Prevalence, Antimicrobial Susceptibility, and Multidrug Resistance of Bacterial Pathogens Isolated from Ornamental Fishes in Latur District, Maharashtra, India

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Abstract

Ornamental fish production and retail trade may facilitate the circulation of opportunistic bacterial pathogens, particularly when fish are held at high densities or exposed to suboptimal water quality and handling conditions. This study document reports bacteriological findings from 100 ornamental fish representing goldfish (*Carassius auratus*), guppy (*Poecilia reticulata*), angelfish (*Pterophyllum scalare*), fighter fish (*Betta splendens*), and molly (*Poecilia sphenops*) sampled from aquarium retail outlets and local breeding units in Latur District, Maharashtra, India. Bacterial growth was reported for 75 of 100 fish (75.0%). Four bacterial groups were reported among the positive samples: *Aeromonas hydrophila* (28 isolates; 37.3%), *Pseudomonas aeruginosa* (20; 26.7%), *Flavobacterium columnare* (16; 21.3%), and *Vibrio cholerae* non-O1/non-O139 (11; 14.7%). Disk-diffusion results for 75 isolates indicated the highest reported resistance to ampicillin (73.3%), followed by oxytetracycline (48.0%). Susceptibility was reported as 82.7% for gentamicin and 76.0% for ciprofloxacin. Thirty-two isolates (42.7%) were classified as multidrug-resistant (MDR), defined in the supplied dataset as resistance to at least three tested antimicrobial classes. *Pseudomonas aeruginosa* showed the highest pathogen-specific MDR proportion (60.0%).

Keywords: ornamental fish; bacterial pathogens; antimicrobial susceptibility; multidrug resistance; Latur; aquaculture biosecurity

1. INTRODUCTION

The ornamental-fish trade connects breeders, wholesalers, retailers, transport systems, and home aquaria. Fish moving through these systems may encounter crowding, repeated handling, transport stress, and changes in water chemistry. These stressors can compromise host defences and increase the likelihood that opportunistic bacteria cause disease. Motile *Aeromonas species* are widely associated with fish disease and can carry virulence determinants and antimicrobial-resistance traits; published studies have also documented multidrug-tolerant bacteria and resistance genes in ornamental fish and their transport water (Verner-Jeffreys et al., 2009; Hossain & Heo, 2021).

Antimicrobial resistance in aquatic environments is a One Health concern because resistant bacteria and resistance determinants may circulate between animals, water, equipment, and people. Studies of ornamental-fish farms have reported diverse bacterial taxa and variable resistance profiles, underlining the importance of local surveillance rather than assuming that susceptibility patterns from one region or bacterial species apply elsewhere (Preena et al., 2020).

For Latur District, Maharashtra, region-specific information can help describe the bacterial organisms recovered from ornamental fish sold or maintained in local retail systems. The present manuscript therefore summarizes the supplied dataset on bacterial recovery, reported antimicrobial susceptibility, and multidrug resistance among five commonly traded ornamental-fish species. The study objectives were to describe the proportion of fish yielding bacterial growth, summarize the distribution of the four reported bacterial taxa, compare susceptibility categories for four antimicrobials, and quantify the reported MDR burden.

2. MATERIALS AND METHODS

2.1 Study area and sampling design

The supplied study record states that 100 symptomatic and asymptomatic ornamental fish were sampled over six months from 10 aquarium retail outlets and local breeding units across Latur city, Ausa, and Udgir blocks of Latur District. Twenty fish were reported for each of five species: goldfish (*Carassius auratus*), guppy (*Poecilia reticulata*), angelfish (*Pterophyllum scalare*), fighter fish (*Betta splendens*), and molly (*Poecilia sphenops*).

2.2 Sample collection, bacterial isolation, and identification

According to the supplied methods, samples were obtained from skin lesions or mucus, gills, and internal organs including liver or kidney where aseptic necropsy was performed. Samples were inoculated onto Nutrient Agar, MacConkey Agar, thiosulfate-citrate-bile salts-sucrose (TCBS) Agar, and Rimler–Shotts medium. The listed identification workflow included Gram staining and biochemical tests such as catalase, oxidase, indole, methyl red, Voges–Proskauer, citrate utilization, and triple-sugar-iron reactions. The four reported taxa were *A. hydrophila*, *P. aeruginosa*, *F. columnare*, and *V. cholerae* non-O1/non-O139.

2.3 Antimicrobial susceptibility testing

The supplied record describes Kirby–Bauer disk diffusion on Mueller–Hinton agar using oxytetracycline (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), and ampicillin (10 µg), with incubation reported at 28 ± 2 °C for 24 h. Susceptibility testing of aquatic bacterial pathogens should follow a validated aquatic-animal method and appropriate organism-specific interpretive criteria. CLSI VET03 describes disk-diffusion and broth-dilution methods for bacteria from aquatic animals, while CLSI VET04 provides the associated performance and interpretive tables (CLSI, 2020a, 2020b).

2.4 Multidrug resistance and data analysis

The supplied manuscript defines MDR as resistance to at least three tested antimicrobial classes. It also defines a multiple-antibiotic-resistance (MAR) index as a/b, where a is the number of antimicrobials to which an isolate was resistant and b is the total number tested (b = 4).

3. RESULTS

3.1 Bacterial recovery across ornamental-fish species

Bacterial growth was reported from 75 of 100 examined fish, giving an overall recovery proportion of 75.0%. The highest species-specific positive proportion was reported for goldfish (18/20; 90.0%), followed by guppy (16/20; 80.0%), angelfish (15/20; 75.0%), fighter fish (14/20; 70.0%), and molly (12/20; 60.0%).

Table 1. Species-Wise Distribution and Bacterial Recovery Rate of Ornamental Fishes Examined in Latur District, Maharashtra.

Fish species	Scientific name	Examined (n)	Positive (n)	Positive (%)
Goldfish	*Carassius auratus*	20	18	90.0
Guppy	*Poecilia reticulata*	20	16	80.0
Angelfish	*Pterophyllum scalare*	20	15	75.0
Fighter fish	*Betta splendens*	20	14	70.0
Molly	*Poecilia sphenops*	20	12	60.0
Total	—	100	75	75.0

3.2 Distribution of reported bacterial isolates

Among 75 reported isolates, *A. hydrophila* was the most frequently reported taxon (28/75; 37.3%), followed by *P. aeruginosa* (20/75; 26.7%), *F. columnare* (16/75; 21.3%), and *V. cholerae* non-O1/non-O139 (11/75; 14.7%). The counts sum to 75 and the percentages sum to 100.0% after rounding.

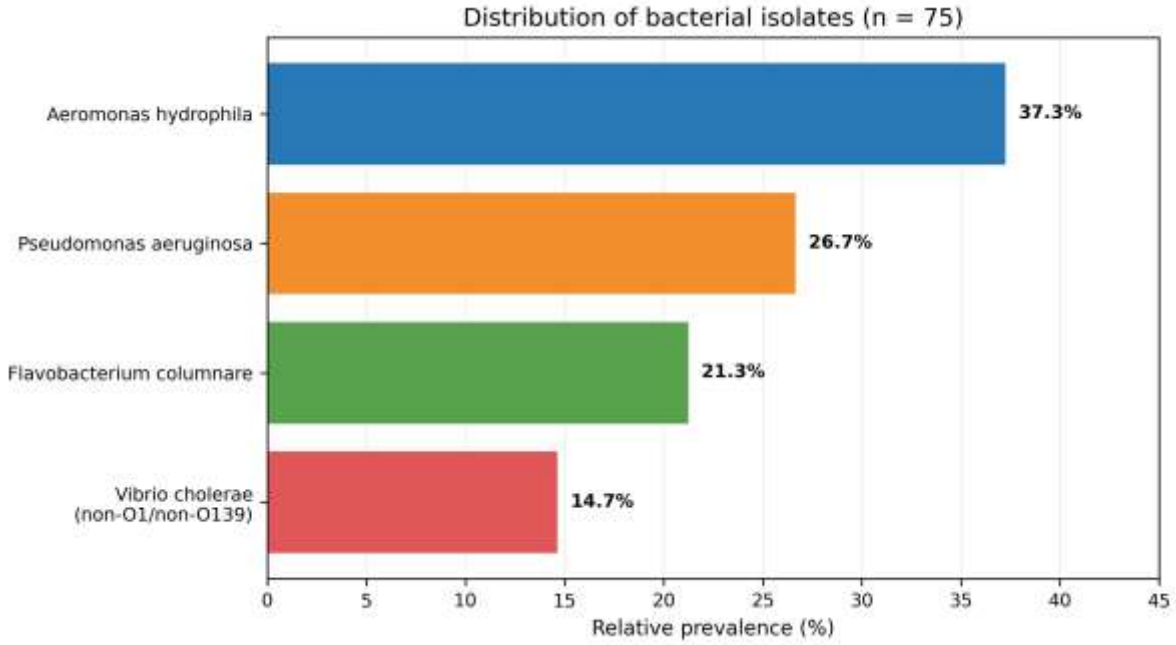


Figure 1. Relative distribution of the four reported bacterial taxa among 75 isolates.

Table 2. Distribution and Relative Prevalence of Bacterial Pathogens Isolated from Ornamental Fishes.

Reported bacterial taxon	Isolates (n)	Relative prevalence (%)	Clinical signs listed in supplied record
Aeromonas hydrophila	28	37.3	Haemorrhagic lesions, ulcers, dropsy
Pseudomonas aeruginosa	20	26.7	Fin/tail rot, scale protrusion, exophthalmia
Flavobacterium columnare	16	21.3	Saddleback lesions, gill erosion, cotton-mouth
Vibrio cholerae (non-O1/non-O139)	11	14.7	Abdominal distension, lethargy, internal hyperaemia

3.3 Antimicrobial susceptibility profile

The reported susceptibility profile varied substantially among the four antimicrobials. Gentamicin had the highest reported susceptible proportion (62/75; 82.7%), followed by ciprofloxacin (57/75; 76.0%). Resistance was highest for ampicillin (55/75; 73.3%) and oxytetracycline (36/75; 48.0%). These are descriptive classifications from the supplied dataset; they do not establish that a drug is clinically effective for a particular fish or pathogen.

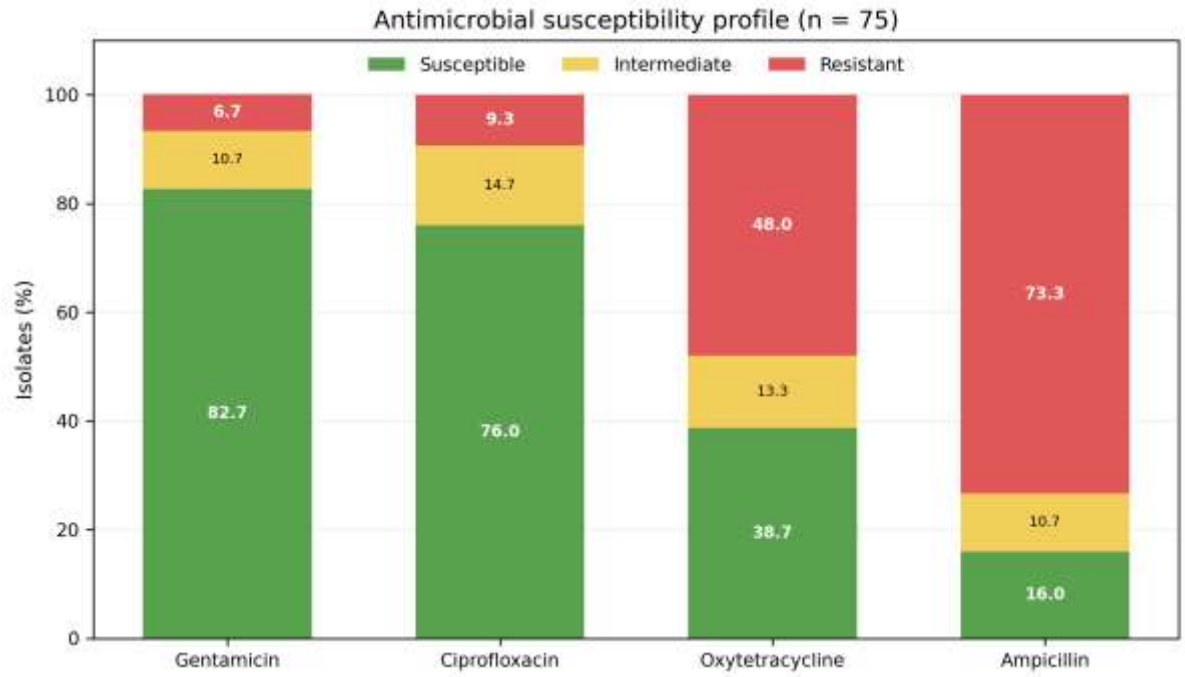


Figure 2. Reported susceptible, intermediate, and resistant proportions for the four tested antimicrobials.

Table 3. Antimicrobial Susceptibility Profiles of Bacterial Isolates Against the Four Tested Antimicrobial Agents.

Antimicrobial (disk content)	Class	Susceptible n (%)	Intermediate n (%)	Resistant n (%)
Gentamicin (10 µg)	Aminoglycoside	62 (82.7)	8 (10.7)	5 (6.7)
Ciprofloxacin (5 µg)	Fluoroquinolone	57 (76.0)	11 (14.7)	7 (9.3)
Oxytetracycline (30 µg)	Tetracycline	29 (38.7)	10 (13.3)	36 (48.0)
Ampicillin (10 µg)	β-lactam / penicillin	12 (16.0)	8 (10.7)	55 (73.3)

3.4 Pathogen-specific resistance and MDR

The the following pathogen-specific resistance percentages: for ampicillin, 82% in *A. hydrophila*, 90% in *P. aeruginosa*, 56% in *F. columnare*, and 55% in *V. cholerae*; for oxytetracycline, 54%, 60%, 31%, and 36%, respectively

3.5 Multidrug resistance

Thirty-two of 75 isolates were reported as MDR (42.7%). The pathogen-specific MDR counts were 13/28 *A. hydrophila* isolates (46.4%), 12/20 *P. aeruginosa* isolates (60.0%), 4/16 *F. columnare* isolates (25.0%), and 3/11 *V. cholerae* isolates (27.3%). *P. aeruginosa* therefore had the highest reported MDR proportion in this dataset. The summary table also reports 15 isolates resistant to one drug and 28 resistants to two drugs; together with 32 MDR isolates, these categories sum to 75.

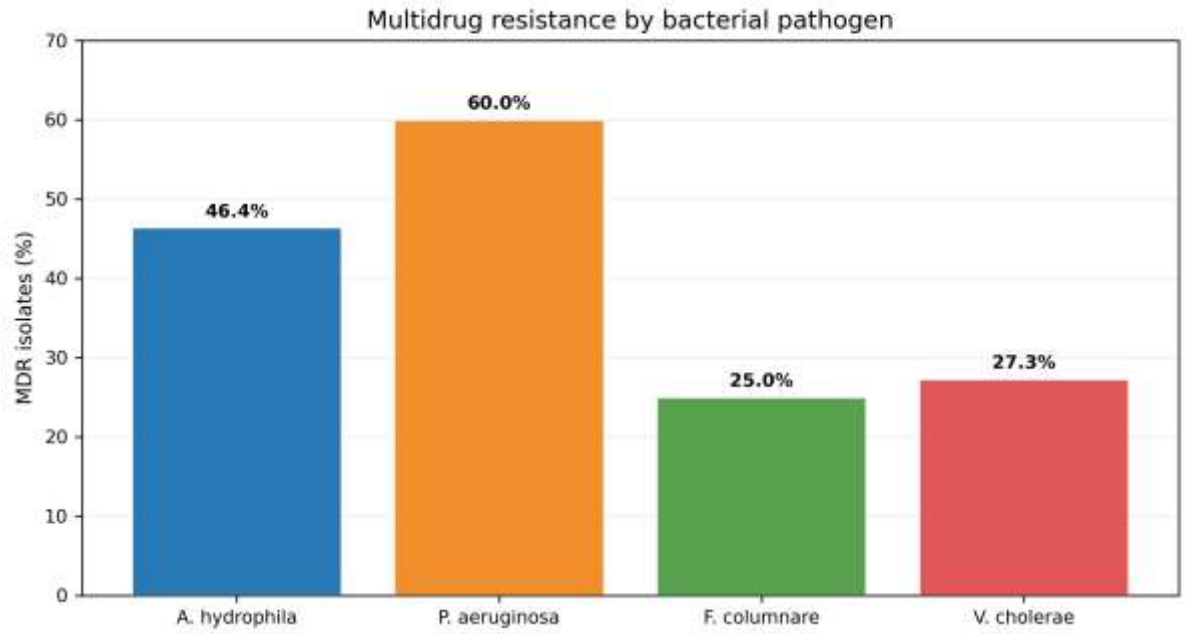


Figure 3. Reported proportion of MDR isolates within each bacterial taxon.

Table 4. Multidrug Resistance (MDR) Distribution and Mean Multiple Antibiotic Resistance (MAR) Index of Bacterial Pathogens

Bacterial taxon	Isolates tested (n)	MDR isolates (n)	MDR (%)	Mean MAR index reported
A. hydrophila	28	13	46.4	0.56
P. aeruginosa	20	12	60.0	0.63
F. columnare	16	4	25.0	0.38
V. cholerae (non-O1/non-O139)	11	3	27.3	0.41
Total	75	32	42.7	0.50

The MAR-index values in the supplied source are retained as reported but could not be independently recalculated without isolate-level resistance profiles.

4. DISCUSSION

The present study revealed bacterial growth in 75 out of 100 ornamental fish examined, representing an overall bacterial recovery rate of 75.0%. Among the five fish species investigated, goldfish (Carassius auratus) exhibited the highest recovery rate (90.0%), followed by guppy (Poecilia reticulata) (80.0%), angelfish (Pterophyllum scalare) (75.0%), fighter fish (Betta splendens) (70.0%), and molly (Poecilia sphenops) (60.0%).

Among the 75 bacterial isolates, Aeromonas hydrophila was the most frequently recovered bacterium, accounting for 28 isolates (37.3%). It was followed by Pseudomonas aeruginosa (20; 26.7%), Flavobacterium columnare (16; 21.3%), and Vibrio cholerae non-O1/non-O139 (11; 14.7%). The predominance of A. hydrophila is consistent with its recognized role as an opportunistic bacterial pathogen associated with fish diseases. Previous investigations have documented multidrug-tolerant bacteria and antimicrobial-resistance determinants in ornamental fish and their transport water (Verner-Jeffreys et al., 2009; Hossain and Heo, 2021).

The antimicrobial susceptibility results demonstrated differences in the reported responses to the four tested agents. Gentamicin showed the highest reported susceptibility, with 62 of 75 isolates (82.7%) classified as susceptible, followed by ciprofloxacin, with 57 isolates (76.0%). In contrast, resistance was highest against ampicillin, affecting 55 isolates (73.3%), followed by oxytetracycline, against which 36 isolates (48.0%) were classified as resistant. Similar concerns regarding antimicrobial resistance among bacteria associated with ornamental-fish systems have been documented in previous studies (Verner-Jeffreys et al., 2009; Preena et al., 2020).

The multidrug-resistance analysis showed that 32 of the 75 isolates (42.7%) were resistant to at least three tested antimicrobial classes. Among the bacterial taxa, Pseudomonas aeruginosa exhibited the highest reported MDR proportion, with 12 of 20 isolates (60.0%), followed by Aeromonas hydrophila, with 13 of 28 isolates (46.4%). Lower proportions were recorded for Vibrio cholerae non-O1/non-O139 (3/11; 27.3%) and Flavobacterium columnare (4/16; 25.0%). These findings demonstrate variation in the reported MDR proportions among the bacterial pathogens isolated from ornamental fish.

The antimicrobial susceptibility results were evaluated using the reported disk-diffusion method. The Clinical and Laboratory Standards Institute provides guidance on antimicrobial susceptibility testing of bacteria isolated from aquatic animals through CLSI VET03 and VET04 (CLSI, 2020a, 2020b). These standards provide a relevant framework for interpreting susceptibility results in aquatic bacterial pathogens.

Overall, the findings document the recovery of four bacterial taxa and the occurrence of antimicrobial resistance among ornamental fish sampled in Latur District, Maharashtra. The predominance of A. hydrophila, the comparatively high reported resistance to ampicillin and oxytetracycline, and the observed MDR proportion highlight the importance of bacterial pathogen surveillance in ornamental-fish systems. Maintaining appropriate stocking densities, improving filtration and water-quality management, quarantining newly introduced fish, and cleaning and disinfecting aquarium equipment are important practices for fish health management.

5. CONCLUSION

This study record describes bacterial recovery from 75 of 100 ornamental fish sampled in Latur District and identifies *Aeromonas hydrophila* as the most frequently reported taxon, followed by *Pseudomonas aeruginosa*, *Flavobacterium columnare*, and *Vibrio cholerae* non-O1/non-O139. The supplied antimicrobial results show the greatest reported resistance to ampicillin and oxytetracycline, while 32 of 75 isolates were classified as multidrug-resistant. *Pseudomonas aeruginosa* showed the highest reported MDR proportion among the four taxa. These findings provide a preliminary local dataset that supports continued bacteriological surveillance and improved husbandry practices in ornamental-fish retail and breeding systems.

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