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Isolation and identification of bacterial gill disease in goldfish, *Carassius auratus* in Madurai

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Abstract

Aquarium fish sometimes suffer from a disease complex called bacterial gill disease. Although it most often affects young fishes, it can affect any type of aquarium fish. The bacterial gill disease (BGD) were diagnosed in goldfish *Carassius auratus* purchased from Madurai. Histologically, the fish from all outbreaks had large numbers of filamentous bacteria associated with the gill tissue. The bacterial species such as *Flavobacteria*, *Aeromonas* and *Pseudomonas* spp. were isolated from the experimental fishes. The results of this present work, concludes that the ornamental fishes from Madurai district indicate that the pathogenic bacteria species were abounded in the aquarium. Epidemics of bacterial diseases are common in dense populations of cultured food or aquarium fish. Predisposition to such outbreaks frequently is associated with poor water quality, organic loading of the aquatic environment, handling and transport of fish, marked temperature changes, hypoxia, or other stressful conditions. Most bacterial pathogens of fish are aerobic, gram-negative rods. Diagnosis is by isolation of the organism in pure culture from infected tissues and identification of the bacterial agent.

Keywords: Aqua farm, *Carassius auratus*, Microbiota, pathogenic bacteria

Introduction

In the global scene, tropical aquarium fish keeping is considered as the second largest hobby next only to photography. The practice of maintaining fish for ornamental purpose is known from Tang dynasty (680 to 908, A.D). Ornamental fish keeping was initiated as a small time hobby using gold fish during the 18th century. In 20th century; aquarium keeping and rearing of ornamental fish got worldwide recognition.

Aquaculture production has been hindered by many factors, including diseases [1] and high stocking density of fishes in ornamental fish ponds increase the number of disease outbreaks. The gills are primarily affected; fish suffering from bacterial gill disease will have respiratory problems, with visibly rapid or labored breathing and swimming close to the surface of the water as if trying to get air. They will show signs of a lost appetite, with little to no eating. The gills will often though not always show signs, with swelling, redness within the gill tissue, and deformed gills. The gills may have patchy growth of bacteria on them and a blotchy appearance. Without treatment, the gill and fin tissues will deteriorate and necrotize. Number of pathogenic bacteria, including *Aeromonas* spp., *Bacillus* spp., *Citrobacter* spp., *Flavobacterium* spp., *Klebsiella* spp., *Proteus* spp., and *Vibrio* spp., in ornamental fish were reported worldwide [2]. Many reports on the bacterial diseases of Indian freshwater fishes have been published earlier [3,4]. Recently reported a mass mortality in ornamental Koi carp due to a bacterial pathogen, *Proteus hauseri*, in India [5]. Among ornamental fishes, *Carassius auratus*, the goldfish, is common and they are the smallest member of the carp subfamily (Cyprininae) including Crucian carps (Genus: *Carassius*) and common carp (*Cyprinus carpio*) [6]. Fish are highly susceptible to a wide variety of bacterial pathogens. Most of them can cause disease and are saprophytic in nature. Generally bacterial fish diseases do not develop easily by exposing a host to an infectious agent [7]. In most cases, the disease occurs by the complex interactions between pathogen and fish under environmental stress that affect the host susceptibility to pathogen and cause disease [8]. Bacterial gill disease typically occurs as a result of poor living conditions, such as overcrowding, poor water quality, high organic debris, increased temperature of the water, and increased ammonia levels. While it is most often the young and/or weak fish that contact the disease, due to their vulnerable immune systems, gill disease can affect fish of any age. The bacteria that cause gill infections are primarily *Flavobacteria*, *aeromonas* and *Pseudomonas* spp. The present study report isolation of pathogenic bacteria in gills from ornamental goldfish (*Carassius auratus*) in Madurai.

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Materials and Methods

Sample collection

Goldfishes shown hemorrhage symptoms were collected from an ornamental fish farm, Madurai. The samples were kept in sterile plastic bags and transported to the laboratory on ice within 3-4 h. Infected tissues viz., gills of the individual fish were excised for isolation of bacteria. Tissues homogenate were inoculated in peptone broth and incubated overnight at 28 °C.

Isolation of Bacteria

Bacteria were isolated using serial dilution method. 1ml of the sample was serially diluted up to 10⁻⁷ dilutions using 9ml of distilled water. 100µl of sample from 10⁻⁵, 10⁻⁶ and 10⁻⁷ dilutions were spread plated into nutrient agar plates and incubated at room temperature for 24 h. From the plates, colonies with different morphology were selected and subculture.

Morphological identification

Pure cultures of the isolates were streaked on Nutrient agar plates for colony development and examine for size, shape, colour, structure, margin, optical properties, consistency, elevation and pigmentation for the colony morphology. From that single colony, 3% KOH string test and gram’s staining performed for the morphological examination. Motility examined by Hanging drop technique.

Biochemical identification

All the isolates were biochemically identified by performing, Indole, Methyl red, Voges proskauer, citrate, Triple sugar iron agar, urease, nitrate, catalase, oxidase, mannitol, motility, lactose fermentation and sugar fermentation tests.

Results

The bacteria that cause gill infections are primarily *Flavobacteria*, *Aeromonas* and *Pseudomonas* spp. The direct initiating cause by these bacteria is not conclusive, but they will often be found as secondary, opportunistic infection.

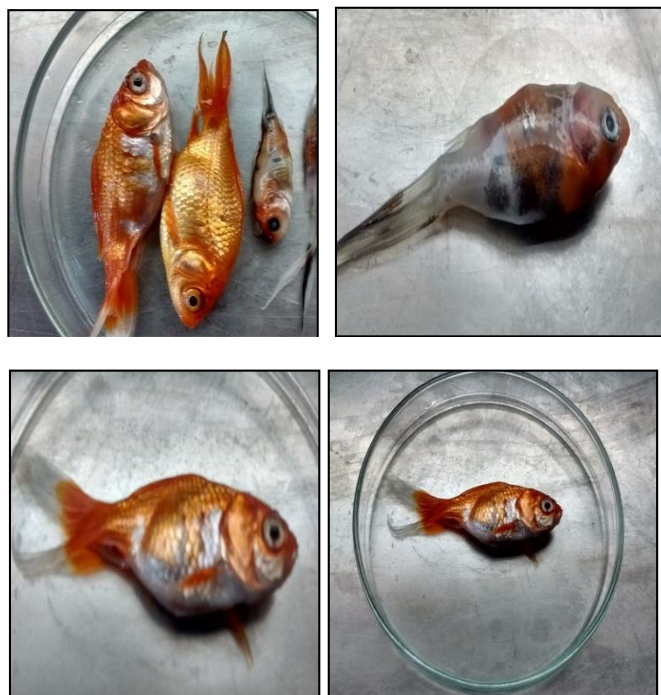


Fig 1: Diseased ornamental gold fish *Carassius auratus*

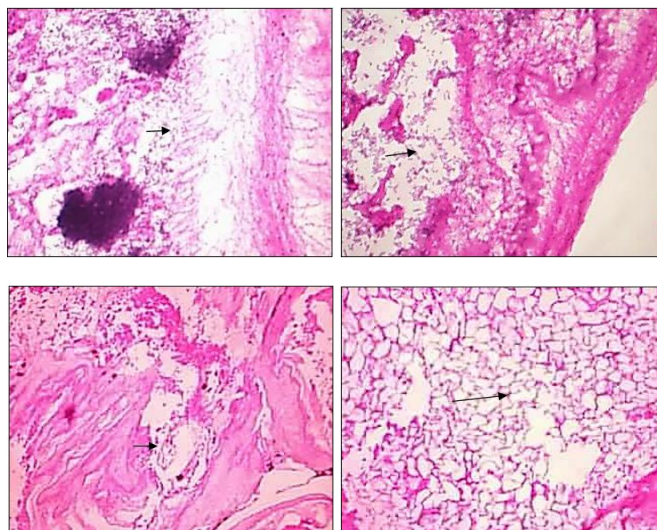


Fig 2: Histopathological examination of the gills of the ornamental gold fish infected with bacteria. The middle part indicates necrosis of gill lamellae

Table 1: Bacteria isolated from Gill

S. No	Ornamental fish	Bacterial species
1	<i>Carassius auratus</i>	<i>Flavobacteria</i> , <i>Aeromonas</i> and <i>Pseudomonas</i> spp.

Table 2: Biochemical identification of each bacterial strain isolated from naturally infected fish.

Test	GF 1	GF2	GF3
Gram staining	-	-	-
Motility	+	+	+
Indole	+	+	+
Methyl Red	+	+	-
Voges Proskauer	+	+	-
Citrate	+	+	+
H ₂ S	-	+	-
Glucose Test	-	-	+
Lactose Test	-	-	-
Sucrose Test	-	-	-
Catalase Test	+	+	+
Oxidase Test	+	+	+
Gelatin	+	+	+
Identification	<i>Flavobacteria</i> ,	<i>Aeromonas</i>	<i>Pseudomonas</i>

Discussion

There are many bacterial aquaculture diseases worldwide, of which many relevant for tropical regions. Some of the pathogens may be contact-zoonotic, good hygiene is crucial for aquafarmers, field technicians, and processors. Proper diagnosis of bacterial diseases is crucial. Many of the diseases can be prevented by good management, including use of appropriate vaccines.

Conclusion

The best way to prevent bacterial gill disease from occurring is by maintaining hygienic living conditions for fish. Keeping the water clean of organic debris, giving the fish plenty of space in which to move, with no overcrowding, maintaining a consistent temperature, and testing the water quality regularly to ensure that it is balanced are all the best practices for keeping fish healthy and stress free. Additionally, filters should be changed every month or checked according to the filter manufacturer's directions.

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