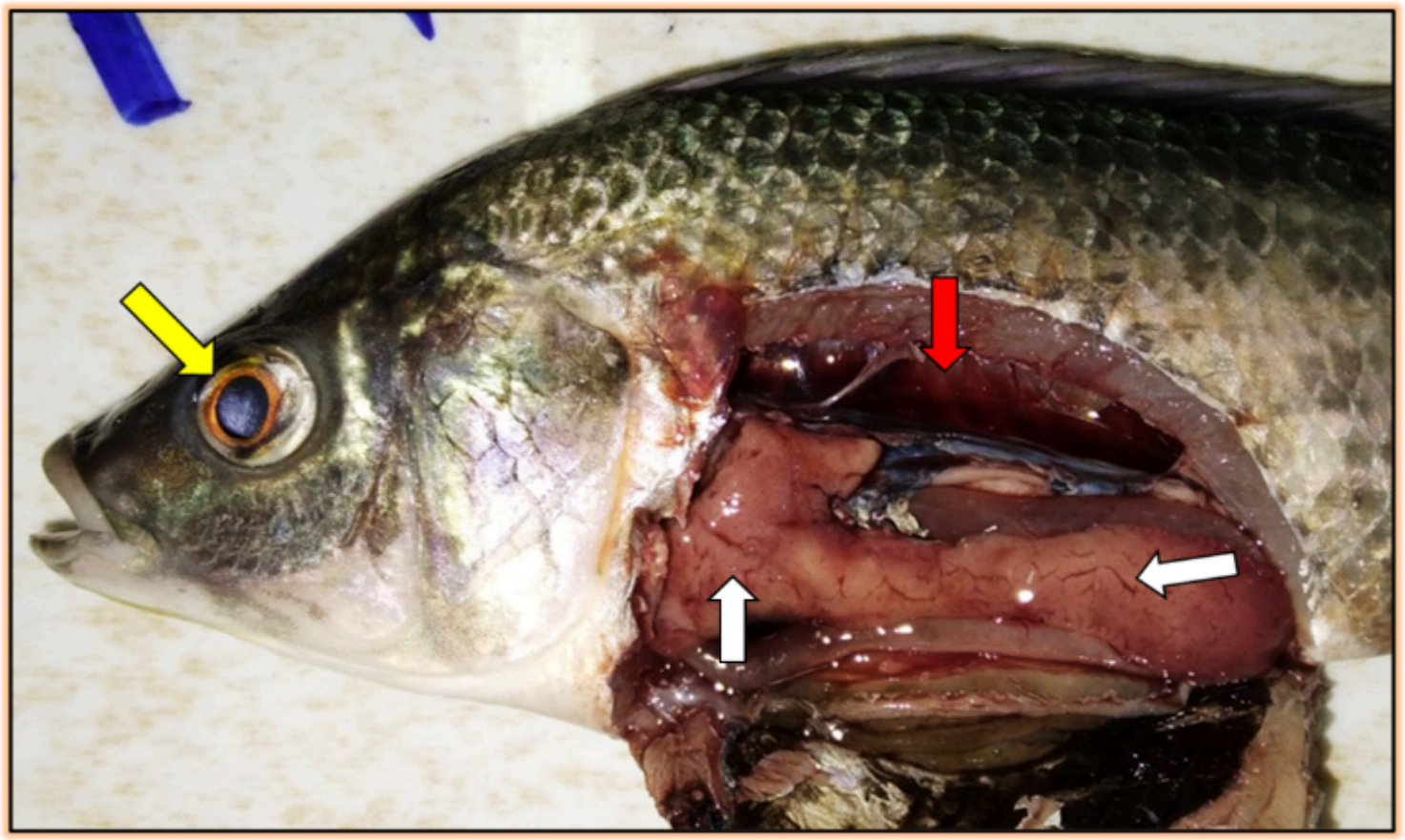

Figure 3

Nile tilapia showed eye obesity (yellow arrow), bloody exudate in the abdominal cavity (red arrow) and pale liver (white arrow)

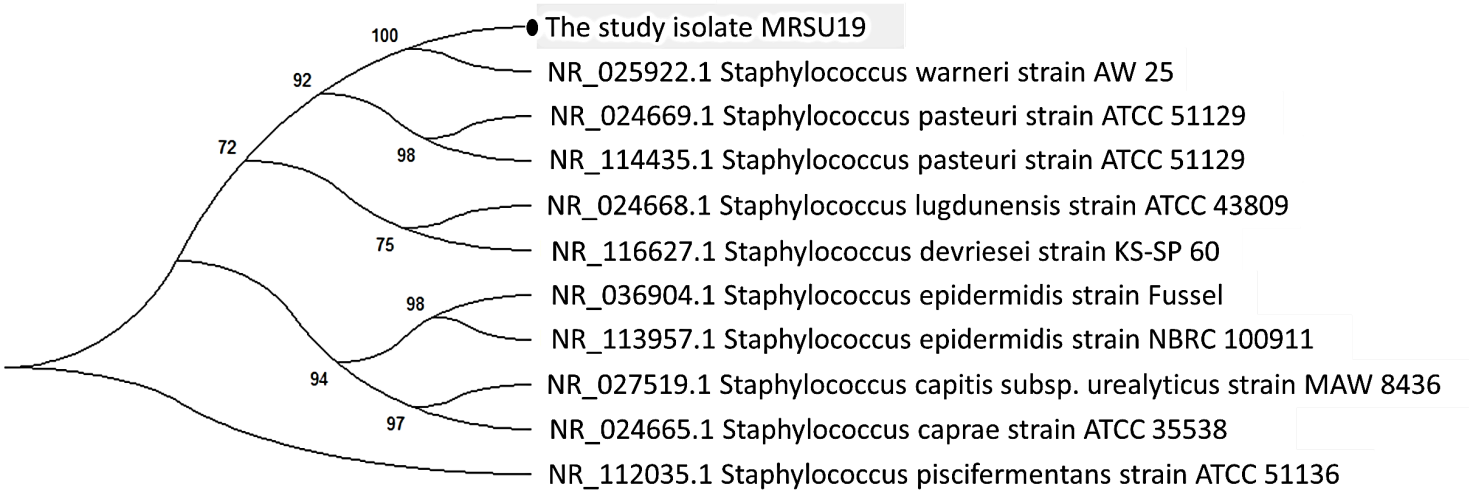

Figure 4

Phylogenetic analysis based on 16S rRNA gene sequences was conducted using the neighbor-joining method to assess the relationship between strain MRSU19 and its closest relatives. Bootstrap support values >50% are shown at the nodes. A total of 11 nucleotide sequences were included in the analysis. Ambiguous positions were removed using pairwise deletion, resulting in a final alignment of 1,412 positions.



Figure 5

Nile tilapia experimentally infected with *S. warneri* displayed clear skin depigmentation.

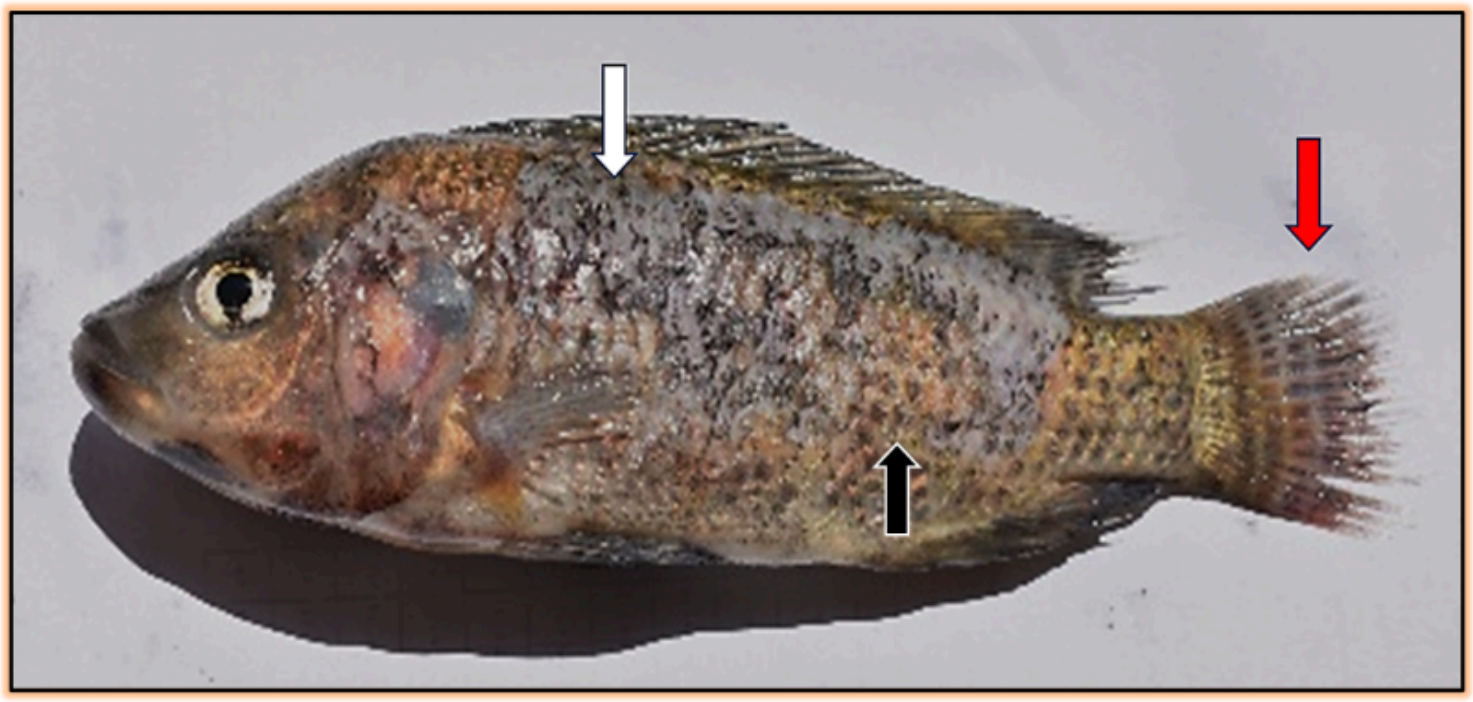

Figure 6

Nile tilapia showing skin depigmentation (black arrow), fin rot (red arrow), skin surface ulceration and scale-loss (white arrow).