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Prevalence and Bacteriological Identification of Bacterial Isolates Associated with Disease in Farmed Rainbow Trout (*Oncorhynchus mykiss*) in Kashmir, India

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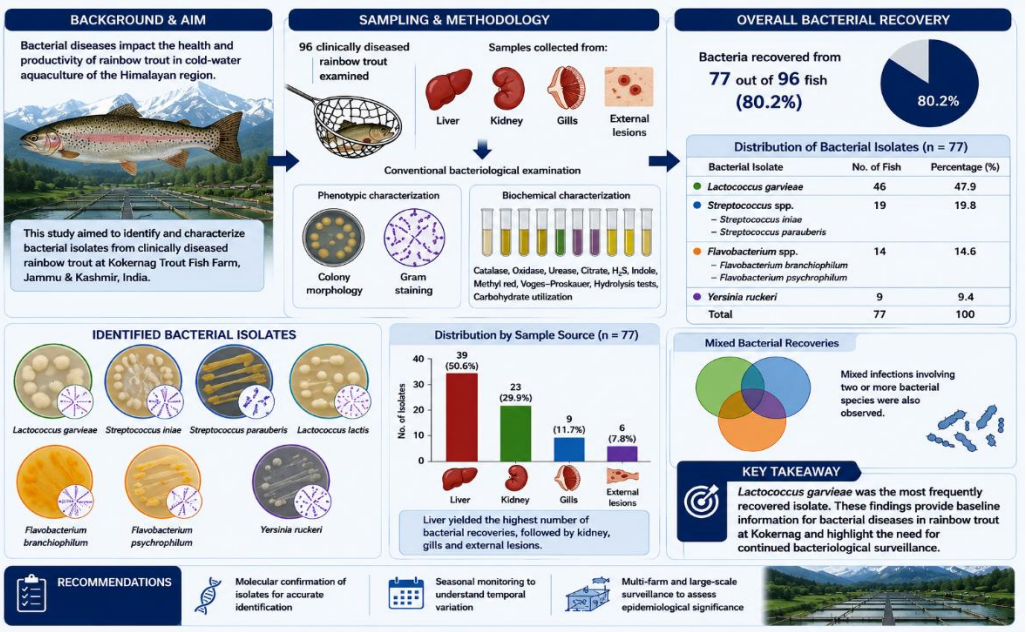
Abstract
Rainbow trout (*Oncorhynchus mykiss*) is an important cold-water aquaculture species in the Himalayan region, where bacterial diseases can adversely affect fish health and production. This study investigated the occurrence and bacteriological characteristics of bacterial isolates associated with clinically diseased rainbow trout at Kokernag Trout Fish Farm, Jammu and Kashmir, India. A total of 96 diseased rainbow trout were examined, and samples from the liver, kidney, gills, and external lesions were subjected to conventional bacteriological examination. Isolates were characterised based on colony morphology, Gram staining, and biochemical tests, including catalase, oxidase, urease, citrate utilisation, hydrogen sulphide production, indole, methyl red, Voges–Proskauer, hydrolysis reactions, and carbohydrate utilisation. One or more bacterial isolates were recovered from 77 fish (80.2%). The presumptively identified bacterial isolates included *Streptococcus iniae, Streptococcus parauberis, Lactococcus garvieae, Lactococcus lactis, Flavobacterium branchiophilum, Flavobacterium psychrophilum*, and *Yersinia ruckeri. Lactococcus garvieae* was the most frequently detected bacterial species, being recovered from 46 fish (47.9%), followed by Streptococcus spp. (19.8%), Flavobacterium spp. (14.6%), and Yersinia spp. (9.4%). The liver yielded the highest number of bacterial recoveries (47), followed by the kidney (37), gills (15), and external lesions (12). Mixed bacterial recoveries were also observed. These findings provide baseline information on bacterial isolates associated with diseased rainbow trout at Kokernag and support the need for continued bacteriological surveillance. Molecular confirmation and broader seasonal and multi-farm investigations are recommended to determine the distribution and broader epidemiological significance of these bacterial isolates in Kashmir trout aquaculture.

Keywords: Rainbow trout; bacterial pathogens; *Lactococcus garvieae*; bacterial prevalence; biochemical characterization

Graphical Abstract



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Introduction

Rainbow trout (*Oncorhynchus mykiss*) is one of the most widely cultured salmonid species and is particularly suited to cold, well-oxygenated freshwater environments. Because of its favourable growth characteristics, nutritional value and high market demand, rainbow trout has become an important component of cold-water aquaculture in several regions of the world (Woynarovich et al., 2011; Austin & Austin, 2016; Singh, 2020). In India, trout farming is concentrated predominantly in the Himalayan states and Union Territories, where suitable climatic and water conditions favour its culture. The Himalayan region has considerable potential for rainbow trout production, with Jammu and Kashmir being an important trout-producing area in India (Sarma & Chandra, 2021; Singh, 2020). Kashmir, with its abundant cold-water resources and extensive network of streams and rivers, represents an important region for trout production.

Despite the favourable environmental conditions, disease remains one of the major constraints to sustainable trout farming. Intensification of aquaculture, high stocking densities, fluctuations in water quality, handling stress and other husbandry-related factors may increase the susceptibility of cultured fish to infectious agents. Among these, bacterial infections are particularly important because they can produce acute or chronic disease, increased mortality and reduced production. The consequences may be particularly severe in hatcheries and intensive farming systems, where pathogens can spread rapidly among susceptible fish (Faruk et al., 2017; Mishra et al., 2018; Daneshamouz et al., 2020). In the Himalayan region, increasing intensification of trout culture has further emphasised the need for improved fish-health management and disease surveillance (Jaies et al., 2020; Sarma & Chandra, 2020).

A variety of bacterial organisms have been associated with disease in rainbow trout. Important bacterial pathogens species belonging to the genera *Lactococcus, Streptococcus, Flavobacterium* and *Yersinia*, among others. *Lactococcus garvieae* has been recognised as an important cause of lactococcosis in cultured fish, while *Streptococcus iniae* and related streptococci are associated with streptococcosis in several aquaculture species. *Flavobacterium branchiophilum* is associated with bacterial gill disease (Good et al., 2015), whereas *Flavobacterium psychrophilum* has been linked with important diseases of salmonids, including bacterial cold-water disease and rainbow trout fry syndrome (Starliper, 2011; Bebak et al., 2007). *Yersinia ruckeri* is the causative agent of enteric redmouth disease, an important bacterial disease of salmonids (Austin & Austin, 2007; Mishra et al., 2018; Daneshamouz et al., 2020). The importance of *L. garvieae* in Indian trout aquaculture has also been demonstrated by its isolation and pathogenicity characterisation in farmed rainbow trout from the northern Himalayan region of India (Shahi et al., 2018). The pathogenicity and genomic characteristics of *L. garvieae* isolates from rainbow trout in India have also been documented (Shahi et al., 2018; Shahi & Mallik, 2020).

The clinical manifestations associated with bacterial infections in rainbow trout can vary according to the pathogen, disease stage and environmental conditions. Diseased fish may exhibit lethargy, anorexia, abnormal swimming, exophthalmia, skin haemorrhages, ulcers, fin damage, gill abnormalities, emaciation and other external or internal lesions (Austin & Austin, 2016; Pękala-Safińska, 2018; Karami et al., 2019; Ortega et al., 2020). However, clinical signs alone are generally insufficient for definitive identification of the causative organism. Bacteriological isolation followed by morphological and biochemical characterisation, therefore, remains an important approach for the preliminary identification of bacterial pathogens (Austin & Austin, 2012; Austin, 2019; Ortega et al., 2020). Daneshamouz et al. (2020), for example, reported clinical abnormalities including exophthalmia, corneal opacity, anorexia, abnormal swimming, lethargy, gill hyperplasia, emaciation and haemorrhages in diseased rainbow trout examined from fish farms in Iran.

The occurrence and distribution of bacterial pathogens may differ among geographical regions because of differences in environmental conditions, water quality, season, culture practices, host susceptibility and diagnostic approaches (Dalsgaard & Madsen, 2000; Karsidani et al., 2010; Duman et al., 2019; Duman et al., 2020). Daneshamouz et al. (2020) examined 103 diseased rainbow trout and 412 tissue samples from 17 farms in Zanjan Province, Iran. Of the 199 bacterial isolates obtained by culture and biochemical examination, 184 (92.4%) were subsequently confirmed by molecular analysis. *Lactococcus garvieae* was the most frequently detected organism (56.3%), followed by *S. iniae* (11.5%), *F. branchiophilum* (9.5%) and *Y. ruckeri* (9.1%). These findings emphasise the importance of location-specific bacteriological investigations because the prevalence and composition of bacterial organisms associated with diseased trout may vary between farming regions (Karsidani et al., 2010; Duman et al., 2020).

Therefore, the present study was designed to investigate bacterial isolates associated with diseased rainbow trout at Kokernag Trout Fish Farm, Jammu and Kashmir, India. The study focused on their isolation, occurrence and phenotypic characterisation based on morphological and biochemical characteristics. By documenting the bacterial organisms recovered from clinically diseased trout at this important trout-farming location, the study aims to provide baseline information for routine bacteriological surveillance, early disease recognition and improved health-management practices in trout aquaculture in Kashmir.

Materials and Methods

Study area

The study was conducted at the Kokernag Trout Fish Farm, Anantnag district, Jammu and Kashmir, India. The farm is located in Kokernag at approximately 33.59° N latitude and 75.29° E longitude, at an elevation of approximately 1,965–2,000 m above mean sea level. The area is characterised by a cool climate and low-temperature, well-oxygenated freshwater resources suitable for Rainbow Trout (*Oncorhynchus mykiss*) culture. The Kokernag Trout Fish Farming Project was established in 1984 and serves as an important facility for the production of quality rainbow trout and brown trout seed in Jammu and Kashmir. The farm was selected as the study site for assessing the occurrence and distribution of bacterial isolates associated with diseased rainbow trout under farm conditions.

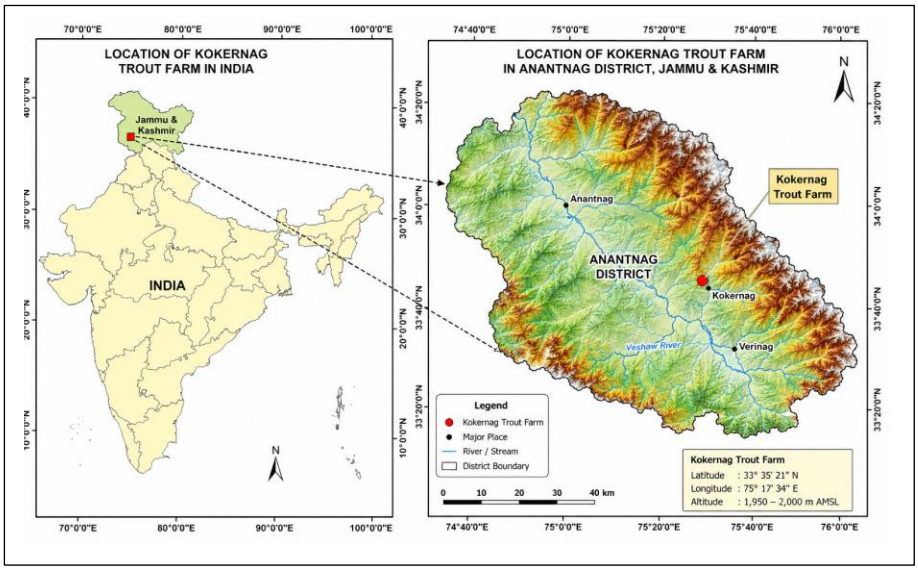

Figure 1: Geographical location of Kokernag Trout Fish Farm, Anantnag, Jammu and Kashmir, India.

Sampling of diseased fish

A total of 96 diseased rainbow trout were examined during the study period. Fish were selected from Kokernag Trout Fish Farm based on visible clinical signs suggestive of bacterial disease. The fish were examined externally for abnormalities, including lethargy, abnormal swimming behaviour, anorexia, skin discolouration, haemorrhagic lesions, ulceration, fin erosion, exophthalmia, abdominal distension and other apparent pathological changes.

The number of diseased fish examined at each sampling site was recorded. Fish were collected aseptically and transported to the laboratory under chilled conditions for bacteriological examination. Samples were processed as soon as possible after collection to minimise changes in the bacterial population and to facilitate recovery of disease-associated bacterial isolates.

Collection of the tissue samples

The collected fish were examined externally and internally under aseptic conditions. Before opening the body cavity, the external surface of each fish was disinfected using the actual disinfectant, concentration and exposure time employed during sampling to minimise contamination from surface-associated microorganisms. Tissue samples were collected aseptically from lesions and selected internal organs showing apparent pathological changes. Where applicable, samples were collected from organs commonly associated with systemic bacterial infections in rainbow trout, including the liver, kidney and gills, as well as from external lesions. Each sample was collected using sterile instruments and inoculated directly onto the appropriate bacteriological media.

Bacterial isolation

Bacterial isolation was performed using conventional bacteriological culture techniques. Tissue samples were aseptically streaked onto appropriate culture media, including blood agar, tryptic soy agar (TSA) and MacConkey agar, depending on the bacterial groups targeted for recovery. The inoculated plates were incubated under conditions appropriate for the recovery of fish-associated bacterial pathogens. Where isolation of slow-growing or cold-water bacterial organisms was required, appropriate selective or specialised media and lower incubation temperatures were used according to the laboratory protocol.

Following incubation, plates were examined for bacterial growth. Distinct colonies were identified on the basis of their visible colony characteristics, including colony size, shape, margin, elevation, surface appearance, pigmentation, opacity and haemolytic activity, where applicable. Representative colonies with different morphological characteristics were selected and purified by repeated subculturing until pure cultures were obtained. Pure bacterial cultures were maintained under appropriate storage conditions for subsequent phenotypic and biochemical characterisation for presumptive identification.

Phenotypic characterisation of bacterial isolates

The purified bacterial isolates were initially characterised on the basis of their cultural and morphological characteristics. Colony morphology was recorded for each isolate, including size, shape, margin, elevation, surface texture, pigmentation, opacity and haemolytic activity on blood agar, where applicable.

Gram staining was performed for preliminary characterisation of the bacterial isolates. The Gram reaction, cellular morphology and arrangement of bacterial cells were recorded. Based on the combined interpretation of colony morphology, Gram reaction and growth characteristics, isolates were provisionally grouped into appropriate bacterial categories. Representative isolates from each morphological group were subsequently subjected to biochemical characterisation for presumptive identification.

Biochemical characterisation

The purified bacterial isolates were subjected to conventional biochemical tests according to their Gram reaction and preliminary phenotypic characteristics. The tests included catalase, oxidase, indole production, urease activity, citrate utilisation, methyl red, Voges–Proskauer, hydrogen sulfide production, casein hydrolysis, gelatin hydrolysis, albumin digestion, esculin hydrolysis and carbohydrate fermentation. The reactions were recorded and compared with standard identification criteria and published descriptions of bacterial organisms associated with rainbow trout.

Presumptive identification of the bacterial isolates was based on the combined interpretation of colony morphology, Gram reaction and biochemical characteristics rather than on a single diagnostic test.

Identification of bacterial pathogens

Bacterial isolates showing characteristic phenotypic and biochemical profiles were assigned to the most appropriate bacterial genus/species based on their combined identification characteristics. The bacterial isolates recovered from diseased rainbow trout were recorded for each individual fish and sampling location. Where more than one bacterial species was recovered from the same fish, each bacterial pathogen was recorded separately, allowing the detection of possible bacterial co-infections.

Determination of bacterial isolation frequency

The proportion of examined fish yielding bacterial isolates was calculated as the number of fish from which one or more bacterial isolates were recovered relative to the total number of fish examined. The fish-level occurrence of individual bacterial isolates was calculated based on the number of fish positive for the respective bacterial organism relative to the total number of fish examined. The percentage of examined fish yielding a bacterial isolate was expressed using the following formula:

Bacterial isolation frequency (%) = (Number of fish yielding one or more bacterial isolates / Total number of fish examined) × 100

Where more than one bacterial isolate was recovered from an individual fish, each isolate was recorded separately for isolate-specific occurrence analysis. However, the fish, rather than individual tissue samples, was considered the primary epidemiological unit for estimating overall fish-level bacterial recovery.

Results

Clinical findings and bacterial isolation

A total of 96 rainbow trout showing apparent clinical abnormalities were examined at Kokernag Trout Fish Farm, Kashmir, India. One or more bacterial isolates were recovered from 77 fish (80.2%). The major clinical manifestations observed included lethargy, abnormal swimming behaviour, skin discolouration, haemorrhagic lesions, ulceration, fin erosion, exophthalmia and abdominal distension. Lethargy and abnormal swimming behaviour were commonly observed clinical manifestations.
Several fish exhibited multiple clinical abnormalities simultaneously.

Bacterial isolation

Bacteriological examination resulted in bacterial recovery from the liver, kidney, gills and external lesions, with the highest number of tissue recoveries recorded from the liver, followed by the kidney, gills and external lesions. Considerable variation was observed among the recovered bacterial colonies with respect to colony size, shape, pigmentation, opacity, surface appearance, margin,

elevation and haemolytic activity. Based on Gram staining, both Gram-positive and Gram-negative bacterial groups were recovered. The Gram-positive isolates were predominantly represented by streptococcal and lactococcal groups, whereas the Gram-negative isolates included *Flavobacterium* and *Yersinia* groups.

Phenotypic characterisation of bacterial isolates

The bacterial isolates were initially differentiated on the basis of cultural and morphological characteristics, followed by Gram staining and biochemical characterisation. The presumptively identified bacterial isolates included Gram-positive cocci, represented by *Streptococcus iniae*, *Streptococcus parauberis*, *Lactococcus garvieae* and *Lactococcus lactis*, and Gram-negative bacteria represented by *Flavobacterium branchiophilum*, *Flavobacterium psychrophilum* and *Yersinia ruckeri*.

The Gram-positive isolates showed characteristic coccoid morphology, whereas the Gram-negative isolates exhibited bacillary morphology. Differences were observed among the isolates in colony characteristics, pigmentation, haemolytic activity, colony texture and growth characteristics.

Biochemical characterisation

The biochemical profiles differed among the isolates and were used to support their presumptive identification. The Gram-positive isolates showed distinct reactions in hydrolysis, methyl red, Voges–Proskauer, and esculin tests, while the Gram-negative isolates showed characteristic catalase, oxidase and hydrolytic reactions. The presumptive *Yersinia ruckeri* isolate showed positive reactions for urease, citrate utilisation, and H_2S production. All isolates produced acid from glucose, fructose and maltose, whereas variable reactions were observed for the other carbohydrates tested. Detailed results are presented in **Table 1.**

Table 1: Phenotypic and biochemical profiles of bacterial isolates recovered from diseased rainbow trout (*Oncorhynchus mykiss*).

Test	*S. iniae*	*S. parauberis*	*L. garvieae*	*L. lactis*	*F. branchiophilum*	*F. psychrophilum*	*Y. ruckeri*
Gram reaction	+	+	+	+	−	−	−
Catalase	−	−	−	−	+	+W	+
Oxidase	−	−	−	+	+W	−	−
Urease	−	−	−	−	−	−	+
Citrate utilisation	−	−	−	−	+	−	+
H_2S production	−	−	−	−	−	−	+
Indole production	−	−	−	−	−	+W	−
Casein hydrolysis	+	+W	+	−	+	+	−
Gelatin hydrolysis	+	+	+	−	+	+	−
Albumin digestion	+	+	−	+	+	+	−
Methyl red	−	+	+	−	−	−	+
Voges–Proskauer	−	+	+	+	−	−	−
Esculin hydrolysis	−	+	+	+	−	+W	−
Acid production from carbohydrates							
Glucose	+	+	+	+	+	+	+
Fructose	+	+	+	+	+	+	+
Lactose	−	+	+	+	−	−	+

Ribose	+	+	+	−	−	−	+
Raffinose	−	−	+W	−	−	−	−
Sorbitol	−	+	−	−	−	−	+
Sucrose	+	+	+	+	−	−	+
Trehalose	+	+	+	+	−	+	+
Arabinose	−	−	−	−	−	−	+
Inositol	+	−	+	−	−	−	+
Maltose	+	+	+	+	+	+	+

Note: + = positive; − = negative; +W = weakly positive.

Prevalence and distribution of bacterial pathogens

Lactococcus garvieae was the most frequently recovered isolate, being detected in 46 of 96 fish (47.9%). This was followed by *Streptococcus* spp. in 19 fish (19.8%), *Flavobacterium* spp. in 14 fish (14.6%), and *Yersinia* spp. in 9 fish (9.4%). Other bacterial isolates were recovered from 5 fish (5.2%). Because more than one bacterial isolate could be recovered from an individual fish, isolate-specific occurrence values were not mutually exclusive and therefore did not sum to the overall proportion of fish yielding bacteria.

L. garvieae represented the predominant bacterial isolate, followed by *Streptococcus* spp., *Flavobacterium* spp. and *Yersinia* spp. The overall distribution of the recovered bacterial groups is presented in **Figure 2.**

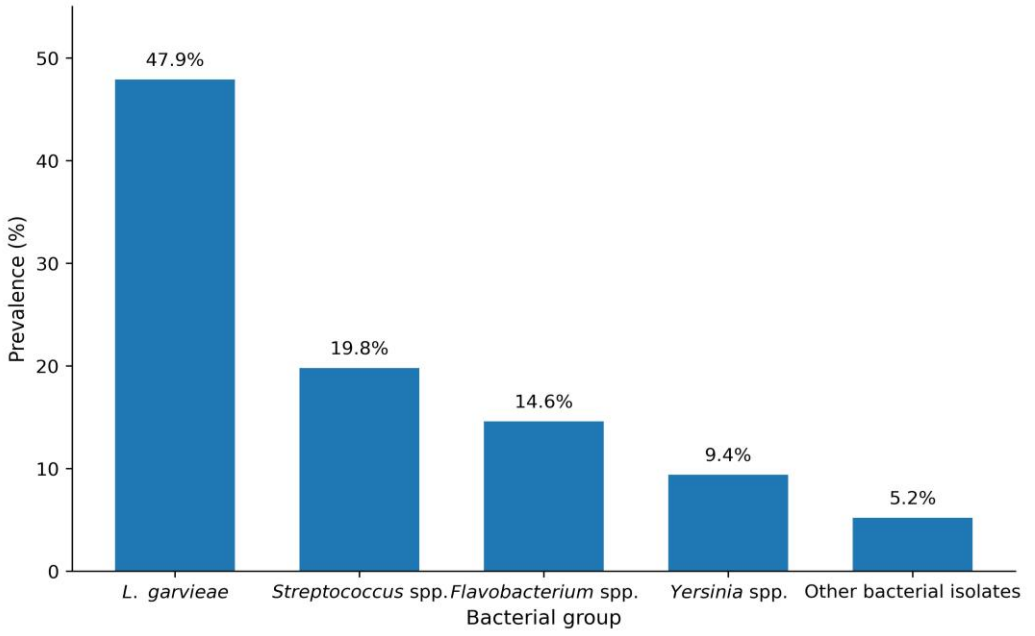

Figure 2. Prevalence of bacterial isolates recovered from diseased rainbow trout (*Oncorhynchus mykiss*) at Kokernag Trout Fish Farm, Jammu and Kashmir, India.

3.6 Organ-wise distribution of bacterial isolates

The frequency of bacterial recovery varied among the examined tissues. The liver yielded the highest number of positive recoveries, followed by the kidney, gills and external lesions. A total of 47 positive recoveries were recorded from liver samples, compared with 37 from kidney, 15 from gills and 12 from external lesions.

Lactococcus garvieae was predominantly recovered from the liver, where it accounted for 31 recoveries, followed by the kidney with 18 recoveries. In contrast, Flavobacterium spp. showed a comparatively greater association with the gills and external lesions, with 10 and 5 recoveries, respectively. Streptococcus spp. was recovered primarily from the kidney and liver, whereas Yersinia spp. was detected mainly from the liver and kidney.

The organ-wise recovery pattern showed that internal organs, particularly the liver and kidney, were important sites for recovery of the predominant bacterial isolates, whereas Flavobacterium spp. showed a greater association with the gill and external tissues. The organ-wise distribution is presented in **Table 2.**

Table 2. Organ-wise distribution of bacterial recoveries from diseased rainbow trout (*Oncorhynchus mykiss*).

Bacterial group	Liver	Kidney	Gills	External lesions	Total reported
Lactococcus garvieae	31	18	—	—	49
***Flavobacterium* spp.**	—	—	10	5	15
***Streptococcus* spp.**	—	—	—	—	—
***Yersinia* spp.**	—	—	—	—	—
Total bacterial recoveries	**47**	**37**	**15**	**12**	**111**

Note: Values represent positive tissue recoveries rather than the number of individual fish. Multiple positive tissue recoveries could originate from the same fish; therefore, tissue-level recovery totals may exceed fish-level occurrence values.

Discussion

Bacterial diseases are important constraints to rainbow trout (*Oncorhynchus mykiss*) production, particularly in intensive culture systems where environmental and management conditions can influence disease occurrence (Austin & Austin, 2016; Singh et al., 2017). Rainbow trout are susceptible to diverse bacterial organisms, including Streptococcus, Lactococcus, Flavobacterium and Yersinia (Starliper, 2011; Austin & Austin, 2016). The recovery of these bacterial groups in the present investigation indicates a diverse profile of bacterial isolates among clinically affected trout at the investigated farm.

The clinical signs observed, including lethargy, abnormal swimming, anorexia, skin discolouration, haemorrhagic lesions, ulceration, fin erosion, exophthalmia and abdominal distension, are commonly reported in diseased cultured salmonids but are not pathogen-specific. Therefore, clinical examination should be complemented by bacteriological investigation for preliminary identification of the associated bacterial isolates (Kumar et al., 2015; Meyburgh et al., 2017).

The recovery of *Streptococcus iniae, Streptococcus parauberis, Lactococcus garvieae, Lactococcus lactis, Flavobacterium branchiophilum, Flavobacterium psychrophilum* and *Yersinia ruckeri* demonstrated considerable bacterial diversity in the investigated trout samples (Dalsgaard & Madsen, 2000; Singh et al., 2017; Daneshamouz et al., 2020). The occurrence of *L. garvieae* is particularly important because this organism has previously been reported from farmed rainbow trout in the northern Himalayan region of India (Shahi et al., 2018). Its detection in the present study highlights the need for continued surveillance of this organism in Himalayan trout farms (Karsidani et al., 2010; Shahi et al., 2018; Shahi & Mallik, 2020).

The recovery of *F. psychrophilum* is noteworthy because it is associated with bacterial cold-water disease and rainbow trout fry syndrome, which are important diseases of salmonids (Barnes & Brown, 2011; Starliper, 2011). Similarly, *F. branchiophilum* is associated with bacterial gill disease and represents another important flavobacterial organism of cultured fish (Loch & Faisal, 2014; Good et al., 2015). The detection of these organisms is relevant to disease surveillance in the cold-water trout production system of Kashmir. The occurrence of *Y. ruckeri* expands the bacterial isolate profile of the investigated trout samples. This organism causes enteric red mouth disease, an important systemic disease of salmonids, particularly rainbow trout (Tobback et al., 2007; Kumar et al., 2015). Its recovery from internal organs in the present study is consistent with its known association with systemic disease. However, organ-specific recovery should be interpreted as a distribution pattern rather than as definitive evidence of tissue tropism, as histopathological examination and quantification of bacterial burden were not performed.

The phenotypic and biochemical characteristics observed in the present study provided useful evidence for the presumptive identification of the bacterial isolates (Janda & Abbott, 2007; Austin & Austin, 2016). Gram reaction, colony morphology, enzymatic reactions, hydrolysis tests and carbohydrate utilisation patterns collectively supported differentiation among the bacterial groups. However, biochemical identification alone may not reliably distinguish closely related bacterial taxa; therefore, molecular confirmation using species-specific PCR or sequencing would strengthen the identification (Clarridge, 2004; Janda & Abbott, 2007; Austin & Austin, 2016). The isolate-specific occurrence values observed in this study should be interpreted in relation to the sampling

design. Since the investigation was conducted at a single trout-farming site in Kokernag, the findings are site-specific and should not be considered representative of all trout farms in Kashmir (Dalsgaard & Madsen, 2000; Singh et al., 2017). Differences in bacterial recovery may result from variation in season, water temperature, fish age, stocking density, farm management and diagnostic methods (Dalsgaard & Madsen, 2000; Schmidt et al., 2000; Duman et al., 2019; Daneshamouz et al., 2020).

From a disease-management perspective, regular monitoring of fish behaviour, feeding activity, external lesions and mortality, combined with bacteriological examination, may facilitate early detection of bacterial disease (Dalsgaard & Madsen, 2000; Austin & Austin, 2016). Accurate identification of bacterial isolates is essential for developing appropriate disease-control strategies.

An important limitation of the present investigation is that bacterial identification was based primarily on cultural, morphological and biochemical characteristics (Janda & Abbott, 2007; Austin & Austin, 2016). Therefore, the isolates should be regarded as presumptively identified unless molecular confirmation is performed (Clarridge, 2004; Janda & Abbott, 2007). Furthermore, bacterial isolation from diseased fish demonstrates an association but does not independently establish causality, particularly in cases of mixed recovery (Starliper, 2011; Austin & Austin, 2016). Molecular identification, histopathology and pathogenicity studies would provide stronger evidence regarding the role of individual bacterial isolates (Janda & Abbott, 2007; Shahi et al., 2018).

Conclusion

The present study demonstrates the occurrence of diverse bacterial isolates associated with clinically diseased rainbow trout at Kokernag. The detection of *L. garvieae*, *Streptococcus spp., F. psychrophilum*, *F. branchiophilum* and *Y. ruckeri* highlights the importance of bacterial disease surveillance in Himalayan trout aquaculture. Further multi-farm, seasonal and molecularly supported studies are needed to determine the broader distribution and epidemiological significance of these bacterial organisms in Kashmir.

Author Contributions

Aamir Majeed: Conceptualisation, methodology, data analysis and interpretation, sample collection, laboratory work, bacterial isolation and identification, writing- original draft. **Muzamil Liakat Mir:** investigation, sample collection, laboratory work, bacterial isolation and identification, data analysis, and writing-original draft **Khalida Hassan:** Scientific guidance, interpretation of results, and critical revision of the manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

Data Availability Statement

The data generated and/or analysed during the present study are available from the corresponding authors upon reasonable request.

References

1. Austin, B. (2017). The value of cultures to modern microbiology. *Antonie Van Leeuwenhoek, 110*(10), 1247–1256. https://doi.org/10.1007/s10482-017-0840-8
2. Austin, B., & Austin, D. A. (2007). *Bacterial fish pathogens: Diseases of farmed and wild fish* (4th ed.). Springer. https://doi.org/10.1007/978-1-4020-6069-4
3. Austin, B., & Austin, D. A. (2016). *Bacterial fish pathogens: Disease of farmed and wild fish* (6th ed.). Springer. https://doi.org/10.1007/978-3-319-32674-0
4. Barnes, M. E., & Brown, M. L. (2011). A review of *Flavobacterium psychrophilum* biology, clinical signs, and bacterial cold water disease prevention and treatment. *The Open Fish Science Journal, 4*, 1–9. https://doi.org/10.2174/1874401X01104010040
5. Bebak, J. A., Welch, T. J., Starliper, C. E., Baya, A. M., & Garner, M. M. (2007). Improved husbandry to control an outbreak of rainbow trout fry syndrome caused by infection with *Flavobacterium psychrophilum*. *Journal of the American Veterinary Medical Association, 231*(1), 114–119. https://doi.org/10.2460/javma.231.1.114
6. Clarridge III, J. E. (2004). Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. *Clinical Microbiology Reviews, 17*(4), 840–862. https://doi.org/10.1128/cmr.17.4.840-862.2004
7. Dalsgaard, I., & Madsen, L. (2000). Bacterial pathogens in rainbow trout, *Oncorhynchus mykiss* (Walbaum), reared at Danish freshwater farms. *Journal of Fish Diseases, 23*(3), 199–209. https://doi.org/10.1046/j.1365-2761.2000.00242.x
8. Daneshamouz, S., Haghi, F., & Zeighami, H. (2020). Detection and identification of bacterial pathogens in rainbow trout (*Oncorhynchus mykiss*) samples from fish farms in Iran. *Thalassas: An International Journal of Marine Sciences, 36*(1), 133–141. https://doi.org/10.1007/s41208-019-00169-9

9. Duman, M., Saticioglu, I. B., & Altun, S. (2019). Molecular characterization and antimicrobial resistance profile of fecal contaminants and spoilage bacteria that emerge in rainbow trout (*Oncorhynchus mykiss*) farms. *Bioscience of Microbiota, Food and Health, 38*(2), 41–48. https://doi.org/10.12938/bmfh.18-007
10. Duman, M., Saticioglu, I. B., Ozdemir, B., Ajmi, N., & Altun, S. (2020). Determination of opportunistic pathogens and antimicrobial resistance characterization isolated from rainbow trout in Turkey. https://doi.org/110.30782/jrvm.743861
11. Faruk, M. A. R., & Anka, I. Z. (2017). An overview of diseases in fish hatcheries and nurseries. *Fundamental and Applied Agriculture, 2*(3), 311–316. https://orcid.org/0000-0002-8143-3582
12. Good, C., Davidson, J., Wiens, G. D., Welch, T. J., & Summerfelt, S. (2015). *Flavobacterium branchiophilum* and *F. succinicans* associated with bacterial gill disease in rainbow trout *Oncorhynchus mykiss* (Walbaum) in water recirculation aquaculture systems. *Journal of Fish Diseases, 38*(4), 409–413. https://doi.org/10.1111/jfd.12249
13. Jaies, I., Shah, F. A., Abubakr, A., Asmi, O., Bhat, B. A., & Balkhi, M. U. H. (2020). PCR based identification of *Saprolegnia parasitica* affecting rainbow trout aquaculture in Kashmir region of Indian Himalayas. *Indian Journal of Animal Research, 54*(9), 1149–1154. https://doi.org/10.18805/ijar.B-3873
14. Janda, J. M., & Abbott, S. L. (2007). 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: Pluses, perils, and pitfalls. *Journal of Clinical Microbiology, 45*(9), 2761–2764. https://doi.org/10.1128/jcm.01228-07
15. Karami, E., Alishahi, M., Molayemraftar, T., Ghorbanpour, M., Tabandeh, M. R., & Mohammadian, T. (2019). Study of pathogenicity and severity of *Lactococcus garvieae* isolated from rainbow trout (*Oncorhynchus mykiss*) farms in Kohkilooieh and Boyerahmad province. *Fisheries and Aquatic Sciences, 22*(1), 21. https://doi.org/10.1186/s41240-019-0135-2
16. Karsidani, S. H., Soltani, M., Nikbakhat-Brojeni, G., Ghasemi, M., & Skall, H. F. (2010). Molecular epidemiology of zoonotic streptococcosis/lactococcosis in rainbow trout (*Oncorhynchus mykiss*) aquaculture in Iran. *Iranian Journal of Microbiology, 2*(4), 198.
17. Kumar, G., Menanteau-Ledouble, S., Saleh, M., & El-Matbouli, M. (2015). *Yersinia ruckeri*, the causative agent of enteric redmouth disease in fish. *Veterinary Research, 46*, 103. https://doi.org/10.1186/s13567-015-0238-4
18. Loch, T. P., & Faisal, M. (2014). Emerging flavobacterial infections in fish: A review. *Journal of Advanced Research, 6*(3), 283–300. https://doi.org/10.1016/j.jare.2014.10.009
19. Meyburgh, C. M., Bragg, R. R., & Boucher, C. E. (2017). *Lactococcus garvieae*: An emerging bacterial pathogen of fish. *Diseases of Aquatic Organisms, 123*(1), 67–79. https://doi.org/10.3354/dao03083
20. Mishra, A., Nam, G. H., Gim, J. A., Lee, H. E., Jo, A., & Kim, H. S. (2018). Current challenges of *Streptococcus* infection and effective molecular, cellular, and environmental control methods in aquaculture. *Molecules and Cells, 41*(6), 495–505. https://doi.org/10.14348/molcells.2018.2154
21. Ortega, C., Irgang, R., Valladares-Carranza, B., Collarte, C., & Avendaño-Herrera, R. (2020). First identification and characterization of *Lactococcus garvieae* isolated from rainbow trout (*Oncorhynchus mykiss*) cultured in Mexico. *Animals, 10*(9), 1609. https://doi.org/10.3390/ani10091609
22. Pękala-Safińska, A. (2018). Contemporary threats of bacterial infections in freshwater fish. *Journal of Veterinary Research, 62*(3), 261–267. https://doi.org/10.2478/jvetres-2018-0037
23. Sarma, D., & Chandra, S. (2021). Coldwater fish farming in Indian Himalayan region: Challenges and opportunities. *Indian Farming, 70*(11). https://epubs.icar.org.in/index.php/IndFarm/article/view/108195
24. Schmidt, A. S., Bruun, M. S., Dalsgaard, I., Pedersen, K., & Larsen, J. L. (2000). Occurrence of antimicrobial resistance in fish-pathogenic and environmental bacteria associated with four Danish rainbow trout farms. *Applied and Environmental Microbiology, 66*(11), 4908–4915. https://doi.org/10.1128/AEM.66.11.4908-4915.2000
25. Shahi, N., Mallik, S. K., Sahoo, M., Chandra, S., & Singh, A. K. (2018). First report on characterization and pathogenicity study of emerging *Lactococcus garvieae* infection in farmed rainbow trout, *Oncorhynchus mykiss* (Walbaum), from India. *Transboundary and Emerging Diseases, 65*(4), 1039–1048. https://doi.org/10.1111/tbed.12843
26. Shahi, N., & Mallik, S. K. (2020). Emerging bacterial fish pathogen *Lactococcus garvieae* RTCLI04, isolated from rainbow trout (*Oncorhynchus mykiss*): Genomic features and comparative genomics. *Microbial Pathogenesis, 147*, 104368. https://doi.org/10.1016/j.micpath.2020.104368
27. Singh, A. K. (2020). Emerging scope, technological up-scaling, challenges and governance of rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) production in Himalayan region. *Aquaculture, 518*, 734826 . https://doi.org/10.1016/j.aquaculture.2019.734826

28. Singh, A. K., Pandey, N. N., & Ali, S. (2017). Current status and strategies of rainbow trout *Oncorhynchus mykiss* farming in India. *International Journal of Aquaculture, 7*. https://doi.org/10.5376/ija.2017.07.0004
29. Starliper, C. E. (2011). Bacterial coldwater disease of fishes caused by *Flavobacterium psychrophilum*. *Journal of Advanced Research, 2*(2), 97–108. https://doi.org/10.1016/j.jare.2010.04.001
30. Tobback, E., Decostere, A., Hermans, K., Haesebrouck, F., & Chiers, K. (2007). *Yersinia ruckeri* infections in salmonid fish. *Journal of Fish Diseases, 30*(5), 257–268. https://doi.org/10.1111/j.1365-2761.2007.00816.x
31. Vendrell, D., Balcázar, J. L., Ruiz-Zarzuela, I., De Blas, I., Gironés, O., & Múzquiz, J. L. (2006). *Lactococcus garvieae* in fish: A review. *Comparative Immunology, Microbiology and Infectious Diseases, 29*(4), 177–198. https://doi.org/10.1016/j.cimid.2006.06.003
32. Woynarovich, A., Hoitsy, G., & Moth-Poulsen, T. (2011). *Small-scale rainbow trout farming*. FAO Fisheries and Aquaculture Technical Paper No. 561. Food and Agriculture Organization of the United Nations.