



Prevalence of Bacterial Diseases in Ornamental Fish Rearing Units of Kolathur, Tamil Nadu, India

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ABSTRACT

Background: Ornamental fishkeeping is a widely embraced hobby globally. In India, Kolathur, Chennai Tamil Nadu is an important ornamental fish hub involved in trading of ornamental fishes. As a lucrative industry, the ornamental fish trade faces significant challenges due to diseases caused by various pathogens, impacting market value and causing economic losses.

Methods: A surveillance study was conducted in Kolathur, Chennai, from December 2022 to August 2023. Ornamental fish samples exhibiting clinical signs of bacterial diseases were collected from the farms and retail outlets. Bacterial isolation and identification techniques, including conventional and PCR detection, were employed to isolate and identify bacterial strains from infected fish samples.

Result: Out of 196 fish samples, 51 (29.4%) were found infected with bacterial pathogens. The prevalent bacterial pathogens included *Aeromonas veronii* (47%), *A. caviae* (17%), *Pseudomonas aeruginosa* (15%), *Streptococcus* sp. (11.3%), *Mycobacterium fortuitum* (5.6%) and *Shewanella* sp. (3.7%). Different fish families exhibited varying susceptibility to bacterial diseases, with cyprinids being the most affected (56.6%). The findings stress the importance of adhering to health regulations to prevent transboundary spread and suggest the development of targeted management strategies to sustain the health of ornamental fish populations.

Key words: Bacterial pathogens, Kolathur, Ornamental fish.

INTRODUCTION

Ornamental fish keeping is widely practised as a popular hobby across the globe, adorning the homes and gardens. A substantial number of households in developed countries embrace this hobby, with roughly 16% in Australia, 13% in UK, 10% in USA and 1.25% in India nurturing ornamental fish within their premises. In India, notably, cities like Kolkata, Mumbai and Chennai serve as pivotal hubs for trading ornamental fishes. In exports Kolkata holds the major share of approximately 90%, followed by Mumbai (8%) and Chennai (2%) contributing to India's ornamental fish trade (Ghosh *et al.*, 2002).

Fish diseases stem from a combination of underlying and perpetuating factors, a concept elucidated by (Chapman *et al.*, 1997). The major cause of diseases in aquatic organism is due to wide spread distribution of bacterial pathogens and stress caused by intensification of practices. On a global scale, the aquaculture industry has witnessed over 13 bacterial genera implicated as major bacterial diseases (Pridgeon and Klesius, 2012). Bacterial infections caused due to fluctuations in key physical and chemical parameters of pond water, such as abrupt changes in turbidity, temperature, salinity, pH, water conductivity and dissolved oxygen levels (Islam *et al.*, 2019; Nadirah *et al.*, 2013).

Kolathur, located in Chennai, Tamil Nadu, India is a ornamental fish trading hub with 1,850 operational ornamental fish producing units operating in Kolathur and adjacent regions of Athur and Devambattu with a worth about US\$ 0.5-1.1 million are traded annually (Shinoj *et al.*, 2021). Due to a high volume and an increasing

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incidence of diseases including the transboundary diseases there is a growing concern within the industry. Whenever live animals are moved, there is potential transfer of the pathogens with the host as well. In order to prevent the spread of diseases across boundaries without unjustified trade restrictions, international regulations regarding the health state of livestock have been implemented (Grant and Arita, 2017). To address this situation, it is essential to conduct a prevalence study to assess the incidence of existing diseases, identify potential emerging diseases and establish screening protocols. Additionally, there is a need to formulate new guidelines and responsibilities in this regard. The implementation of aquatic animal health control measures is tailored based on the health status of both importing and exporting countries, particularly concerning the presence of World

Organisation for Animal Health (formerly as OIE) listed pathogens that pose a high risk if introduced to a pathogen-free zone. This underscores the potential of ornamental fish to serve as transboundary carriers of significant diseases (Ariel, 2005). Hence, the present study was carried out to understand the occurrence of bacterial diseases in ornamental fishes at Kolathur, Tamil Nadu, India.

MATERIALS AND METHODS

Sample collection

Ornamental fish samples showing clinical signs lethargy, abnormal swimming behaviour, ulceration on the body, eroded scales, tail rot and fin rot were collected as a part of the surveillance study conducted during Dec 2022 to Aug 2023 in the ornamental fish farms and outlets of Kolathur (GPS: 13.126004°E; 80.209976°N), Chennai. The fishes were transported to the wet lab of State Referral Laboratory Aquatic Animal Health, TNJFU, Madhavaram in live condition. The samples were aseptically dissected and the disease signs in internal organs were recorded. The skin, gill and fin scrapings were collected from the live infected fish samples and the wet mounts were examined under the microscope for parasite infestation.

Isolation of bacterial pathogens

For isolation of bacterial pathogens, swab samples were collected aseptically from the ulcers, haemorrhages on the skin and inoculated in appropriate bacteriological media (Tryptic soy broth) (Himedia, India) and incubated at 35±2°C for 20-24 h. The infected tissue from internal organs were homogenised in PBS, inoculated in liquid media (Himedia, India) and incubated at 35±2°C for 20-24 h. Further isolation was carried out on specific bacteriological media *viz.*, *Aeromonas* isolation medium base for *Aeromonas* sp. and *Pseudomonas* sp., Streptococcus selective agar *Streptococcus* sp. and Thiosulfate citrate bile salt (TCBS) agar for *Shewanella* sp. (Himedia, India).

For isolation of *Mycobacterium* sp. tissue samples from the infected fish were homogenized and centrifuged at 3500 rpm for 15 min, the pellets were suspended in 1 ml of 12% sulfuric acid, incubated at room temperature for 20 min. The pellet was added with distilled water followed by centrifugation. Again, the pellet was resuspended in 200 µl of distilled water and inoculated into Middlebrook 7H9 broth with an indicator (Himedia and incubated for 7-10 days at 37°C). Conventional identification of *Mycobacterium*

sp. involves observation of pink granules in the broth and microscopically examining the Ziehl-Neelsen stained bacterial cultures. The bacterial isolates were identified by following conventional biochemical methods *viz.*, Gram staining, catalase, oxidase, indole, methyl red, voges proskaur, citrate utilisation, nitrate reduction and Pyrazinamidase test. Prevalence data was generated by the application of the formula:

$$\text{Prevalence \%} = \frac{\text{No. of bacteria}}{\text{Total no. of samples}} \times 100$$

Subsequently, graphical representation was created based on the obtained prevalence values.

PCR detection of bacterial pathogens

Total genomic DNA was isolated from pure cultures of bacterial pathogens using bacterial total DNA extraction kit (Qiagen, Germany) following the manufacturer's instructions. The extracted DNA from isolates were amplified with the primers targeting 16s rDNA (Weisburg *et al.*, 1991), aerolysin gene for detection of *Aeromonas* sp. (Nawaz *et al.*, 2010) and heat shock protein for detection of *Mycobacterium* sp. (Kim *et al.*, 2005). The PCR amplified products were sequenced (Eurofins, India), analysed for their similarity with the sequences available in the GenBank, NCBI, their identity were confirmed and submitted for the Accession nos.

RESULTS AND DISCUSSION

Among the total number of 196 fishes screened 51 (29.4%) fish samples were found infected with bacterial pathogens. About 53 bacterial isolates which included *Aeromonas veronii* (n=25), *A.caviae* (n=9), *Pseudomonas aeruginosa* (n=8), *Streptococcus* sp. (n=6), *Shewanella* sp. (n=2), *Mycobacterium fortuitum* (n=3) were isolated from infected ornamental fishes. Table 1.1 and 1.2 represents the biochemical results for the isolated bacterial pathogens and species were confirmed by PCR and sequence analysis has been carried out. PCR amplification (Fig 1.1, 1.2,1.3) and sequence analysis confirmation of the isolates for the infected fishes are shown in Table 2.

Bacterial species-wise prevalence in fish samples

The analysis of the prevalence pattern of the bacterial pathogens in ornamental fishes of Kolathur showed that *Aeromonas* sp. (64.15%) (*A. veronii*-47%; *A. caviae*-17%)

Table 1.1: Biochemical characterization of bacterial pathogens isolated in this study.

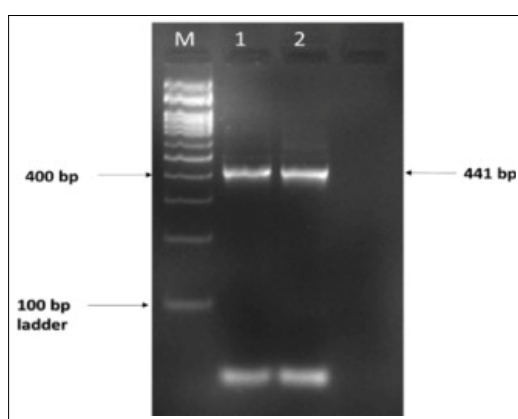
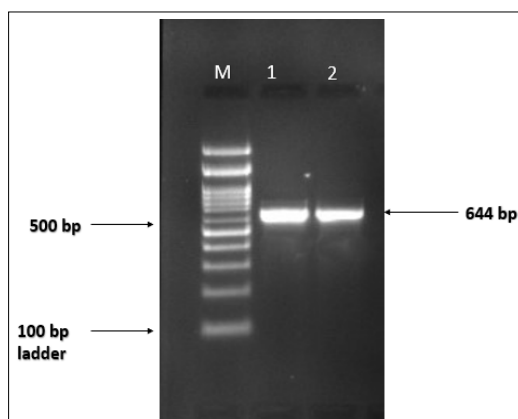
Bacterial pathogen	Gram staining	Catalase	Oxidase	Indole	Methyl red	Voges proskauer test	Citrate utilisation test
<i>Aeromonas</i> sp.	-	+	+	+	+	+	+
<i>Pseudomonas</i> sp.	-	+	+	-	-	-	+
<i>Shewanella</i> sp.	-	+	+	+	-	-	+
<i>Streptococcus</i> sp.	+	-	-	-	+	-	-

Table 1.2: Biochemical characterization of *Mycobacterium* sp. isolated in this study.

Bacterial pathogen	Acid-fast staining	Catalase	Pyrazinamidase	Nitrate reduction
<i>Mycobacterium</i> sp.	+	+	+	+

Table 2: GenBank accession nos. (NCBI) of the bacterial pathogens isolated in the study.

Pathogens	NCBI accession. no
<i>Aeromonas veronii</i>	OR487464
<i>A. caviae</i>	OR763411
<i>Pseudomonas aeruginosa</i>	OR511870
<i>Streptococcus</i> sp.	OR487864
<i>Shewanella</i> sp.	OR487862
<i>Mycobacterium fortuitum</i>	OR487463

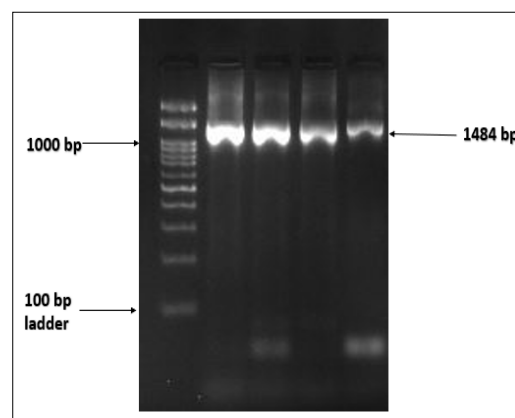
**Fig 1.1:** PCR amplification aerolysin gene of *Aeromonas* sp.**Fig 1.2:** PCR amplification of *hsp 90* gene of *Mycobacterium* sp.

was the highly prevalent pathogen followed by *Pseudomonas aeruginosa* (15%), *Streptococcus* sp. (11.3%), *Mycobacterium fortuitum* (5.6%) and *Shewanella* sp. (3.7%) (Fig 2).

Fishes are susceptible to various infectious diseases caused by bacteria, virus, fungi and parasites and non-infectious diseases. Among the various diseases reported in aquaculture, bacterial fish diseases are considered important as they affect the development and expansion of

ornamental fish farming practices due to reduced growth and mortality with the reported economic losses of about 15% (Mohd-Aris *et al.*, 2019;). Bacterial pathogens that are gram-negative are known widely to infect ornamental fishes (Sorum, 2005). It is well known that the occurrence of new diseases are trading of live fishes is being reported from all around the world (Dias *et al.*, 2012). Transportation of live fishes without proper screening is responsible for the cause of infectious diseases (Cardoso *et al.*, 2019).

Aeromonas sp. being the major infectious agent in ornamental fish industry and opportunistic in nature (Hettiarachchi and Cheong, 1994; Chandrarathna *et al.*, 2018). They are highly diverse and probably shows different infection levels among fish hosts (Nwaj-Fyzul *et al.*, 2007) and causing severe infection in immune-compromised individuals (Wu *et al.*, 2019). *A. veronii* has been reported to be an important pathogen of *Aeromonas* genus causing disease outbreaks with clinical signs like haemorrhagic septicemia (*Trichogaster* sp.), dropsy (*Astronotus ocellatus*) tail and fin rot (*Carassius auratus*) (Sreedharan *et al.*, 2013; Praveenraj *et al.*, 2023) as well as contributing mass mortality in ornamental carps (Chen *et al.*, 2019). Infected fish species and contaminated water are regarded as the routes of transmission for *Aeromonas* sp. (Thornton *et al.*, 1999; Ozcan, 2023). In the present study, *Aeromonas* sp. associated with mass mortality and dropsy of Koi carp, Goldfish and Flower horn. Hatha and Nifty (2012) reported a high prevalence of *Aeromonas* sp. (37.5%) in ornamental fishes at Kerala. *A. caviae* formerly is known as *A. punctata*, causes infectious abdominal dropsy in fishes. Recently, Anjur *et al.* (2021) reported that *A. caviae* is the most dominant cause of bacterial disease in ornamental fishes. In contrast our study recorded *A. veronii* as the dominant pathogen (47%) in ornamental fish farms of Kolathur. This variation continues to elucidate the factors contributing to differences in pathogen prevalence, aiding in the

**Fig 1.3:** PCR amplification of 16S rDNA bacterial pathogens.

development of targeted and effective strategies for disease management in ornamental fish populations.

Pseudomonas aeruginosa is an opportunistic human pathogen known to affect fishes as they are opportunistic in certain environmental condition (Yoon *et al.*, 2010). Causing various pathological signs viz., high mortality, scale desquamation, hemorrhagic skin ulceration, abdominal distension (ascites) and exophthalmia (Thomas *et al.*, 2014; El-Bahar *et al.*, 2019; Raj *et al.*, 2004). Nair *et al.* (2021) reported mass mortality in *Etroplus maculatus* in *P. aeruginosa* infected fishes and it is regarded as a surface pathogen. In this study, *P. aeruginosa* infected fishes showed clinical signs which included abdominal dropsy, fin and tail rot in both tiger barb and goldfish with a prevalence of 15%. Previously, reported prevalence of this diseases in ornamental fishes were 2.13% in Poland (Saengsitthisak *et al.*, 2020), 5% in Italy (Sicuro *et al.*, 2020) and 7.1% in Thailand (Walczak *et al.*, 2017) in ornamental fishes.

Streptococcosis is a septicemic disease affecting both cultured and wild populations of freshwater and marine fish species throughout the world (Kitao, 1981; Austin and Austin, 1999). It is known to exhibit clinical signs, viz., erratic swimming; loss of buoyancy control, lethargy, ascites, darkening with high mortality rate (>50%) over a period of 3 to 7 days (Yanong and Francis-Floyd, 2002). Other clinical signs reported are weakness, loss of equilibrium in ram cichlid *Mikrogeophagus ramirezi* (Lazado *et al.*, 2018) and mortality in doctor fish *Garra rufa* (Ruane *et al.*, 2013). In this study, Goldfish, Koi carp and Devil cichlids infected with *Streptococcus* sp. exhibited lethargic movement, loss of equilibrium and erosion in scales with a prevalence of 11.3%. *Streptococcus* sp. prevalence of 1.3% has been recorded earlier in aquarium outlets in Malaysia (Nie *et al.*, 2015).

Fish Mycobacteriosis is one of the most devastating diseases in aquaculture in recent years. Mycobacteriosis causes chronic systemic infections in fish and is capable of causing skin lesions in human handlers due to its zoonotic nature (Ucko *et al.*, 2002). Mycobacteriosis caused due to *M. fortuitum* and *M. marinum* has been reported in fishes (Puk *et al.*, 2018). *M. fortuitum* was described for the first time by Da costa cruz in 1938. Clinical signs viz., lethargic swimming, scoliosis, skin ulceration and loss of pigmentation have been reported in gold fish and other fishes (Lawhavinit *et al.*, 2005; Gokul Raj *et al.*, 2019; Dissanayake *et al.*, 2017). Which are highly challenging to treat (Bartralot *et al.*, 2005). In this present study, *M. fortuitum* infection was reported in Zebra fish, Goldfish and Guppies that showed lethargic movement and mortalities with a prevalence of 5.66%. Mycobacteriosis in ornamental fishes with a total prevalence of 46.8% have been recorded in Italy (Zanoni *et al.*, 2008) and 50.1% in Poland (Puk and Guz, 2020). In India, the prevalence of mycobacterial infections in freshwater ornamental fish presents a significant concern. This is because, apart from causing infection in fish, these non-tuberculous mycobacteria have

the potential to spread from fish and induce both localized and widespread infections in humans (Shukla *et al.*, 2014).

Shewanellosis is an emerging disease in freshwater fishes caused by the bacteria of *Shewanella* sp. It has been generally isolated from sea water samples and putrefied fish (Stenstrom and Molin, 1990). The first documentation of disease outbreaks in freshwater fish attributable to this bacterium were reported in Poland in 2004. Recently *Shewanella* sp. has been reported as an opportunistic bacterial pathogen in fish and isolated from moribund goldfish in Turkey (Altun *et al.*, 2014), common carp and other ornamental fishes exhibiting necrotic lesions and ulceration on skin. In this study, *Shewanella* sp. infected koi carp exhibited deep ulceration on skin and exudated around the kidney with a prevalence of 3.77%. Walczak *et al.* (2017) reported a prevalence of 7.1% in ornamental fishes from an aquarium shop in Poland. In the recent times, there is an escalation in the spread of *Shewanella* sp. infections among freshwater fishes. Hence, there is an urgent need to undertake comprehensive studies on this bacterium concerning emerging risks (Pazdzoir *et al.*, 2019).

Fish family-wise prevalence of bacterial diseases

The prevalence of bacterial pathogens in various fish families is presented in the Fig 3. Analysis of the pattern of prevalence of bacterial diseases in fish family-wise showed that cyprinids were the most susceptible to bacterial diseases with a disease prevalence rate of 56.6% caused by *Aeromonas* sp., *Pseudomonas* sp., *Mycobacterium fortuitum* and *Shewanella* sp. This may be attributed to the high contribution of Cyprinids, Gold fish and koi carp to the total number of fishes (93/196), being the most popular ornamental fishes in India and other countries. The next most susceptible fish family was cichlid, with a total disease prevalence of 15% (18/196), caused by *Aeromonas* sp., *Streptococcus* sp. and *Pseudomonas* sp. Live bearers of Poeciliidae family showed overall disease prevalence of 13.2% (21/196). Bacterial disease prevalence showed the least occurrence in fish families viz., Osphronemidae (27/196), Characidae (7/196) and Loricariidae (7/196) with an overall prevalence being less than 5% caused by *Aeromonas* sp. and *Pseudomonas* sp. Whereas, no bacterial diseases were observed in fishes of families Adrianichthyidae, Pomacentridae, Gobiidae and Ambassidae.

In our research, we observed higher prevalence of bacterial diseases in egg laying fishes belonging to families viz., Cyprinidae, Osphronemidae, Cichlidae, Characidae, Loricariidae, Adrianichthyidae, Pomacentridae, Gobiidae and Ambassidae (egg layers). The total prevalence rate of diseases in egg layers was 87%, while live bearers of family Poeciliidae exhibited a total bacterial diseases prevalence of 13%. These findings highlight that bacterial diseases prevalence can vary among different fish families, potentially due to differences in their inherent

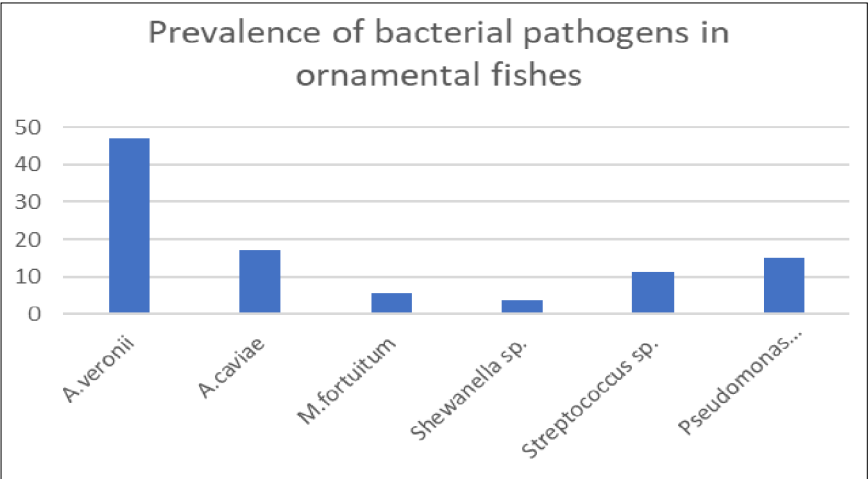


Fig 2: Prevalence of bacterial pathogens in ornamental fishes of Kolathur, TN.

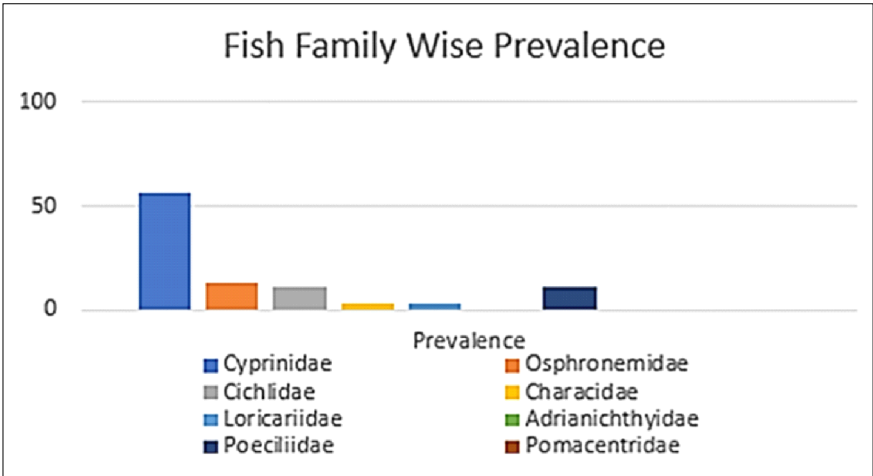


Fig 3: Fish family-wise prevalence of bacterial pathogens in ornamental fishes.

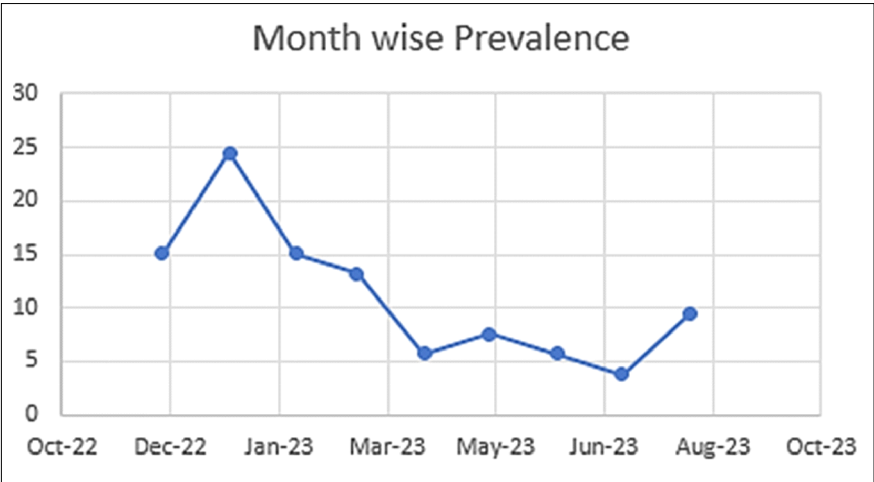


Fig 4: Month wise prevalence of bacterial diseases in ornamental fishes.

disease susceptibility/resistance, environmental factors, behaviour, physiology and inherent susceptibility to specific diseases. Of particular interest is the comparison with Sicuro *et al.* (2020) in which they have reported an exceptionally bacterial diseases prevalence of 68% in the Poeciliidae family (live bearers). Potential explanations may include variations in sampling methods, geographic regions, or the specific species within the Poeciliidae family was studied.

The fish samples were collected weekly twice during a total period of nine months (Dec 2022- Aug 2023). The most and prevalence of bacterial diseases was observed to increase from the month of December and peaked during January and reduced thereafter. As January is considered as a winter season, some pathogens thrive well in colder condition and certain diseases exhibited prolonged incubation, leading to severe infection in ornamental fishes. Conversely, May 2023 exhibited the lowest prevalence at 3.7%, signifying a lower proportion of infected hosts during that time, reinforcing the comparatively reduced microbial load (Fig 4).

CONCLUSION

In conclusion, this comprehensive study conducted in the ornamental fish farms in Kolathur, Chennai, illuminates the intricate dynamics of bacterial diseases affecting ornamental fish, underlining the critical need for robust disease surveillance and management practices in the ornamental fishkeeping industry. The identified bacterial pathogens, particularly the prevalence of *Aeromonas veronii*, *A. caviae*, *Pseudomonas aeruginosa*, *Streptococcus* sp., *Mycobacterium fortuitum* and *Shewanella* sp. in various species of fishes provide valuable insights into the specific health challenges faced by various fish families. The observed variations in bacterial disease prevalence during various months emphasize the influence of seasonal and environmental factors. These findings not only contribute to the understanding of disease patterns but also underscore the necessity for collaborative research and adherence to health regulations to prevent the transboundary spread of ornamental fish diseases. The study's implications extend to the development of targeted management strategies for the ornamental fish industry, aiming to mitigate economic losses and sustain the health of these cherished aquatic pets. For disease control and prevention emphasis should be given on proper sanitation and bio-security measures in the aquaculture production systems.

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Conflict of interest

The authors declare that they have no competing interest.

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