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Occurrence and Antimicrobial Susceptibility of *Aeromonas* spp. Isolated from Schizothoracine Fishes of the River Ganga and its Tributaries in Uttarakhand, India

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Abstract: The present study examined the pathogenic bacteria in Schizothoracine fish species inhabiting the cold-water of the River Ganga and its major tributaries in Uttarakhand, India. Over a one-year period (2023-2024), samples were collected across seven key sites along the mentioned river during winter and summer seasons. A total of 563 fish specimens were sampled throughout this period and examined for the detection of bacterial pathogens and their infection in fishes. The study revealed the presence of presumptive *Aeromonas* spp. in the samples. The findings revealed that only a few samples from specific sites harboured pathogenic *Aeromonas* spp. isolates. However, no disease outbreaks were observed among the examined fish species. The absence of observable outbreaks is likely due to relatively low levels of pathogenic bacterial contamination in the snow-fed tributaries of the river Ganga. This study provided one of the first assessments of the occurrence of pathogenic *Aeromonas* species in wild *Schizothorax* genus inhabiting the Himalayan stretches of the River Ganga and its tributaries.

Keywords: Cold-water fishes • *Schizothorax* • Pathogenic bacteria • *Aeromonas* • Gram-negative bacteria • Bacterial diseases • motile aeromonas septicemia (MAS).

Introduction

The snow-fed central Himalayan river (Karmakar, 2000) Ganga (Ganges) is considered one of the holiest sacred rivers for Hindus from time immemorial (Bhutiani et al., 2016) and contributes 25% of the country's water resources (Paul, 2017). The tributaries of the river Ganga in Uttarakhand are dominated by snow trout (*Schizothorax and Schizothoraichthys Species*), mahseer, hill trout (*Barilius*), *Garra and Glyptothorax* (Alam et al., 2025). *The* Schizothoracine group is widely distributed across numerous rivers, lakes and tributaries throughout the Himalayas (Regmi et al., 2023; Du et al., 2024; Gul & Rashid, 2025) and were predominantly caught (60%) during fishing in 2023-2024. Additionally, Schizothoracine fishes inhabit a wide range of lotic and lentic habitats (Bhatt & Manish, 2023), particularly cold, fast-flowing, oxygen-rich waters, high-elevation environments and exhibit specific physiological behaviours and morphological adaptations. All these sensitive characters make Schizothoracine fishes more susceptible to various stresses, such as poor physico-chemical water parameters, pathogenic and parasitic diseases.

A variety of bacterial, viral, fungal and parasitic pathogens have been identified in fish inhabiting cold-water ecosystems worldwide. Among these, bacterial pathogens pose a significant threat to fish populations, particularly in the challenging, extreme conditions prevalent in these environments. The epidermis, gills and gastrointestinal (GI) tract are the three principal routes of pathogen entry into fish (Ringø et al., 2010). Although fish develop both specific and non-specific defences against microbial pathogens and certain illnesses. Most cold-water bacterial pathogens are gram-negative, with a thin peptidoglycan layer and an outer cell membrane, which includes common genera such as *Pseudomonas, Edwardsiella, Aeromonas, Flavobacterium* and *Vibrio.* Whereas, gram-positive genera such as

Mycobacterium, *Renibacterium and Streptococcus* are the primary pathogens affecting fish inhabiting the cold-water environments (Austin & Allen-Austin, 1985; Kumar et al., 2023). *Aeromonas* comprises gram-negative, rod-shaped (bacilli), opportunistic, facultative, anaerobic, motile bacteria (Hudson, 2004) that cause motile aeromonas septicemia (MAS) in cultured and freshwater fish (Semwal et al., 2023). Most species are characterised by rod-like morphology, oxidase-positive (Mulia et al., 2023) and survive in the water column, fish organs, gills and gastrointestinal tract (Semwal et al., 2023), thus causing both external lesions (ulcer, fin/tail rot) and systemic infection, like organ necrosis, dropsy and anaemia in fish (Shabana et al., 2025). Furthermore, *Aeromonas hydrophila, A. veronii, A. sobria* and *A. caviae* are the most common species of the genus *Aeromonas* found in fish (Janda & Abbott, 2010).

Recent studies indicate that the Ganga River exhibits bacteriophage activity, potentially reducing bacterial loads (Behera et al., 2022; Desmecht et al., 2026). However, it is important to note that anthropogenic activities continue to contribute to the increasing pollution of this vital water source. Thus, the research area generated significant interest, particularly regarding the potential presence of pathogenic bacteria in wild fish populations in the River Ganga and its tributaries in Uttarakhand, India. A limited number of scientific studies have addressed the bacterial fish pathogens in the river Ganga. Therefore, Schizothoracine fishes, which are prevalent in the river tributaries, were selected as the experimental subjects for the detection of pathogenic bacteria. The objective of this study was to identify pathogenic bacterial isolates from the Schizothoracine fishes in the Ganga River in the Uttarakhand region of the central Himalaya. Selective culture media, Gram staining and a series of biochemical tests and susceptibility assessments were used to confirm that numerous isolates belonged to the genus *Aeromonas*.

Materials and methods

Study location: A total of 7 sampling sites were designated along the River Ganga and its tributaries in the state of Uttarakhand, India, for sample collection. All sampling sites span a diverse elevation range from 384 metres to 2531 metres (Figure 1a). During sampling, parameters such as temperature, pH and dissolved oxygen (DO) were systematically measured at each site (Table 1) to ensure accurate, reliable data collection and comprehensive analysis.

Clinical Observation

Fish samples were examined for external lesions, injuries or other abnormalities and these findings were recorded. Fish species were identified, (Jayaram, 1981; Nelson et al., 2016; Tilak, 1987) weighed and measured their length. Fishes were then sanitised with 70% alcohol and prepared for dissection.

Culture, isolation and purification

Samples obtained from the kidney, liver, heart, gills and ascites of infected fish were directly streaked onto selective media Aeromonas Strach DNA Agar Base (HiMedia M1284) with aseptically rehydrated contents of Ampicillin supplement (HiMedia FD082). Culture plates were incubated aerobically at 27°C for 24-72 hours. Dominant isolates were streaked and re-streaked on the same culture media for purification. Pure isolates were stored in Soyabean Casein Gigest Agar (Tryptone Soya Agar) (HiMedia M290) at 4°C for further study.

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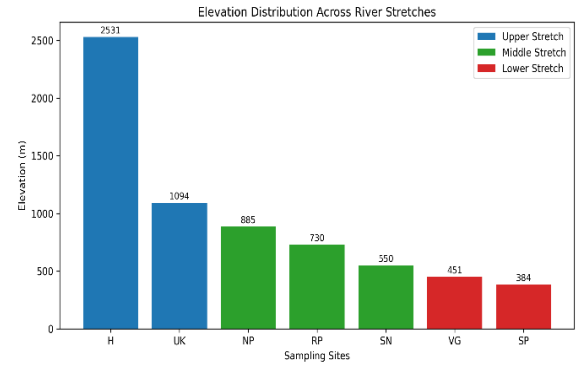

1a **1b**

H = Harsil; UK = Uttarkashi; NP = Nandaprayag; RP = Rudraprayag; SN = Srinagar; VG = Vyas Ghat; SP = Shivpuri.

Figure 1: Figure 1a showed the elevation difference among the seven sampling sites across the river in metres (m) and Figure 1b showed the fish samples of Schizothoracine fishes

Table 1: Physico-chemical parameters and presence of *Aeromonas* isolates in summer and winter seasons across 7 sampling sites

Sites	Seasons	Temp. (˚C)	pH	DO (mg ˉ1)	*Aeromonas*
Harsil	Winter	2.43 ± 0.15	7.13 ± 0.06	13.83 ± 0.21	0
Harsil	Summer	11.23 ± 0.60	7.37 ± 0.21	10.23 ± 0.49	0
Uttarkashi	Winter	7.0 ± 2.0	7.23 ± 0.15	13.10 ± 0.26	0
Uttarkashi	Summer	13.10 ± 0.66	7.60 ± 0.20	10.50 ± 0.46	1
Nandaprayag	Winter	10.97 ± 0.57	6.97 ± 0.15	10.47 ± 0.23	0
Nandaprayag	Summer	17.07 ± 0.67	7.37 ± 0.21	9.6 ± 0.20	1
Rudraprayag	Winter	10.90 ± 0.36	7.60 ± 0.20	10.00 ± 0.17	0
Rudraprayag	Summer	21.77 ± 0.25	7.87 ± 0.12	9.67 ± 0.40	1
Srinagar	Winter	11.90 ± 0.36	8.7 ± 0.15	8.23 ± 0.35	1
Srinagar	Summer	21.80 ± 0.20	8.37 ± 0.21	8.27 ± 0.40	1
Vyas Ghat	Winter	11.53 ± 0.25	7.7 7 ± 0.15	10.80 ± 0.27	0
Vyas Ghat	Summer	21.80 ± 0.20	7.27 ± 0.15	10.23 ± 0.25	0
Shivpuri	Winter	12.70 ± 0.36	7.53 ± 0.15	11.30 ± 0.44	0
Shivpuri	Summer	22.17 ± 0.32	7.97 ± 0.12	9.97 ± 0.15	1

Temp. = Temperature; SD = Standard deviation; DO = Dissolved Oxygen in milligram per liter; pH = Potential of Hydrogen ions; 0 = absence; 1= presence

Pathogenic examination

The morphological characters, such as shape, size, colour and colony diameters of the purified colonies were recorded. Additionally, Gram staining, motility of the isolates and biochemical identification tests, including catalase, oxidase, urease, indole, H_2S production, Voges-Proskauer (VP) and sugar fermentation, were determined (Holt et al., 1994; Austin & Austin 2016; Cappuccino & Welsh 2020) .

Antibacterial susceptibility test

A total of 16 *Aeromonas* isolates were tested for susceptibility to 9 commercially available antibiotics using the disc diffusion method, and the susceptible, intermediate and resistant patterns were quantified (Abbey & Deak, 2019). The antibacterial drugs and their concentration were given as Ampicillin (10 µg), Ciprofloxacin (5 µg), Gentamicin (10 µg), Levofloxacin (5 µg), Oxytetracycline (30 µg), Penicillin (10 µg), Streptomycin (10 µg),

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Tetracycline (30 µg) and Azithromycin (10 µg).

Observations and Results

Clinical signs: Of the 563 total samples collected across the 7 sites, approximately 35-40 fish showed clinical signs, including abdominal ascites, skin depigmentation, fin necrosis, haemorrhage and liver swelling (Figure 2a), during the summer season at the stations UK, NP, RP, SN and SP. During the winter season, fish samples were collected from all stations and examined. A very few lesions or signs of disease were observed. However, no bacterial cultures grew on selective media, except for those collected from the SN station.

2a **2b**

Figure 2: Figure 2a indicated the swelling in the liver, Figure 2b indicated the Gram-staining image of *Aeromonas* isolates

Identification of genus *Aeromonas*

Aeromonas species were cultured on selective agar media and identification was performed using a combination of morphological, physiological and biochemical tests. The genus *Aeromonas* grew in yellowish colonies, with a diameter range of 0.5-3.1mm, on selective media Aeromonas Strach DNA Agar Base (HiMedia M1284) with aseptically rehydrated Ampicillin supplement (HiMedia FD082). Microscopically, the bacteria appeared as Gram-negative, motile and straight rod-shaped bacilli (Figure 2b). The relevant biochemical tests were performed and yielded the following results (Table 2), indicating that the pure culture belonged to different isolates of the genus *Aeromonas*. The results of the previous study (Holt et al., 1994; Austin & Austin, 2016; Cappuccino & Welsh, 2020; Semwal et al., 2023) *Aeromonas* was utilised as a control for comparative analysis.

Table 2: Morphological, physiological and biochemical characteristics of *Aeromonas* isolates collected from the Schizothoracine group during the winter and summer seasons from different sampling sites.

Biochemical tests	**Results during winter 2023 and summer season 2024**																
Sites	Harsil (H)		Uttarkashi (UK)		Nandaprayag (NP)		Rudraprayag (RP)		Srinagar (SN)		Vyas (VG)		Ghat		Shivpuri (SP)		(Semwal et al., 2023)
Seasons	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer			Winter	summer	
Growth on selective media	-	-	+		-	+	-	+	+	+	-	-			-	+	
Growth at 4°C	-	-	-		-		-		-	-	-	-				-	-
Growth at 27°C	-	-	+		+		+		+	+	-	-				+	+
Growth at 37°C	-	-	+		+		+		+	+	-	-				+	+
Shape			Rod		Rod		Rod		Rod	Rod						Rod	Rod
Motility			+		+		+		+	+						+	+
Grams Stain			-		-		-		-	-						-	-
Oxidase test			+		+		+		+	+						+	+

Catalase test	+	+	+	+	+	+	+
O/F test	F	F	F	F	F	F	F
Indole test	+	+	+	+	+	+	+
Methyl red test	-	-	-	-	-	-	-
Voges-Proskauer test	+	+	+	+	+	+	+
Citrate Utilisation Test	+	+	+	+	+	+	+
Urease test	-	-	-	-	-	-	-
Nitrate red. Test	+	+	+	+	+	+	+
H_2S production	+	+	+	+	+	+	+

+ = positive isolates; - = negative isolates; O/F = oxidative/fermentation; °C = Degree Celsius

Drug sensitivity test

Using the disc diffusion method, 9 antibacterial drugs with different concentrations were tested to determine the sensitivity of *Aeromonas* isolates (Table 3). The 16 isolates were divided into 3 categories, viz., sensitive, intermediate and resistant, based on the percentage of antibiotic sensitivity. Results indicated that the isolates were highly sensitive to both gentamicin and Azithromycin (56.25%), followed by Levofloxacin (37.50%), Penicillin (31.25%) and Oxytetracycline (18.75%). Whereas Tetracycline was the most resistant (87.50%) and Gentamicin was the least resistant (12.50%).

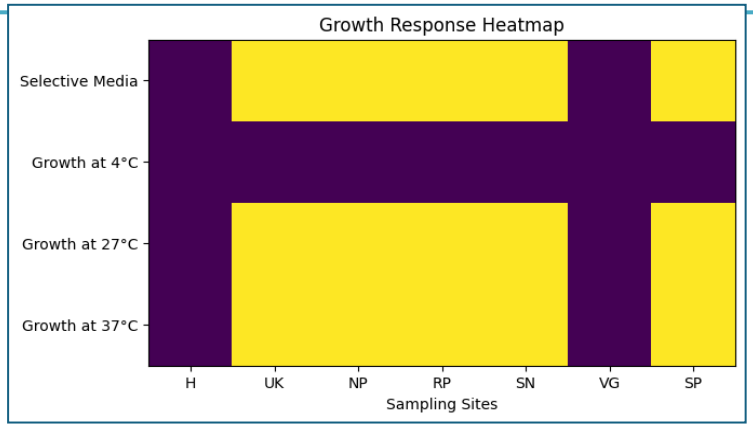
Table 3: Disc diffusion susceptibility test for 16 isolates of *Aeromonas* spp.

Antibacterial drugs	**Concentration of drugs**	***Aeromonas* isolates**					
		Sensitive		Intermediate		Resistant	
		Number	Percentage	Number	Percentage	Number	Percentage
Ampicillin	10 µg	1	6.25%	2	12.5	13	81.25%
Ciprofloxacin	5 µg	4	25%	1	6.25%	11	68.75%
Gentamicin	10 µg	9	56.25%	5	31.25%	2	12.50%
Levofloxacin	5 µg	6	37.50%	5	31.25%	5	31.25%
Oxytetracycline	30 µg	3	18.75%	6	37.50%	7	43.75%
Penicillin	10 µg	5	31.25%	5	31.25%	6	37.50%
Streptomycin	10 µg	1	6.25%	3	18.75%	12	75%
Tetracycline	30 µg	1	6.25%	1	6.25%	14	87.50%
Azithromycin	10 µg	9	56.25%	4	25%	3	18.75%

µg = microgram; % = percent

Discussion

The *Aeromonas* isolates have recently been identified in the Schizothoracine group (*Schizothorax plagiostomus and Schizothorax richardsoni)* collected from the Ganga River and its tributaries in Uttarakhand, India. The heatmap (Figure 3) derived from Table 2 indicated that during culture on selective media, bacterial growth was observed at 27°C within 24 hours, with maximum growth at 37°C after 48 hours (yellow), and no growth at 4°C (dark purple). Furthermore, no growth of *Aeromonas* isolates was observed in samples collected from stations H and VG (dark purple), indicating less water contamination. This seasonal variation in bacterial occurrence may be correlated with elevated water temperatures (12-22°C) observed in summer and low elevation, which foster favourable conditions for bacterial growth and proliferation.



H = Harsil; UK = Uttarkashi; NP = Nandaprayag; RP = Rudraprayag; SN = Srinagar; VG = Vyas Ghat; SP = Shivpuri.

Figure 3: The heatmap showed the growth responses at different temperatures and a selective media across 7 sites (dark purple = absence, Yellow = presence).

The number of different morphological and biochemical characteristics of the isolates (Table 1) was like that of the genus *Aeromonas*, supported by the studies (Austin et al., 1989; Borty et al., 2016; Mallik et al., 2020; Kazemipour & Kheirkhah, 2020; Mazumder et al., 2021; Mulia et al., 2023; Semwal et al., 2023; Shabana et al., 2025). *Aeromonas* is distributed among fish species like *Anabus testudineus* (Borty et al., 2016), grass carp (Yang et al., 2018), *Schizothorax prenanti* (Fu & Wang, 2020), *Channa striata* (Samayanpaulraj et al., 2020) and *Oncorhynchus mykiss* (Kazemipour & Kheirkhah, 2020) supported the current study, which is also found in *Schizothorax plagiostomus and Schizothorax richardsoni* inhabiting the cold-water Himalayan regions. The disc diffusion susceptibility test was conducted on 16 isolates (Table 2) and analysed (Figure 4), revealing that Tetracycline, Ampicillin and Streptomycin exhibited high resistance (≥70%), while Ciprofloxacin showed moderate resistance (50-69%). In contrast, the remaining antibiotics demonstrated low resistance (<50%) against the *Aeromonas* isolates, finding that aligns with the authors' observations (Borty et al., 2016; Stratev & Odeyemi, 2016; Borty et al., 2016; Baron et al., 2017; Yang et al., 2018; Mallik et al., 2020; Gallani et al., 2020; Xue et al., 2022; Semwal et al., 2023; Nair et al., 2024). Across all 9 antibiotics (n = 16 isolates each), the mean resistance rate is 50.7% ± 24.8 SD and the mean sensitivity is just 27.1% ± 18.8 SD, indicating that *Aeromonas* isolates were highly resistant to Antibiotics.

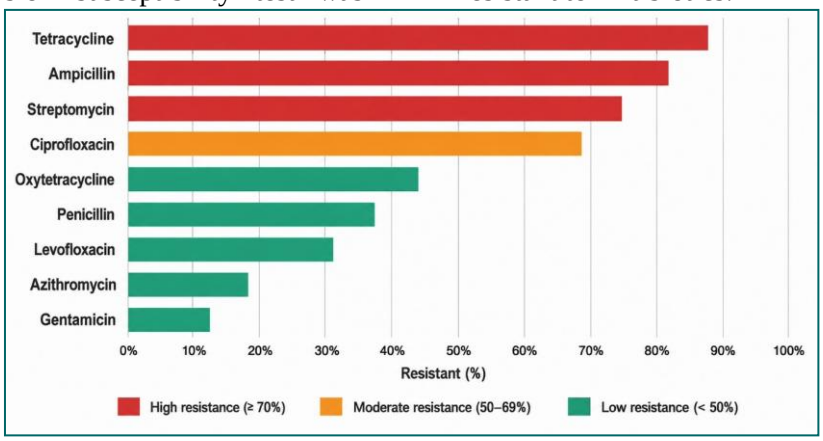


Figure 4: Analysis of 16 isolates of *Aeromonas* through disc diffusion susceptibility methods

Although *Aeromonas* caused huge mortality and outbreaks of disease, such as motile aeromonas septicemia (MAS). It is considered an opportunistic and highly pathogenic genus (Fernández-Bravo & Figueras, 2020; Shabana et al., 2025; Lavanya et al., 2025; Zhang et al., 2025). *Aeromonas* isolates were detected in 6 of 14 sampling sites (42.86% prevalence). Seasonal prevalence was considerably higher during summer (71.43%, 5/7) than winter (14.29%, 1/7). However, Fisher's exact test (Table 1) indicated that the difference in prevalence between seasons was not statistically significant ($p = 0.103$). The Mann–Whitney U test (Table 1) indicated significant differences in environmental parameters between *Aeromonas*-positive and *Aeromonas*-negative samples. Water temperature was significantly higher in *Aeromonas*-positive samples (17.97°C) compared to *Aeromonas*-negative samples (11.07°C) ($U = 5.5$, $p = 0.020$). Additionally, pH was significantly higher in *Aeromonas*-positive samples (7.98) compared with *Aeromonas*-negative samples (7.35) ($U = 5.0$, $p = 0.017$). Conversely, dissolved oxygen concentrations were significantly lower in *Aeromonas*-positive samples (9.37 mg L^{-1}) than in *Aeromonas*-negative samples (11.25 mg L^{-1}) ($U = 44.0$, $p = 0.012$). Notably, no disease outbreaks were documented in the samples, which can be attributed to the satisfactory water quality parameters, including pH, dissolved oxygen and temperature, as well as reduced levels of sewage contamination. Furthermore, the presence of bacteriophages in the tributaries of the Ganga River reduced the bacterial load (Dimri et al., 2019; Behera et al., 2022; Desmecht et al., 2026). Therefore, it could be hypothesised that bacteriophages present in the river Ganga control pathogenic bacteria and help reduce bacterial diseases in the fish fauna.

Conclusion

This study provides significant insights into the presence, prevalence, seasonal variation and identification of *Aeromonas*-like spp. in the *Schizothorax* genus. The integration of selective media, morphological, Gram staining and biochemical methods has demonstrated effectiveness in accurately identifying bacterial isolates as *Aeromonas* spp. Additionally, investigations into antimicrobial resistance patterns in combination with environmental stressors would significantly enhance our understanding of the pathogenic potential of *Aeromonas* isolates in cold-water fishes. The occurrence of *Aeromonas* isolates in the summer season, at low dissolved oxygen levels, at low elevation, within the temperature range of 12°C to 22°C. *Aeromonas* is acknowledged as an opportunistic pathogen with the potential to cause disease outbreaks, especially in connection with intermittent human activities in the tributaries of the Ganga River in the coming decades. Overall, the study represents one of the initial assessments of pathogenic *Aeromonas* species in the wild *Schizothorax* genus residing in the Himalayan regions of the River Ganga and its tributaries. The findings contribute essential baseline data for pathogen surveillance concerning native Himalayan mahseer-like fish species, thereby supporting future conservation and health management initiatives.

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