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IDENTIFICATION AND CHARACTERIZATION OF *Aeromonas caviae* ISOLATED FROM INFECTED GIFT TILAPIA (*Oreochromis mossambicus*) NURSERY

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Abstract

Aeromonas caviae CMST AAHL was isolated and identified from the infected Tilapia moribund samples. Dominant bacterial microbiota was isolated from infected fishes and challenged with healthy GIFT Tilapia fishes and monitored the survival. Among the different strains challenged, GT3, GT4 and GT5 had influenced to lower survival and higher virulence factors such as proteolytic, haemolytic, gelatinase and cellulose activity. The three virulent strains are also able to produce biofilm. Histopathaological studies also revealed that, the affected tissue parts had found inflammation and necrosis. Antibiotic sensitivity tests confirmed that, GT3, GT4 and GT5 were susceptible for gentamycin, tetracycline and chloramphenicol. Based on the strong virulence and pathetic effect of the GT3 strain, the strain was genomically identified by 16S rRNA sequencing and confirmed as *A. caviae* and deposited the GenBank and the accession number is OQ804404.1.

Keywords: *Aeromonas caviae,* Aquaculture, Fish Disease, *Oreochromis mossambicus,* Virulence Factors

Introduction

Tilapia has made significant contributions to food security in various countries such as China, Egypt, the Philippines, Brazil, Thailand and Bangladesh. One of the most reliable fish species for securing a position as a substitute for food security is tilapia, particularly given its expanding population and affordable protein. An estimated 4.0 million tons of tilapia are produced worldwide, with a 3.0 billion dollar market value. The FAO report projects that by 2030, 7.3 million tons of tilapia will be produced worldwide (Bhendarkar & Kalbande, 2022). Among this species, GIFT Tilapia can truly be a gift for aquaculture due to various benefits such as grow in wide range of environmental condition, well-resistant to illness and poor water quality and enhancing socio-economic and welfare performance of the related aquaculture systems (Balarin & Haller, 1982; Dey, 2000). Compared to the most widely cultivated strain, the GIFT strain was found to grow 60% faster on average and have 50% higher harvest survival (Sultana et al., 1997).

Microbial diseases such as bacterial, viral and parasitic infections are the important disease outbreaks in GIFT tilapia culture. Among the bacterial infections, *Aeromonas* spp especially *A. caviae* is one of the important pathogens affect GIFT tilapia. *A. caviae*, a facultative anaerobic Gram-negative bacterium belonging to Aeromonadaceae family, present in food, drinking water, sewage, and environmental water and infect a wide range of hosts, including fish and humans (Rey et al., 2009). They can infect many freshwater cultivable fish species including *Clarias batrachus, Oncorhynchus mykiss, Cyprinus carpio, Ctenopharyngodon idellus* and the crayfish *Procambarus clarkii* etc (Thomas et al., 2013; Zepeda-Velázquez et al., 2017; Haipeng et al., 2014) and the symptoms are surface swimming, listlessness, inappetence, hemorrhagic septicemia, ulceration, hepatosplenomegaly, eye disease. (Thomas et al., 2013; Baldissera et al., 2017; Ogara et al., 1998).

Numerous virulent factors, such as cell surface structures that allow bacteria to bind to a large number of cells and biomolecules in a lectin-like manner, must be produced by these microbes in order for them to colonize and invade a host (Janda & Dujey, 1988). A wide variety of virulence factors, such as extracellular toxins, enzymes, and other molecules that contribute to bacterial pathogenicity, are produced by Aeromonas species (Kanai & Wakabayashi, 1984). The harmful toxins like hemolysin, aerolysin and cytotonic enterotoxins reported by Sen and Rodgers (2004) and five 5 virulence genes such as alt, ast, act, aer and hly were reported by Dallal et al. (2016). The present study intends to isolate and characterize the bacterial pathogens from infected GIFT tilapia nursery tanks during disease outbreak.

Material and Method

Infected GIFT Tilapia samples:

Disease infected fishes (7±1 cm length) associated with high mortality was collected from a biofloc fish farm located at Swamithoppu village, Kanyakumari district (8°07′00.37″N, 77°29′28.12″ E), Tamilnadu, India during winter season. The fishes fed with normal commercial pellet feed and the water temperature in the culture system is 28± 0.5 °C and oxygen level is maintained of 6.5 to 7.5 mg/l. The affected fish exhibited abnormal behaviour like lethargy and erratic swimming, and clinical signs of skin lesions, red spots on body surface, scale loss and blind eyes. The collected fish sample was immediately transported to laboratory in sterilized polythene bags in ice cold condition. The fish sample was identified using standard reference manuals (Zacharias *et al*., 2013).

Bacterial isolation:

Infected GIFT Tilapia fishes were washed with sterile distilled water to remove any external undesired dusty materials. The muscle tissue of infected fish was dissected under sterilized conditions. Further, the tissue was homogenized using sterile distilled water and centrifuged at 12,000 rpm for 10 minutes. After centrifugation, the supernatant was taken and serially diluted with sterile distilled water. From the serially diluted samples, 100 µl of 10^{-5}, 10^{-7}, 10^{-9} and 10^{-11} diluted samples were spread plate onto nutrient and aeromonas agar and incubated at 28 °C for 24 hours. Growth of bacterial colonies on the plates was examined and counted (Velmurugan et al., 2015). Dominant and morphologically different colonies were chosen and further streaked on nutrient agar plates and Aeromonas isolated agar to get pure cultures which were then maintained for future use. The microbial colonies were purified using the quadrant streaking method. Glycerol stocks of individual isolates were maintained in deep freezer for further use.

Challenge studies by dominant bacterial microbiota:

Healthy disease free GIFT tilapia fishes had the mean weight of 10 ± 1 g (10 fish × 3 tanks) were intraperitoneally injected with 100 µl of live bacterial cells (GT1 to GT5) individually at the LD_{50} dose concentration of 10^7 CFU/fish^{-1} . As a control, 100 µl of 1X PBS (without bacterial cells) was injected into a group of thirty fishes. Percentage survival was examined every day for ten days in control and experimental groups. After the challenge study, all bacterial challenged fishes were treated with sodium hypochlorite solution and buried in soil.

Bacterial identification by phenotypic level:

Infected GIFT tilapia was used to isolate the dominant bacterial microbiota, GT1 to GT5, which were then chosen for phenotypic identification. The identification protocols including morphology, biochemical and physiological confirmative tests were based on the methodologies described by Bergey's Manual of Systematic Bacteriology (Vos et al., 2009) and the Bacteriological Analytical Manual (Tallent et al., 2012).

Virulence factor studies by dominant bacterial microbiota:

Protease activity

The selected bacterial isolates were single streaked in skim milk agar plates and kept it in incubation at 37°C for 24 hours. After incubation time the plates were observed for zone formation around the colonies. Presence of clear zone around the colonies indicated as positive result and no zone around the colonies was taken as negative result (Jasna Beganovic et al., 2013).

Hemolytic activity

The blood agar was prepared by adding 1.5 ml of blood in 100 ml of nutrient agar. After solidification the isolates were streaked on the plates and allowed it for incubation up to 24 hours at 37°C. After 24 hours, the hemolytic activity was observed by the presence of zone formation around the colonies and non-hemolytic activity was observed by the absence of zone formation around the colonies (Tallapragada et al., 2018).

Lipolytic activity

The selected bacterial isolates were single streaked on the sprit blue agar media and incubated at 37 °C for 24 hours (Tuynenburg Muys and Willemse, 1965). After the incubation period, presence of zone indicated as positive result and the absence of zone indicated negative result.

Gelatinase activity

Overnight culture of the bacterial isolates were streaked on nutrient gelatin agar plates and incubated at 37 ºC for 24 hours. After incubation, the plates were flooded with Frazier’s reagent which produced clear zone around the colonies indicated positive result (Clarke, 1953).

Cellulase activity

Active culture of selected bacterial isolates were streaked on carboxymethyl cellulose agar and incubated at 37 ºC for 48 hours. After incubation, the plates were stained by 0.1 % Congo red stain followed by washing with 1 M NaCl. Positive result indicated the formation of clear zone of hydrolysis (Gohel et al., 2014).

Biofilm formation

In test tubes, 10 mL of Luria Bertani (LB) broth was used to cultivate pure bacterial microbiota (GT1 to GT5). For twenty-four hours, the inoculated tubes were incubated at 37°C. Following the incubation period, the tubes' contents were carefully tapped out. Phosphate Buffer Saline (PBS) at pH 7.3 was also used to wash the tubes and they were then left to dry at room temperature. After drying, 0.1% (w/v) crystal violet (cv) was used to stain the tubes. The tubes were cleaned with distilled water to get rid of any excess stain. By looking for visible film on the tube's wall and bottom, biofilm formation in tubes was further verified. The control group did not receive any bacterial cell inoculation (Christensen *et al*. 1982).

Histopathology

The infected GIFT tilapia fishes' eye, gill, gut, and muscle tissues were removed and preserved for 24 hours in 10% buffered formalin. All fixed tissues underwent a 24-hour process that included xylene clearing, graded alcohol processing, impregnation, and paraffin wax embedding. As a decalcification procedure, 0.5% trichloroacetic acid (TCA) was applied overnight to cut blocks of the embedded tissues. After being thoroughly cleaned under running water, the decalcified tissue was sectioned (5–6 µm). Following deparafinized sections' rehydration in graded alcohol, they underwent H&E staining, dehydration, xylene clearing, and DPX mounting. Using a compound microscope (Cos Lab, India), stained sections was examined for pathological signs were captured on camera and documented (Bernet et al., 1999).

Antibiotic susceptibility test

Antibiotic susceptibility was performed against selected antibiotics such as gentamycin, tetracycline, erythromycin, ampicillin and chloramphenicol. The selected bacterial isolates were swabbed evenly over the plate, and then the antibiotic discs were placed above the isolated strains swabbed area and kept the petriplates for incubation at 37°C for 24 hours. After incubation, the clear zone around the discs was measured using a ruler and the results were tabulated (Tallapragada et al., 2018).

Genomic level identification

Based on the strong virulence and pathetic effect to the challenged fishes, strain GT3 was genomically identified by 16SrRNA sequencing. To these 100 ng of genomic DNA which was extracted from the GT3 using MEDOX- Easy genomic DNA extraction kit (Chennai, India) and the 16S rRNA gene was amplified using the universal primer (F: 5-AGAGTTTGATCCTGGCTCAG-3′; R:5-TACGGCTACCTTGTTACGACTT-3) with the standard PCR conditions of initial denaturation 94°C for 5 min and each cycle with denaturation at 94°C for 45 second, annealing at 55°C for 30 second; extension at 72°C for 1.5 min; Final extension at 72°C; end 4°C for 5 min. The PCR products was run in 0.8 % agrose gel electrophoresis and viewed under UV transilluminator. Further the amplicon was purified and sequenced and the sequences were compared with reference sequences using BLASTn in NCBI database. Gene sequences were aligned using the Clustal W (Thompson et al., 1994). The phylogenetic tree was constructed by the Neighbour Joining method by using MEGA 6.0 (Saitou and Nei, 1987). The gene sequences of strains were submitted to the National Centre for Biotechnological Information (NCBI) to get accession number.

Data analysis

One way Analysis of Variance (ANOVA) was carried out using the SPSS statistics data package. Means were compared at 0.05 % level and subsequent post-hoc multiple comparison with SNK test.

Results

The fishes survived 95% up to ten days when no bacterial challenge was given. The survival was significantly ($P <= 0.0001$) decreased to 80 and 70 % in the fish groups challenged with GT2 and GT1 respectively. Further the fishes succumbed to death cent percentage at the 6th day after challenged with GT3 strain. The groups GT4 and GT5 challenged fishes survived 10 and 5 % only at 6th days after challenge. Among the different strains (GT1 to GT5) challenged to the fishes, GT3, GT4 and GT5 had effectively killed the fishes due to its virulence factors. One way ANOVA with SNK post-hoc multiple comparison analysis revealed that the survival was significantly differed ($P < 0.05$) among the different stains challenged with the fishes (Fig. 2).

Figure 1. Infected GIFT Tilapia sample collected from grow out tanks during mass out break

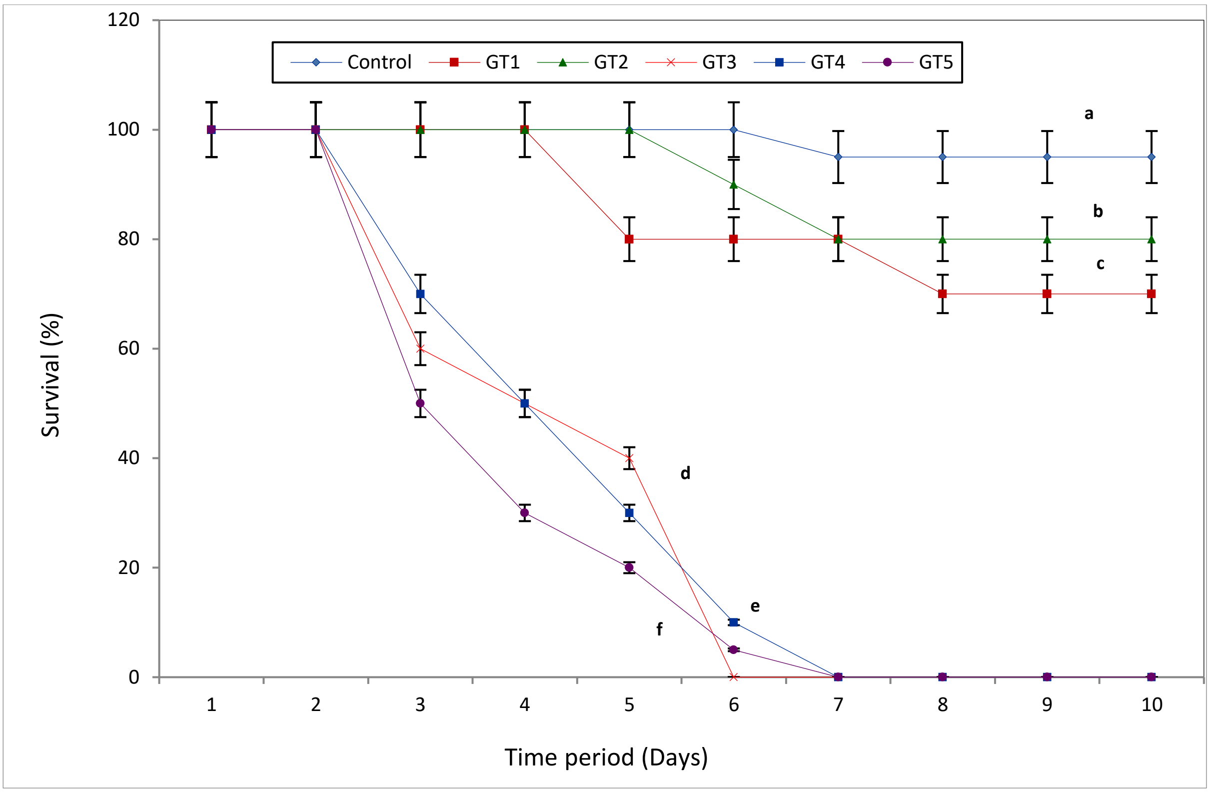

Figure 2. Survival (%) of GIFT Tilapia juveniles challenged with the dominant bacterial micro biota isolated from infected GIFT Tilapia. Bars with different lowercase letters are statistically differed from each other (one-way ANOVA, $P < 0.05$ and subsequent post-hoc multiple comparison with SNK test).

The phenotypic identification including morphological and biochemical characteristics were given in the table 1. Among the different strains, all the strains were motile and rod shaped. The strains GT1 and GT2 were Gram positive and GT3 to GT5 were Gram negative. The higher virulent strains, GT3 to GT5 were indole, methyl Red, Voges Proskauer, citrate, oxidase and catalase positive. The virulent strains, GT3 to GT5 were able to ferment the carbohydrates including glucose, sucrose, mannitol and maltose and unable to ferment lactose. Also the three strains were able to hydrolyze starch and gelatin respectively.

The virulence factors tested against the different stains, the GT1 and GT2 strains had mostly less and no activity for proteolytic, haemolytic, lipolytic, gelatinase and cellulose. GT3 strain had strong positive for proteolytic, haemolytic, lipolytic and gelatinase activity. GT5 strain had strong positive for proteolytic, haemolytic and gelatinase activity (Table 2).

Table 1. Phenotypic identification of selected bacterial micro biota isolated from isolated from infected GIFT Tilapia

Sl. No	Biochemical tests	Dominant Bacterial micro biota isolated				
		GT1	GT2	GT3	GT4	GT5
1	Motility	Motile	Motile	Motile	Motile	Motile
2	Gram staining	+	+	-	-	-
3	Cell shape	Rod	Rod	Rod	Rod	Rod
4	Indole	-	-	+	+	+
5	Methyl Red	+	+	+	+	+
6	Voges Proskauer	-	-	+	+	+
7	Citrate	-	+	+	+	+
8	Oxidase	+	+	+	+	+
9	Catalase	+	+	+	+	+
10	Nitrate reduction	-	-	+	+	+
11	Carbohydrate Fermentation					
	Glucose	+	-	+	+	+
	Sucrose	+	-	+	+	+
	Lactose	+	-	-	-	-
	Mannitol	+	+	+	+	+
	Maltose	+	-	+	+	+
12	TSI	-	+	K/A	K/A	K/A
13	Starch hydrolysis	+	-	+	+	+
14	Gelatin hydrolysis	-	-	+	+	+

+: Positive; -: Negative; K/A: Alkaline slant/Acidic butt

Table 2. Virulence factor studies by dominant bacterial micro biota isolated from infected GIFT Tilapia

Sl. No	Bacterial isolates	Virulence factors				
		Proteolytic	Haemolytic	Lipolytic	Gelatinase	Cellulase
1	GT1	+	+	+	-	-
2	GT2	++	-	+	-	+
3	GT3	+++	+++	++	+++	++
4	GT4	++	+++	++	++	+
5	GT5	+++	+++	++	+++	++

+++: Higher activity; ++: Medium activity; +: Less activity and -: No activity

Based on the strong straining in the bottom of the test tube was indicated as biofilm positive. The bacterial culture not inoculated test tube (Blank control) had no deposition in the bottom and anywhere in the test tube. The strains GT1 and GT2 also had no deposition observed in the bottom of the test tube. The test tubes with inoculating the GT3, GT4 and GT5 strains had able to produce bio films. The test tubes had the presence of strong dark staining viewed (Fig. 3).

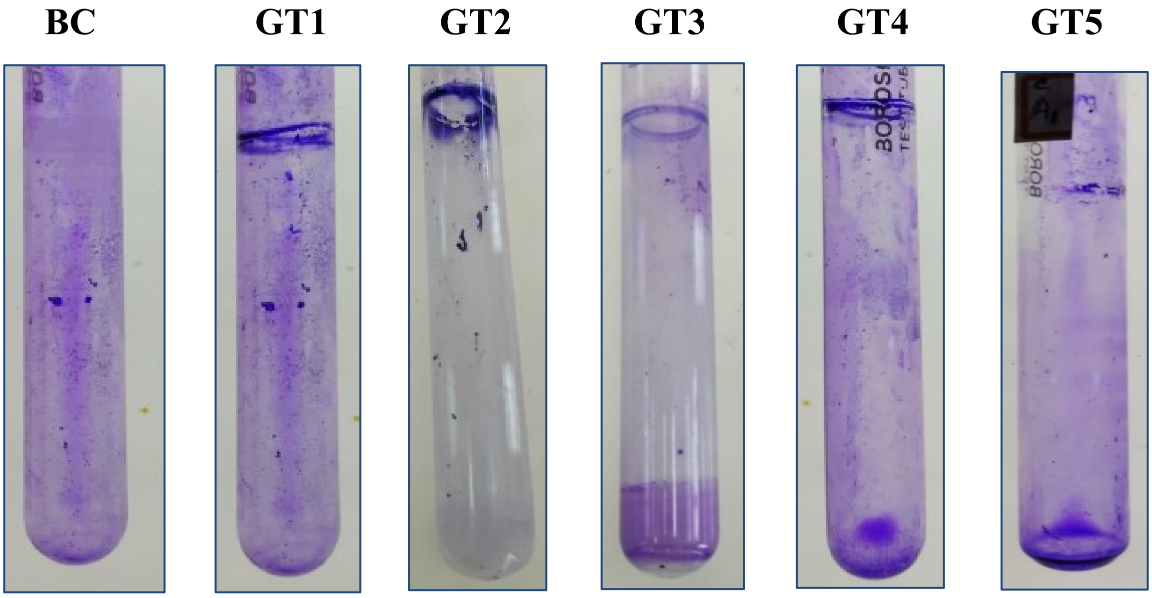

Figure 3. Biofilm formation of bacterial microbiota isolated from isolated from infected GIFT Tilapia. Strong straining in the bottom of the test tube was indicated as biofilm positive

Among the different tissues such as eye, gill, gut and muscle, there was a significant damage and abnormal changes detected like inflammation and necrosis etc. In eye, the inner plexiform layer of the eye structure was damaged. In gills, inflammation and necrosis were observed in the gill tissues and also epithelial swelling was present, likely caused by necrosis or edema. In gut, significant damage and abnormal changes in the villi structure were detected. The outer epithelium of the intestine showed swelling. In muscle tissues, myodegeneration or necrosis of the muscle cells was observed, leading to spacing between the cells (Fig. 4).

Among the different antibiotics such as gentamycin, tetracycline, erythromycin, ampicillin and chloramphenicol against the five strains, GT3, GT4 and GT5 were susceptible for gentamycin, tetracycline and chloramphenicol. Those strains were resistance against erythromycin and ampicillin. The GT1 strain was resistant against erythromycin and the GT2 resistant against gentamycin and ampicillin respectively (Table 3).

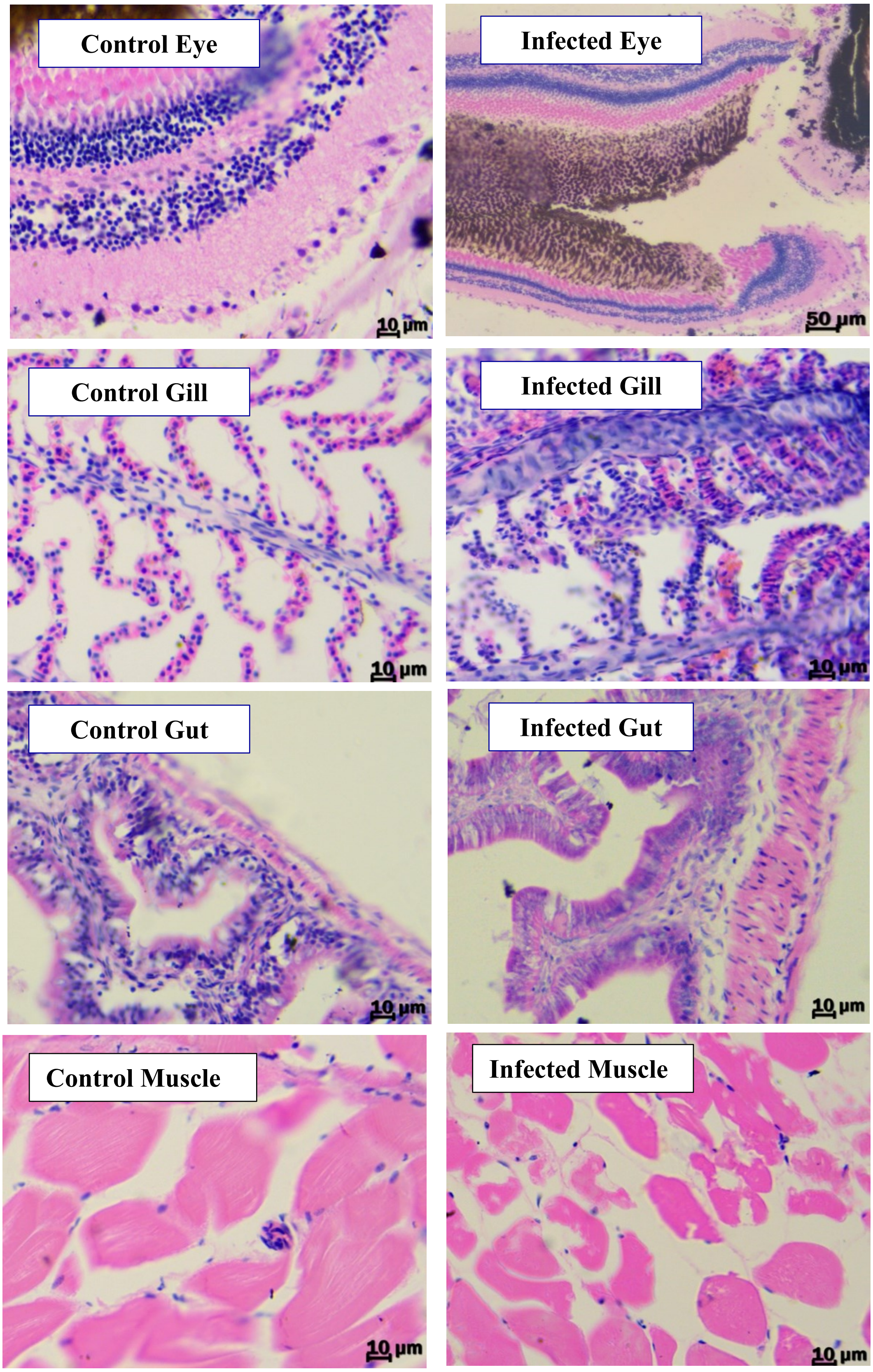

Figure 4. Histopathological immages of infected samples with 40 X magnification from infected GIFT Tilapia during disease outbreak.

Table 3. Antibiotic susceptibility tests for the bacterial micro biota isolated from infected GIFT Tilapia

		Bacterial isolates				
Sl. No	Antibiotics	GT1	GT2	GT3	GT4	GT5
1	Gentamycin	+	-	+	+	+
2	Tetracycline	+	+	+	+	+
3	Erythromycin	-	+	-	-	-
4	Ampicillin	+	-	-	-	-
5	Chloramphenicol	+	+	+	+	+

+: Susceptible; -: Resistance

NCBI Blast search analysis revealed that the causative pathogen GT3 was confirmed as *Aeromonas caviae.* Phylogenetic and evolutionary analysis of the 16S rRNA sequence revealed that *A. caviae* CMST AAHL shared 99 % similarity with *Aeromonas veronii* B5KU041799.1 and *Aeromonas taiwanensis* 933OP115620.1 etc (Fig. 5). The sequence was deposited in the NCBI database and strain name and GenBank accession number was *Aeromonas caviae* CMST AAHL OQ804404.1.

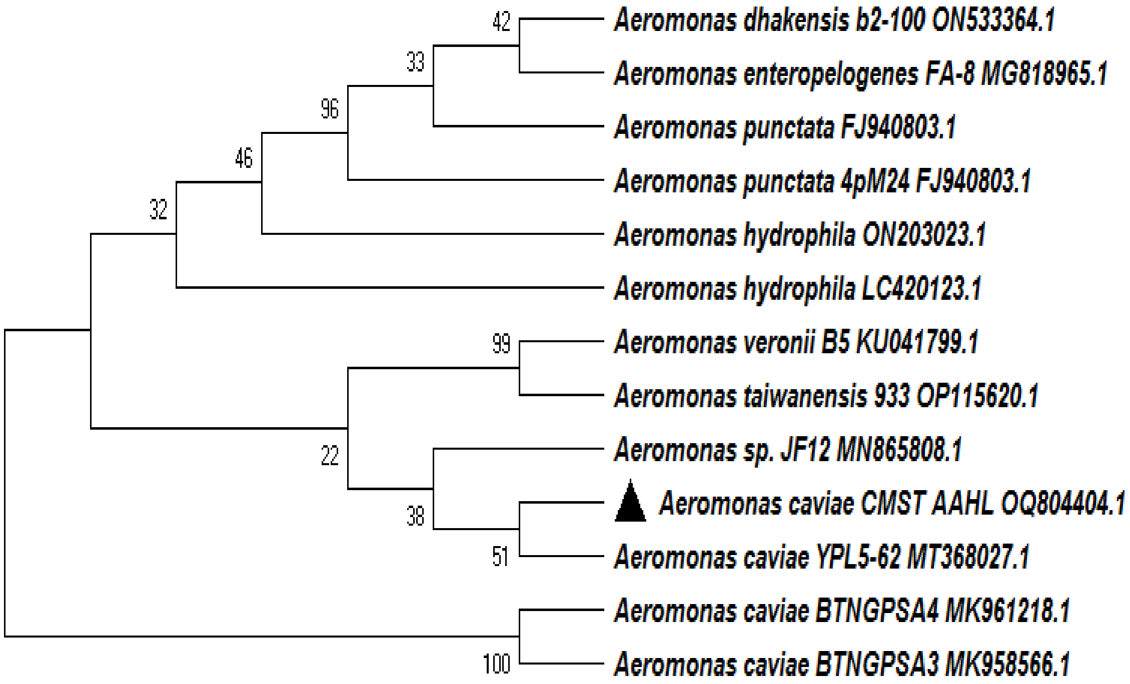

Figure 5. Graphical phylogenetic tree analysis of *Aeromonas caviae* CMST AAHL (GenBank Accession No.: OQ804404.1) by neighbor-joining method based on 16S rRNA gene sequence data compared with other species. Percentage bootstrap values are shown at the branching points of 1000 replicates. The lengths of the branches indicate the degree of difference between the genes represented by the nodes. The phylogenetic tree was constructed using Mega-X software.

Discussion

There are several opportunistic pathogens found in the Aeromonas genus that infect both aquatic and terrestrial animals, including human beings (Woo et al., 2022; Xue et al., 2022). *A. caviae* is one of the pathogens that is widely found in a variety of aquatic environments, including wastewater, aquaculture water, and drinking water (Baldissera et al., 2018; Carvalho et al., 2012). The infection of *A. caviae* causes the clinical symptoms of aeromoniasis on fish which include liquefying kidney, enlarged liver and spleen, ulcerations, surface swimming, listlessness, inappetence, abdominal distension and general signs of hemorrhagic septicemia (Ogara et al., 1998). *A. caviae* mostly affects the liver and kidney of the host animals, however the pathogenic effects on these tissues are not fully understood (Igbinosa et al., 2012). Therefore, more research is needed to completely understand the mechanisms underlying disease pathophysiology, such as how oxidative stress works and how infectious diseases emerge and spread (Castro et al., 2017).

The subsequent challenge study showed that Indian large carp (*Labeo rohita*) and Nile tilapia (*O. niloticus*) exhibited the characteristic symptoms and gross lesions of Aeromonas infection (Saharia et al., 2018; Soto-Rodriguez et al., 2018). Aeromonas species can cause acute-to-chronic infections in fish, as evidenced by the fact that infected fish died as early as 24 hours and as late as 216 hours. The rate of mortality increased with increasing bacterial concentration because more toxins were released, potentially damaging the internal organs of the fish (Dong et al., 2017). In the present study, *A. caviae* challenged the fish showed haemorrhages and abnormal behaviour including as swimming vertically, resting at the bottom and mortality observed on the second day of the experiment. Similarly, previous study reported that *O. niloticus* challenged by *A. caviae* showed symptom of lethargy, aberrant behaviour and 60% of mortality observed in tilapia on 4-day post-injection (dpi) (Roy et al., 2018).

The virulence of Aeromonas species is multifunctional and multifactorial because they generate multiple virulence factors to cause disease, such as hemolysins, lipases, proteases, biofilm formation, and controlling the production of virulence factors by quorum sensing (Rasmussen-Ivey et al., 2016). The virulence factors of *Aeromonas* spp. play a role in a complex network of pathogenic pathways, contribute to their effectiveness in developing a broad range of infection in both aquatic animals and humans (Sen & Rodgers, 2004). The results of this study revealed that *A. caviae* strain could hydrolyze the protein using protease enzymes when examined on skim milk agar. Similarly, (Yuan et al., 2022) reported that *A. caviae* generated extracellular protease enzyme showed a transparent hydrolysis the protein on the skim milk agar plate. Protease activity influences host nutritional sequestration and immune response in addition to aiding in the process of bacterial multiplication (Dallaire-Dufresne et al., 2014). Aeromonas can survive in a variety of habitats because extracellular proteases hydrolyze proteins in cell-free conditions and give it metabolic versatility (Zacaria et al., 2010).

Most of the β -hemolytic producing bacteria were either *A. sobria* or *A. hydrophila*, but infrequently *A. caviae* (Singh & Sanyal, 1992). Similarly, (Nakano et al., 1990) reported that most *A. hydrophila* and *A. sobria* isolates were highly hemolytic, but only 11% of the isolates of *A. caviae* were able to lyse sheep erythrocytes. In contrast, isolated strains such as *A. caviae* tested in our study showed positive result for hemolysin (β -hemolysin) production when cultured on blood agar. Pathogenic bacteria commonly produce hemolytic proteins, and β - hemolysins are considered indirect bacterial virulence factors. *A. caviae* is a significant enteropathogen, with hemolysin being one of its virulence factors (Nowrotek et al., 2021).

Furthermore, *A. caviae* iso-lates in this study demonstrated the ability to hydrolyze lipids by lipase enzyme when cultured on sprit blue agar media for 24 hours at 37°C. Some *Aeromonas* sp generate lipases that aid in the lysis of the plasma membrane of the host erythrocytes during invasion. Free fatty acids produced by lipolytic activity results in affecting immune system function (Sakai, 1985). (Lee & Ellis, 1990) reported that lipase is an extracellular substance that is important in the pathogenicity of *A. salmonicida* in fish. Additionally, the results of this study revealed that fish isolates of *A. caviae* exhibited strong gelatinase activity on nutritional gelatin agar. This result consistent with earlier research, the strain of *A. caviae* that was isolated from the patient's contact lens produced gelatinase enzyme. These enzymes are believed to play essential roles in Aeromonas pathogenicity and have been linked in gastroenteritis (Sechi et al., 2002).

Pathogenic bacteria have a particular ability to develop biofilms. Aeromonads produce biofilms on host epithelial cells to protect themselves from host immune system and to increase their pathogenicity (Beatriz Angeles-Morales et al., 2012). This study found that *A. caviae* isolates can generate biofilm when cultivated on LB broth in test tubes for 24 hours at 37°C. Likewise, (Chenia & Duma, 2017) studied the biofilm-forming capabilities of *A. jandaei, A. allosaccharophila, A. culicicola*, and *A. hydrophila* isolated from seawater at various temperatures and nutrient levels. The adhesive ability and virulence factor of *A. hydrophila* are also altered by changes in the environment condition (Casabianca et al., 2015).

The result of the histopathological analysis revealed that the A. caviae isolate can cause visible tissue damage in the eye, gill, gut and muscle of the tilapia fish. The eye tissues of the challenged tilapia showed histological changes, including the inner plexiform layer of the eye structure. The gill tissues show inflammation and necrosis, and also epithelial swelling was present. In the gut, significant damage and abnormal changes in the villi and myodegeneration or necrosis are observed in muscle tissues. This is supported that, the earlier studies on the tissue damage observed in *Silurus meridionalis* and *Rhamdia quelen* infected with *A. caviae* (Baldissera et al., 2017). Thus, the haemolysin produced by *A. caviae* has been demonstrated in previous studies to be highly pathogenic and capable of rupturing tissue and dissolving red blood cells (Baldissera et al., 2018).

There have been few investigations on antibiotic sensitivity of *A. caviae.* In this study, *A. caviae* strain isolated from tilapia was highly sensitive to gentamycin, tetracycline and chloramphenicol and resistance to erythromycin and ampicillin. Similarly, *A. caviae* WH21406 was isolated from *Micropterus salmoides,* which highly sensitive to enrofloxacin, norfloxacin, streptomycin and amikacin, but susceptible to florfenicol, neomycin sulphate, compound sulfamethoxazole, doxycycline and tetracyclines (Xue et al., 2022). *A. caviae* strains from various fish have varying antibiotic sensitivity profiles. As a result, this work provides a scientific basis for the diagnosis, prevention, and treatment of *A. caviae* infections in fish.

Conclusion

The present study, *A. caviae* CMST AAHL was identified and characterized from the infected GIFT Tilapia. Based on the virulence factors proteolytic, haemolytic, gelatinase, cellulose activity and biofilm forming ability of the *A. caviae* CMST AAHL strain, mass mortality was happened in the nursery tanks.

Ethical approval

The study protocol was approved by the Institutional Review Board of CENTRE FOR MARINE SCIENCE AND TECHNOLOGY, MANONMANIAM SUNDARANAR UNIVERSITY, Rajakkamangalam, Kanyakumari, Tamilnadu, India.

Data availability statement

The authors declare that data can be provided by corresponding author upon reasonable request.

Conflicts of interest

There is no conflict of interests for publishing this study on behalf of all authors by corresponding author, Thavasimuthu Citarasu.

Funding organizations

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Contribution of authors

Vijayan Saranya: Experimental works and alaysis. Eswaramoorthy Thirumalaikumar: Writing and editing. Ganapathi Uma: Writing–reviewing and editing. John Radha Anusha: Writing–reviewing and editing. Thavasimuthu Citarasu: Conceptualisation, investigation, writing original draft, writing–reviewing and editing. All authors have read and agreed to the published version of the manuscript.

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