International Journal of Fisheries and Aquatic Research
www.fishjournals.com
ISSN: 2456-7248
Received: 23-02-2024, Accepted: 20-03-2024, Published: 10-04-2024
Volume 9, Issue 1, 2024, Page No. 16-21

Drug-resistant Bacterial Pathogens: Isolation and characterization in freshwater fish *Catla catla*

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Abstract
This study aimed to identify and characterize the causative agent of microbial illnesses in a freshwater aquacultured *Catla catla*. Along with water samples, a total of 10 fish samples exhibiting clinical indications of illnesses such as hemorrhagic septicemia were gathered. Using morphological, biochemical, and molecular methods, bacteria were characterized. Antibiotics sensitivity test showed that isolate FP04, FP07 and FP10 were shown very little sensitivity towards most of antibiotics. FP04 shown complete resistant towards Cotramoxazole and Tetracycline, FP07 shown resistance against Cotramoxazole while FP10 was fully resist to Nitrofurantoin antibiotic. All water quality measurements, with the exception of temperature, ammonia, and free CO2, were within normal limits. The existence of antibiotic-resistant bacteria in *C. catla* afflicted by hemorrhagic septicemia is confirmed by this investigation. By consuming these aquatic goods and by-products, humans may become infected with these antibiotic-resistant bacteria. Therefore, for an efficient disease control plan, the sensitivity of these bacteria to antibiotics should be regularly assessed.

Keywords: *Catla catla*, drug resistant fish pathogens, isolation, identification

Introduction
Over the past few decades, the global aquaculture sector has grown at an average annual pace of 6.6%, exceeding the increase of the human population (Nadarajah and Flaaten, 2017) [23]. The industry is essential to the growth of the economy. Despite this industry's significant contribution, aquaculture's performance is frequently dictated by how well diseases are managed (Israngkura and Sae-Hae, 2002, Hasimuna *et al*., 2023) [17]. These days, Maharashtra's several districts are concentrated on the polyculture of large Indian carps (Das and Ferosekhan, 2022). The most well-liked carp species is *Catla catla*, a native fish of the region's riverine ecosystem (Bais, 2018) [4]. It is a common food fish in India and plays a significant role in the region's polyculture system because of its faster growth rates and better economic worth. Fish raised in fish farms are frequently more vulnerable to certain pathogens, particularly when the weather is colder. *Pseudomonas* sp. caused outbreaks of severe illnesses such as hemorrhagic septicemia, gill necrosis, abdominal distension, splenomegaly, friable liver, and congested kidney have been linked to significant economic losses (Ardura *et al*., 2013 [3] and Algammal *et al*., 2020) [2].
Variable clinical indications, including as hemorrhages on the skin, fins, and other body parts, are indicative of the condition. The pathogens also attack the fish's internal organs and muscles if the disease lasts for extended periods of time (Overstreet and Hawkins, 2017) [27]. As a result, efforts were conducted in this study to identify the most resistant fish pathogens as well as to isolate the fish pathogens linked to the *Catla catla*. These steps were followed by characterizing the isolates and determining their pattern of antibiotic sensitivity. To ascertain the efficacy of regularly used antibiotics for the treatment of this condition, antibiotic sensitivity was also investigated.

Materials and methods
Collection of Fish and Water Samples
Attendance at outbreaks and the collecting of fish samples from the Vishnupuri Dam in Vishnupuri, Nanded, Maharashtra, were included in the study. This dam is utilized for commercial fish farming and water storage. The postmortem lesions of generalized septicemia surrounding the anus, together with the expansion of the gall bladder and the yellowish hue of the air bladder, were the criteria used to select the moribund fish samples (n = 10). Fish samples were taken, placed in sterile plastic bags, kept cold, and sent straight to the lab for analysis. Water quality measures, such as temperature, dissolved oxygen, CO2, pH, total alkalinity, total hardness, ammonia, and nitrite, were tested on water samples that were taken from several dam locations.

Analysis of water
Temperature and pH were measured at the sampling site (Golterman *et al*., 1978) [13]. While other water quality indicators including Dissolved Oxygen (DO), Carbon Dioxide (CO2), Total Alkality, Total Hardness, Ammonia Concentration and Nitrate concentration were measured in the lab with standard protocol. The association between the water quality indicators and hemorrhagic septicemia was then determined by comparing the observed values of the parameters with standard values (GOP, 2006 and Nurul *et al*., 2016) [26].

Isolation of Bacterial Pathogens
Fish samples collected from the aquaculture site were proceed for disinfection of fish skin with a 70% alcohol and absolute betadine solution, aseptic dissection took place in the lab. The liver, kidneys, spleen, and muscles of the infected fish were streaked in duplicate onto Nutrient Agar (HiMedia, Ltd.) and incubated for a full day at 37°C.

Twenty-four hours after incubation, colonies of bacteria were grown on plates containing cultured media. Through reinoculation onto brain heart infusion agar, suspected colonies were refined. For additional analysis, the pure culture was moved to nutrient agar slants and refrigerated at -4°C.

Characterization of the isolates
Isolated pure strains were subjected to characterize using morphological approach containing size, shape, colour, margine, opacity, consistency, motility of the isolates, biochemical assays *viz*. IMViC test, sugar fermentation test, enzyme tests etc in accordance with Bergey's manual of systematic bacteriological classification (Holt, 1986 [18], Chen *et al*., 2022 [7] and Jadhav *et al*., 2023) [19].

Antibiotic Sensitivity Assay
The standard disc diffusion method was used to determine the antibiotic sensitivity test against Ampicillin (10 mg), Ciprofloxacin (10 mg), Colistin (300 mg), Cotrimoxazole (25 mg), Gentamycin (10 mg), Nitrofurantoin (300 mg), Streptomycin (10 mg), and Tetracycline (30 mg) using an antibiotic Octadisc OD005 (HiMedia, India Ltd.). An active culture of isolates was spread over the nutrient agar plates, and an antibiotic octadisc was aseptically applied. The plate was then incubated for 24 hours at 37°C. Zone of inhibition was used to assess the isolates' susceptibility to antibiotics. Zone measurement scale (HiMedia, India Ltd.) was used to determine the diameter of the entire zone of inhibition. (CLSI, 2009 and Baisthakur *et al*. 2022) [5].

Identification of the Isolates
The isolates shown most resistance against the antimicrobial agents are selected for the identification using molecular approach *viz*. 16S rRNA gene sequencing (Janda and Abbott, 2007 [20] and Johnson *et al*., 2019) [21].

Bioinformatics
The sequences of 16S rRNA gene of FP04, FP07 and FP10 were subjected to BLAST on NCBI portal. The similar sequences were compared to found most similar organism on NCBI. Phylogeny of the isolate and most similar organisms were constructed using most likehood option in MEGA X program. By studying the phylogeny tree, isolates were identified up to species level.

Observation and Results
Collection of Fish and Water Samples
A total 10 samples of *Catla catla* fishes were collected from the water reservoir of vishnupuri Dam, Nanded (Figure 1). Water samples were also collected from the same reservoir by selecting different locations.

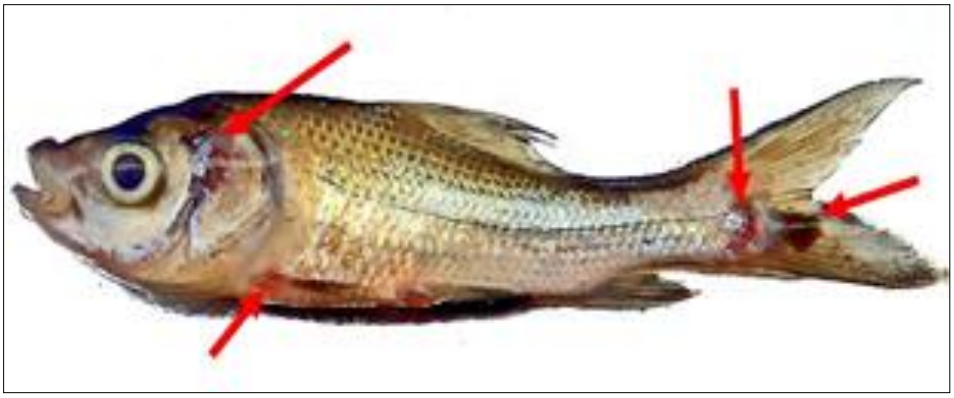

Fig 1: Collected *Catla catla* fish with skin septicemia.

Analysis of Water sample
Water quality parameters are presented in Table 1. Most of the recorded parameters were within recommended limits for this species (Nagar *et al*., 2019) [24] except ammonia and nitrate level of water was slightly high than normal recommended range.

Isolation of Bacterial pathogens
A total 12 purified isolates were recovered from *Catla catla* fish samples obtained from the aquaculture. The results for morphological and biochemical tests are summarized in Table.2. Pure colonies obtained from infected organs of *Catla catla* were identified as *Pseudomonas species and Aeromonas species* after morphological and biochemical tests as mentioned in the Bergey's Manual of Systematic Bacteriology (Holt, 1986) [18].

Antibiotic Sensitivity Assay
Antibiotics sensitivity test showed that isolate FP04, FP07 and FP10 were shown very little sensitivity towards most of antibiotics. FP04 shown complete resistant towards Cotramoxazole and Tetracycline, FP07 shown resistance against Cotramoxazole while FP10 was fully resist to Nitrofurantoin antibiotic. Remaining isolates were sensitive towards the antibiotics. Complete determination of the antibiotic sensitivity assay of the isolates obtained from the *Catla catla* fish samples were shown in Tatble.3.

Identification of the Isolates
As per the data of 16S rRNA gene sequence of most resistant strains FP04, FP07 and FP10 were identifies as *Pseudomonas putida* FP04, *Pseudomonas aeruginosa* FP07 and *Aeromonas hydrophilia* FP10 respectively as shown in the phylogeny tree constructed using neightbor joining method (Figure 2a, 2b and 2c).

Bioinformatics
The 16S rRNA gene sequence obtained from the sequencing were submitted to the NCBI GeneBank and got Accession No. as *Pseudomonas putida* FP04 (PP455756), *Pseudomonas aeruginosa* FP07 (PP455758) and *Aeromonas hydrophilia* FP10 (PP455760).

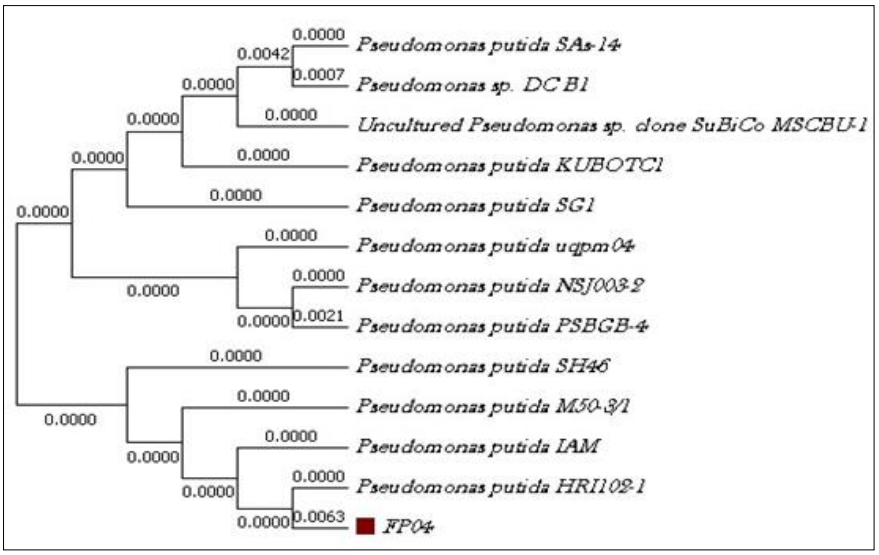

Fig 2a: Phylogeny of isolate FP04 shows most resemblance with *Pseudomonas putida* as per neighbor joining method.

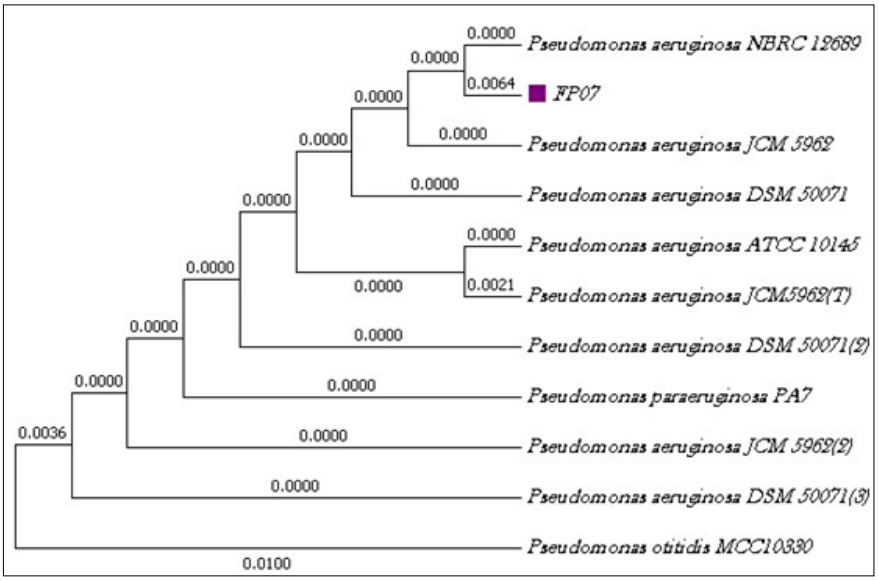

Fig 2b: Phylogeny of isolate FP07 shows most resemblance with *Pseudomonas aeruginosa* as per neighbor joining method.

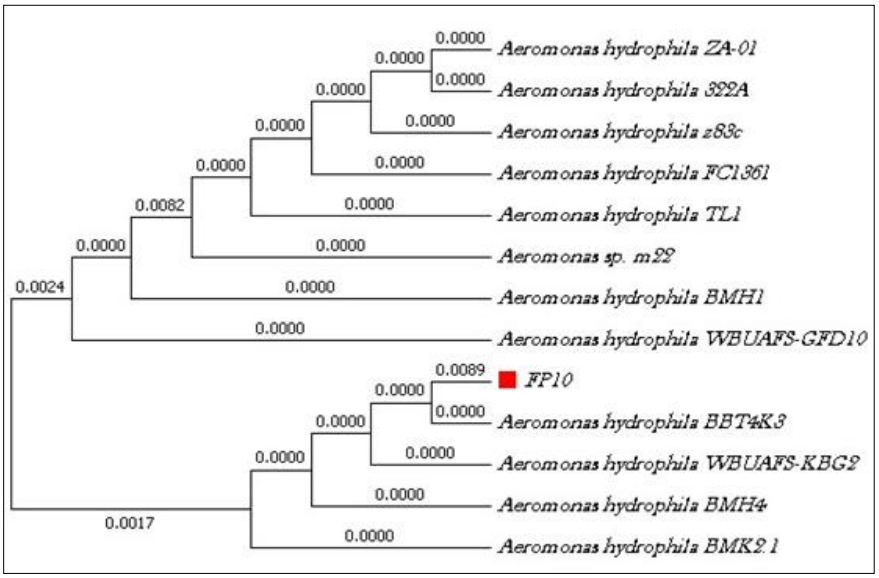

Fig 2C: Phylogeny of isolate FP10 shows most resemblance with *Aeromonas hydrophila*as

per neighbor joining method.

Table 1: Water quality parameters of Vishnupuri dam, Nanded, Maharashtra.

Water Quality Parameteres		
Parameters	**Measured Values**	**Recommended values or range**
Temperature (°C)	16.0±1.12	minimum limit = 14
Dissolved Oxygen (ppm)	4.8±1.02	5
Carbon Dioxide (ppm)	16±1.04	≤ 20 ppm
pH	6.7±0.33	7.0 to 8.0
Total Alkalinity (ppm)	71±1.42	50 to 100 ppm
Total Hardness (ppm)	55±2.16	50 to 100 ppm
Ammonia (ppm)	0.14±0.21	Less than 0.05 ppm
Nitrite (ppm)	0.23±0.13	Less than 0.5ppm

Table 2: Morphological and Biochemical Characteristics of bacterial isolates obtained from *Catla catla.*

Sr. No.	Biochemical Characteristics	Isolate											
		FP1	**FP2**	**FP3**	**FP4**	**FP5**	**FP6**	**FP7**	**FP8**	**FP9**	**FP10**	**FP11**	**FP12**
1	Colony Size (mm)	3	2	4	3	2	2	3	5	3	2	1	2
2	Colony Shape	Round	Round	Round	Round	Round	Round	Round	Oval	Round	Round	Round	Round
3	Colony Colour	White	Pale	White	Green	White	White	White	White	White	White	White	Pale
4	Colony Margine	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire
5	Colony Elevation	Convex	Flat	Convex	Convex	Flat	Convex	Convex	Flat	Flat	Convex	Convex	Convex
6	Colony Opacity	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque
7	Consistency	Sticky	Btrs	Sticky	Sticky	Btrs	Btrs	Btrs	Sticky	Btrs	Btrs	Btrs	Sticky
8	Cell Motility	-	+	+	+	+	+	+	+	+	-	+	+
9	Gram's Nature	+ Cocci	- Rods	- Rods	- Rods	- Rods	- Rods	- Rods	- Rods	- Rods	+ Cocci	- Rods	- Rods
10	Indole	-	-	-	-	-	-	+	-	-	-	-	+
11	Methyl Red	+	-	-	-	-	-	+	-	-	+	-	+
12	Voges Proskeuer	+	-	-	-	-	-	+	-	-	+	-	+
13	Citrate Utilization	+	+	-	-	+	+	+	+	-	+	+	+
14	Oxidase	-	+	+	+	+	+	+	+	+	-	+	+
15	Catalse	+	+	+	+	+	+	+	+	+	+	+	+
16	Nitrase Reductase	+	+	-	-	+	+	+	+	-	+	+	+
17	Urease	+	+	-	-	+	+	-	+	-	+	+	-
18	Hemolysis	β	β	β	β	β	β	β	β	β	β	β	β
19	Fermentation of:												
	a. Arabinose	-	-	-	-	-	-	-	-	-	-	-	-
	b. Galactose	+	-	+	+	-	-	-	-	+	+	-	-
	c. Glucose	+	-	+	+	-	-	+	-	+	+	-	+
	d. Lactose	+	-	-	-	-	-	-	-	-	+	-	-
	e. Sucrose	+	-	-	-	-	-	+	-	-	+	-	+
	f. Mannitol	+	+	+	+	+	+	+	+	+	+	+	+
	g. Xylose	-	-	+	+	-	-	-	-	+	-	-	-
20	Pigment Production	+	+	+	+	+	+	-	+		+	+	-
21	Coagulase	+	-	-	-	-	-	-	-	-	+	-	-
22	Starch Hydrolysis	+	-	-	-	-	-	+	-	-	+	-	+
23	Casein Hydrolysis	+	+	-	-	+	+	-	+	-	+	+	-
24	Cetrimide Test	-	+	+	+	+	+	-	+	+	-	+	-

*Btrs: Butyrous

Table 3: Antibiotic sensitivity of Isolated fish Pathogens: FP04, FP07 and FP10 different antibiotics.

Sr. No.	Antibiotics (µg/disc)	FP1	FP2	FP3	Zone of Inhibition (in mm) FP4	FP5	FP6	FP7	FP8	FP9	FP10	FP11	FP12
1	Ampicillin 10	11	09	05	02	11	01	02	07	06	02	09	10
2	Colistine 10	08	10	10	03	10	04	01	09	12	01	11	13
3	Ciprofloxacin 10	10	12	13	05	12	04	03	12	14	07	12	09
4	Cotrimoxazole 25	07	11	08	**00**	09	02	**00**	11	08	02	09	10
5	Gentamycin 10	16	12	13	02	12	05	08	13	12	04	08	11
6	Nitrofurantoin 300	06	08	07	03	10	03	04	10	08	**00**	10	09
7	Streptomycin 10	14	15	11	01	13	06	05	11	12	05	11	12
8	Tetracycline 30	11	09	14	**00**	12	02	02	09	11	03	13	09

Discussion

Stressful environmental conditions, such as variations in temperature, ammonia levels, pH, and oxygen levels, may impair fish's capacity to mount a robust immune response. Bacterial infections, such as those caused by *Aeromonas* and *Pseudomonas* spp., frequently occur after severe periods of low temperature and oxygen levels (Camus *et al*., 1998, Algammal *et al*., 2020 [2] and Semwal *et al*., 2023) [29]. According to reports, pH, free CO2, and ammonia are more crucial for fish survival than dissolved oxygen alone. pH controls the majority of metabolic reactions. (Mathur *et al*., 2008). Nonetheless, free CO2 (16 ppm) throughout the

current investigation was within the recommended range, leading to a neutral pH (7.0). Total hardness (55 ppm) and total alkalinity (71 ppm), which were lower values, showed that the water body was moderately productive throughout the sampling period. The current research found that the water's temperature was 16 °C, which indicates a normal range of temperatures. The minimum temperature at which this species may survive luxuriously is 14 °C. In addition, the levels of nitrite (0.23 ppm) and dissolved oxygen (4.8 ppm) were both within the usual range (Mirza and Bhatti, 1995); nevertheless, the ammonia concentration (0.14 ppm) was greater than the advised level.

The two main fish infections, *Aeromonas* and *Pseudomonas*, have previously been isolated from several sick fish organs (Li and Cai, 2011 and Pessoa *et al*., 2022) [28]. The isolates from C. catla that caused hemorrhagic septicemia in the current investigation were determined to be *Pseudomonas putida* FP04, *Pseudomonas aeruginosa* FP07, and *Aeromonas ahydrophila*. We can presume that these bacterial pathogens could be a significant factor in fish disease in this area. Additionally, it is indicated that bacterial isolates from various origins might be identified using standard morphological, biochemical, and molecular techniques (FranscoDurate, *et al*., 2019). Research on the antibiotic profile of *Pseudomonas* sp. and *Aeromonas* sp. showed that these three antibiotics were highly resistant to Tetracycline, Nitrofurantoin, and Cotimoxazole, whereas Ampicillin, Ciprofloxacin, Gentamycin, and Streptomycin showed very low resistance. In previous study of Suresh *et al*., had reported the drug resistant *Pseudomonas aeruginosa* from fresh water fishes and discussed about the drug resistance against oxytetracycline, cotrimoxazole, doxycycline, enrofloxacin, ciprofloxacin, cefotaxime, ceftazidime and ampicillin. Beulah *et al*., 2021 [6] also reported the drug resistant bacterial pathogens isolated from fresh water fish: *Catla catla* fingerlings.

Antibiotic resistant Micrococcus were isolated from freshwater farm *Catla catla* which was susceptible to antibiotics such as Amoxycillin, Azithromycin, Cefixime, Ciprofloxacin, Chloramphenicol, Furadantin, Gentamicin, Levofloxacin, Tetracycline and Vancomycin while the sensitive to Amoxycillin and Azithromycin antibiotics (Darak *et al*., 2021) [10]. As in a previous study 2% strains were found resistant to chloramphenicol and only one strain was resistant to chloramphenicol out of sixty-nine strains tested (Goni-Urriza *et al*., 2000 [14]; Akinbowale *et al*., 2007) [1]. While a few strains of *Aeromonas sp.* were sensitive to chloramphenicol (Guz and Kozinska, 2004 [16]; Suhet *et al*., 2011) [30]. One study in Japanese veterinary hospital also revealed the drug resistance in *Pseudomonas aeruginosa* obtained from animal source got resistance against ciprofloxacin, cefotaxime, gentamicin, amikacin, fosfomycin and ceftazidime antibiotics (Yukawa *et al*., 2017) [32].

Three drug-resistant bacterial pathogens were isolated from infected *Catla catla* in the current investigation. Nonetheless, a number of *Aeromonas* species and other bacterial pathogens were isolated from affected fish in earlier, related investigations. The isolation of *Pseudomonas* and *Aeromonas* in the current investigation may have resulted from the introduction of these pathogens into the nearby water bodies through the contaminating of water with household waste. Aside from this rationale, human pathogenic strains in the nearby water bodies may also originate from the government hospital in the Vishnupuri neighborhood of Nanded city. As a result, effective management of household and medical waste as well as the avoidance of contaminated water discharge into nearby drainage systems that eventually meet local water bodies and reservoirs can aid in the reduction of issues related to fish farming and the management of diseases in general (Englande *et al*., 2015) [11]. Such drug resistant pathogen contaminated water may reach to agriculture sector and up to food material (Baisthakur *et al*., 2022) [5].

In conclusion, the fish afflicted with hemorrhagic septicemia yielded P. putida FP04, P. aeruginosa FP07, and A. hydrophila FP10. Since the isolation temperature in this study was 37 °C and the media employed may not have been ideal for the development of other infections, it is impossible to completely rule out the potential that other pathogens may have been involved. The veterinary public health should act quickly to stop the spread of P. putida FP04, P. aeruginosa FP07, and A. hydrophila FP10 to consumers due to their zoonotic potential and medication resistance. It is also advised to carry out a broad-spectrum investigation on the same pattern in order to identify the pocket of fish diseases brought on by the aforementioned pathogens in other regions of the nation.

References

1. Akinbowale OL, Peng H, Grant P, Barton MD. Antibiotic and heavy metal resistance in motile Aeromonads and Pseudomonads from rainbow trout (Oncorhynchus mykiss) farms in Australia. Int J Antimicrob Agents,2007:30:177-182.
2. Algammal AM, Mabrok M, Sivaramasamy E, *et al*. Emerging MDR-Pseudomonas aeruginosa in fish commonly harbor oprL and toxA virulence genes and blaTEM, blaCTX-M, and tetA antibiotic-resistance genes. Sci Rep,2020:10(1):15961. doi:10.1038/s41598-020-72264-4.
3. Ardura A, Linde AR, Garcia-Vazquez E. Genetic detection of Pseudomonas spp. in commercial Amazonian fish. Int J Environ Res Public Health,2013:10:3954-3966.
4. Bais B. Fish scenario in India with emphasis on Indian major carps. Int J Avian & Wildlife Biol,2018:3(6):409-411. doi:10.15406/ijawb.2018.03.00130.
5. Baisthakur PO, Kadam TA, Bhosale HJ, Ramangire MA. Antibiotic Resistant Bacterial Pathogens Associated with Raw Vegetables from Nanded City, 2022.
6. Beulah G, Divya D, Kumar NSS, *et al*. Purification and characterization of bioactive compounds extracted from Suaeda maritima leaf and its impact on pathogenicity of Pseudomonas aeruginosa in Catla catla fingerlings. AMB Express,2021:11(1):135. doi:10.1186/s13568-021-01295-5.
7. Chen H, Zhao Y, Chen K, *et al*. Isolation, Identification, and Investigation of Pathogenic Bacteria From Common Carp (Cyprinus carpio) Naturally Infected With Plesiomonas shigelloides. Front Immunol,2022:13:872896. doi:10.3389/fimmu.2022.872896.
8. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: Nineteenth informational supplement. CLSI

document M100-S19. Wayne, PA: Clinical and Laboratory Standards Institute, 2009.
9. Darak OS, Barde RD. Pseudomonas fluorescens associated with Bacterial Disease in Catla catla in Marathwada Region of Maharashtra. Int J Adv Biotechnol Res,2015:6(2):189-195.
10. Darak OS, Baisthakur PO, Barde RD. Antibiotic Resistance in Micrococcus sp. Isolated from Freshwater Farms Catla catla. Efflatounia,2021:5(2):130-136.
11. Englande AJ Jr, Krenkel P, Shamas J. Wastewater Treatment &Water Reclamation. Reference Module in Earth Systems and Environmental Sciences. B978-0-12-409548-9.09508-7. doi:10.1016/B978-0-12-409548-9.09508-7.
12. Franco-Duarte R, Černáková L, Kadam S, *et al*. Advances in Chemical and Biological Methods to Identify Microorganisms-From Past to Present. Microorganisms,2019:7(5):130. doi:10.3390/microorganisms7050130.
13. Golterman HL, Clymo RS, Ohnsted MAM. Methods for physical and chemical analysis of fresh water. Blackwell Scientific Publication: 1978.
14. Goni-Urriza M, Pineau L, Capdepuy M, Roques C, Caumette P, Quentin C. Antimicrobial resistance of mesophilic Aeromonas spp. isolated from two European rivers. J Antimicrob Chemother,2000:6:297-301.
15. GOP. Pre-feasibility study for fish farming, Inland and coastal. Production, processing and marketing. http://www.pc.gov.pk/studies.html. Accessed June, 2007.
16. Guz L, Kozinska A. Antibiotic susceptibility of Aeromonas hydrophila and Aeromonas sobria isolated from farmed carp (Cyprinus carpio L.). Bull Vet Inst Pulawy,2004:48:391-395.
17. Hasimuna OJ, Maulu S, Nawanzi K, *et al*. Integrated agriculture-aquaculture as an alternative to improving small-scale fish production in Zambia. Front Sustain Food Syst,2023. doi:10.3389/fsufs.2023.1161121.
18. Holt JG. Bergey's Manual of Systematic Bacteriology. Williams and Wilkins, 1986.
19. Jadhav VG, Baisthakur PO, Joshi RD, Jadhav AS. Isolation, Screening and Characterization of Exopolysaccharide Producing Pseudomonas spp. from the Soil sample. J Adv Zool,2023:44(S-3):540-548.
20. Janda JM, Abbott SL. 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils, and pitfalls. J Clin Microbiol,2007:45(9):2761-2764. doi:10.1128/JCM.01228-07.
21. Johnson JS, Spakowicz DJ, Hong BY, *et al*. Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis. Nat Commun,2019:10:5029. doi:10.1038/s41467-019-13036-1.
22. Li Y, Cai SH. Identification and pathogenicity of Aeromonas sobria on tail rot disease in juvenile tilapia (Oreochromis niloticus). Curr Microbiol,2011:62:623-627.
23. Nadarajah S, Flaaten O. Global aquaculture growth and institutional quality. Mar Policy,2017:84:142-151. doi:10.1016/j.marpol.2017.07.018.
24. Nagar P, Mishra A, Kumar P, Tiwari H, Ram RN. Effect of water flow on growth of Catla catla (Hamilton, 1822) reared in recirculatory aquaculture system. Indian J Fish,2019:66(3):14-147.
25. Nalle D, Dhotre M. Studies on Aeromonas infection in Indian major carp *viz*. Catla (Catla catla), Rohu (Labeo rohita) and Common carp (Cyprinus carpio).
26. Nurul IAI, Mohammad NAA, Shohaimi S, Saad MZ, Siti ZA. Associations of water quality and bacteria presence in cage cultured red hybrid tilapia, Oreochromis niloticus×O. mossambicus. Aquaculture Reports,2016:4:57-65. doi:10.1016/j.aqrep.2016.06.004.
27. Overstreet RM, Hawkins WE. Diseases and Mortalities of Fishes and Other Animals in The Gulf of Mexico. In: Ward C, eds. Habitats and Biota of the Gulf of Mexico: Before the Deepwater Horizon Oil Spill. Springer: 2017. doi:10.1007/978-1-4939-3456-0_6.
28. Pessoa RBG, de Oliveira WF, Correia MTDS, Fontes A, Coelho LCBB. Aeromonas and Human Health Disorders: Clinical Approaches. Front Microbiol,2022:13:868890. doi:10.3389/fmicb.2022.868890.
29. Semwal A, Kumar A, Kumar N. A review on pathogenicity of Aeromonas hydrophila and their mitigation through medicinal herbs in aquaculture. Heliyon,2023:9(3):e14088. doi:10.1016/j.heliyon.2023.e14088.
30. Suhet MI, Schocken-Iturrino RP, Amaral LA. Hemolytic activity and resistance to antimicrobials by Aeromonas species isolated from intensive rearing of Nile Tilapia (Oreochromis niloticus). ARS Veterinaria,2011:27:36-44.
31. Suresh K, Pillai D, Soman M, Sreenivas A, Paul R. Isolation and identification of antimicrobial susceptibility, biofilm formation, efflux pump activity, and virulence determinants in multi-drug resistant Pseudomonas aeruginosa isolated from freshwater fishes. J Water Health,2023:21(12):1858-1870.
32. Yukawa S, Tsuyuki Y, Sato T, Fukuda A, Usui M, Tamura Y. Antimicrobial Resistance of Pseudomonas aeruginosa Isolated from Dogs and Cats in Primary Veterinary Hospitals in Japan. Jpn J Infect Dis,2017:70(4):461-463. doi:10.7883/yoken.JJID.2016.536.