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Health Challenges in Semi-Open Mountain Reservoir Aquaculture: A Case Study in *Oncorhynchus mykis*

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Abstract

Bacterial diseases remain a significant threat to salmonid aquaculture worldwide, especially in semi-open cage culture systems that are exposed daily to natural aquatic environments. This study investigates recurring bacterial disease events in Rainbow Trout (*Oncorhynchus mykiss*) raised in a semi-open cage culture facility in the Bicaz mountain reservoir in Romania. Longitudinal monitoring was conducted through clinical assessment of fish health, post-mortem examination of fish that died during recurrent mortality events, bacterial isolation followed by MALDI-TOF MS identification, and determination of antimicrobial susceptibility profiles of the isolated bacteria from 2023 to 2026. These investigations demonstrated the presence of classical pathogenic bacteria found in salmonids as well as a diverse range of opportunistic environmental bacteria. Recurrently identified bacteria included *Aeromonas hydrophila*, *Yersinia ruckeri*, *Aeromonas salmonicida*, *Aeromonas veronii*, *Pseudomonas fluorescens*, *Hafnia alvei*, *Enterobacter aerogenes*, *Providencia alcalifaciens*, and *Flavobacterium psychrophilum*. Mixed bacterial infection resulted in several of the bacterial disease outbreaks, indicating that there are likely to be complex microbial relationships within this semi-open aquaculture environment. Susceptibility profiles of antibiotics tested against the isolated bacteria indicated that some antibiotic classes had varying degrees of susceptibility between different bacterial disease outbreaks and also varied between susceptible and resistant strains of the same bacterial species. Susceptibility profiles were most commonly noted for florfenicol and fluoroquinolone, while beta-lactams, macrolides, and lincosamides displayed variable levels of resistance or diminished susceptibility patterns, particularly among *Aeromonas* spp. strains. Therefore, the results highlight the dynamic epidemiological characteristics of bacterial infections occurring in semi-open mountain aquaculture systems and underscores the importance of longitudinal bacteriological surveillance under commercial farming conditions. Permanent interaction between cultured fish and the aquatic microbiota may influence the recurrence and microbial complexity of bacterial outbreaks in reservoir-based salmonid aquaculture.

Keywords: rainbow trout; semi-open aquaculture; bacterial outbreaks; antimicrobial susceptibility; opportunistic bacteria; mountain reservoir aquaculture

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1. Introduction

Salmonid aquaculture represents one of the most important sectors of global fish production, while *Oncorhynchus mykiss* continues to dominate cold-water farming systems due to its high adaptability and considerable economic value [1,2]. The intensification of inland aquaculture has contributed to an increasing incidence of infectious diseases, which are currently considered among the major constraints affecting the sustainability of salmonid production. Their impact is frequently associated with significant economic losses, recurrent mortality episodes, and, consequently, increased antimicrobial use in fish farming systems [3,4]. Bacterial diseases in salmonids result from complex interactions between the pathogen, the host, and the surrounding farming ecosystem. Technological stress factors, together with the microbiological characteristics of the aquatic environment, play an essential role in the emergence and progression of infectious outbreaks in aquaculture systems [5,6].

In Eastern Europe, salmonid aquaculture conducted in mountain reservoir systems remains insufficiently documented from an epidemiological perspective, despite its recognized regional economic importance. In Romania, the Bicaz reservoir has gradually developed into an important artificial aquatic ecosystem favorable for salmonid farming in semi-open floating cage systems. These systems allow the direct utilization of natural water resources and support the efficient development of fish farming operations under mountain environmental conditions [7].

However, the continuous interaction between farmed fish and the natural reservoir environment may also generate certain secondary effects, including nutrient enrichment of the water, the risk of fish escape from floating cages, and, under specific conditions, localized environmental degradation processes such as eutrophication [7,8]. The characteristics of this farming system create a continuous interaction between cultured fish populations and the natural ecosystem of the aquatic reservoir. Under these conditions, the circulation of microorganisms between the water column, sediments, wild fauna, and cultured fish stocks is facilitated. Such interactions are considerably more limited in controlled recirculating aquaculture systems. Consequently, fish farms located in mountain reservoirs are more exposed to seasonal variability, thermal fluctuations, and changes in the microbial load of the water, factors that may negatively influence the immune status of salmonids [9,10]. Among the major bacterial agents involved in salmonid pathology are *Yersinia ruckeri*, the etiological agent of enteric redmouth disease [11–14], *Aeromonas salmonicida*, responsible for furunculosis [15], *Vibrio anguillarum*, associated with classical vibriosis [16], and *Flavobacterium psychrophilum*, the causative agent of bacterial cold-water disease and rainbow trout fry syndrome [17]. These infections significantly affect fish farm productivity and are frequently associated with septicemic evolution and variable mortality rates.

Recent studies have increasingly highlighted the involvement of opportunistic bacteria in disease outbreaks affecting salmonid aquaculture. Species such as *Aeromonas hydrophila* [18,19], *Aeromonas veronii* [14,20], *Pseudomonas fluorescens* [21], *Hafnia alvei* [22], and various other *Enterobacteriaceae* may be involved in complex bacterial coinfections, making the interpretation of both epidemiological and therapeutic aspects considerably more difficult [23,24]. This increasing diversity of bacterial genera further confirms the expanding influence of environmental microbiota on fish health and supports the hypothesis that epidemiological interactions occurring in salmonid aquaculture may be considerably more complex than those associated with classical bacterial infections alone [25,26].

From both economic and sanitary perspectives, there is therefore increasing interest in monitoring the intestinal microbial load. This may favor the persistence and recurrence of infectious outbreaks in fish farms operating within semi-open aquaculture systems [4,27].

Through the interaction between environmental factors and the immunological status of the organism, the diversity and pattern of the intestinal microbiota are determined, directly influencing fish health. By scanning temperature variations within the optimal range associated with population density control, fish sensitivity is reduced, and thus the risk of bacterial coinfection proliferation is also lowered [28–30].

However, there are still a few longitudinal studies documenting the microbiological dynamics of outbreaks evolving in salmonid aquaculture reared in semi-open mountain reservoirs, especially in the Eastern European region.

With the intensification of fish farming, the use of antimicrobials in aquaculture has become increasingly pronounced, favoring the emergence of antimicrobial resistance, with both becoming currently a major concern for aquatic animal health and public health [31,32]. Through the repeated administration of antimicrobial treatments, the selection of resistant bacterial strains and the dissemination of resistance genes into natural aquatic ecosystems has been favored [33–35]. In semi-open farming systems, where there is permanent contact between fish farms and the natural environment, monitoring antimicrobial susceptibility profiles is of particular importance both for the therapeutic management of outbreaks and for the surveillance of the epidemiological circulation of resistant bacteria in aquatic ecosystems [31,36–38]. At the global level [38], but especially at the European Union level, through its action plans based on the "*One Health*" approach [39], explicit strategies have been implemented to combat antimicrobial resistance in aquaculture.

Although the economic and sanitary importance of semi-open fish farms is recognized, there is a deficit of information regarding the microbial agents that trigger recurrent outbreaks, as well as the evolution of their antimicrobial susceptibility profile, in salmonid farms located in the mountain lakes of Romania. To the best of our knowledge, no longitudinal microbiological studies have been published to date regarding the evolution of recurrent bacterial outbreaks triggered by classical bacterial agents and opportunistic bacteria in rainbow trout farms located in semi-open mountain reservoirs in Romania.

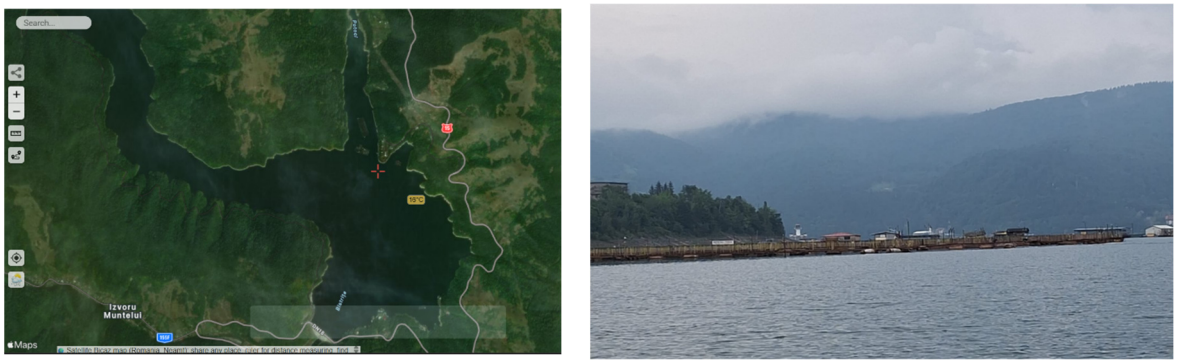
In this context, our study investigated recurrent bacterial outbreaks that occurred over a four-year period in *Oncorhynchus mykiss* reared in a semi-open aquaculture system located in the Bicaz mountain reservoir. The investigated commercial farm has been operating since 2018 and currently owns approximately 50 fish cages. The study was based on the hypothesis that recurrent bacterial outbreaks are characterized by a dynamic combination of classical salmonid pathogens and opportunistic environmental bacteria, whose antimicrobial susceptibility profiles may vary over time and among bacterial species. Accordingly, the objectives of this study were to: (i) microbiologically characterize the bacteria isolated during disease outbreaks, (ii) analyze their antimicrobial susceptibility profiles, and (iii) evaluate the epidemiological dynamics of recurrent outbreaks in a mountain aquaculture system that has been insufficiently documented to date. In conclusion, our findings highlight the dynamic and polymicrobial nature of bacterial outbreaks in semi-open mountain aquaculture systems and underscore the importance of longitudinal bacteriological surveillance as a basis for improving the prudent and evidence-based use of antimicrobials in aquaculture.

2. Materials and Methods

2.1. Study Area and Farming Conditions

The study was conducted within a commercial semi-open rainbow trout farming system, located in the area of the Bicaz mountain reservoir in Neamț County, northeastern Romania (approximate coordinates: 47°00′ N, 26°06′ E), known as one of the most important mountain reservoirs used for aquaculture activities in Romania (Figure 1a). The farm uses a semi-open cage culture system, represented by floating net cages placed directly in

the water mass of the aquatic reservoir (Figure 1b). Within the farm, *Oncorhynchus mykiss* specimens at different developmental stages are raised, from juvenile fish to adults, until they reach commercial size.



(**a**) (**b**)

Figure 1. Geographical location and general view of the semi-open cage aquaculture system, located in the Bicaz mountain reservoir, Romania: (**a**) approximate location of the investigated growing area (satellite image adapted from Google Maps); (**b**) floating cage units used for rainbow trout (*Oncorhynchus mykiss*) production.

The cages used in the farm have variable sizes, with diameters ranging between 12 and 20 m. Since the growing activity takes place in a lentic ecosystem, no data were available regarding the water flow rate at the cage level. The average annual water temperature in the farming area is estimated at approximately 10–12 °C, conditions characteristic of mountain ecosystems and which are favorable for the optimized growth of salmonids. Water temperature was routinely monitored by the fish farm as part of its standard operational procedures and was not included among the variables analyzed in the present study. The population of the stocks at maximum capacity may vary depending on the age category and the production stage, with estimated densities of up to 100,000 specimens/cage for juvenile categories and up to 50,000 specimens/cage for larger-sized batches.

Fish feeding is carried out automatically using BOPIL distribution systems or mobile water transport systems equipped with automated feeding equipment. The rearing structures and the technology applied in the farm are in compliance with the current biosecurity legislative requirements in Romania.

2.2. Outbreak Description and Fish Sampling

The biological samples were analyzed microbiologically during the period 2023–2026 and were collected when disease outbreaks reached a mortality rate of approximately 5% of the fish stock within an affected cage. Daily mortality was monitored through routine daily inspection of the floating cages by farm personnel, and the mortality data were recorded in the farm records. Sample collection was not carried out according to a predetermined schedule but was determined by the farm's epidemiological context, with each disease outbreak being followed by microbiological examination. A total of 345 fish were analyzed, with the sample size for each outbreak ranging from 15 to 25 individuals, with or without external lesions, all of which were sampled before the initiation of any antimicrobial treatment. Sampling was conducted based on the size of the affected cage Recently dead fish were defined as fish collected shortly after death, before the onset of advanced postmortem changes, to ensure the quality of bacteriological examination. The collection, handling, and transport of samples to the laboratory were carried out under appropriate conditions for microbiological examination using sterile containers and a controlled cold chain.

2.3. Clinical Examination and Necropsy

All fish specimens sent for analysis were subjected to external and internal examination after performing necropsy. External changes and lesions associated with bacterial infections were monitored, including cutaneous hemorrhages, ulcerations, pigmentation changes, exophthalmia, or abdominal distension. During necropsy, the hemorrhagic, inflammatory, and degenerative processes that evolved internally were monitored, although in the case of septicemia, the lesional picture provides little data. Biological samples were collected aseptically, under specific conditions of the microbiological diagnostic laboratory. Heart blood samples were aseptically collected from all examined fish. When lesions were observed during necropsy, additional samples were collected from the liver, spleen, kidney, gastrointestinal tract, or dermal ulcers. All bacteriological examinations were performed to establish the etiology of the disease or the cause of mortality.

2.4. Bacteriological Investigation

The collected samples were processed using conventional bacteriological methods, performed in the Microbiology Laboratory of the Regional Center for Advanced Research on Emerging Diseases, Zoonoses and Food Safety within the "Ion Ionescu de la Brad" University of Life Sciences in Iași, Romania. The biological material was inoculated onto usual and selective culture media, namely nutrient broth (Oxoid, Basingstoke, UK), Mueller–Hinton agar + 5% defibrinated horse blood, Brucella blood agar (Oxoid, UK), and Thiosulfate Citrate Bile Sucrose Agar (TCBS; Oxoid, UK). All samples were cultured in duplicate and incubated at 25 °C for 48–72 h in a microbiological incubator (Binder, Tuttlingen, Germany) to facilitate the development of mesophilic and psychrotolerant bacteria associated with salmonid pathology.

The isolated bacterial strains were evaluated culturally, morphologically, and biochemically, and bacterial confirmation was performed by MALDI-TOF MS (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry) (Bruker, Bremen, Germany). The obtained spectra were analyzed against the BDAL reference database (Bruker Daltonics, Germany) and the results were additionally verified by comparison with the CDC spectral database (Centers for Disease Control and Prevention, USA). Routine quality control of the MALDI-TOF MS system was performed using reference ATCC strains according to the laboratory's standard operating procedures. Only identifications with MALDI Biotyper score values $\geq$2.0 were accepted for species-level identification. Molecular characterization of antimicrobial resistance genes and genomic analyses at the strain level were not included in the objectives of the present field investigation.

2.5. Antimicrobial Susceptibility Testing

The antimicrobial susceptibility profile of the bacterial isolates was evaluated using the Kirby–Bauer disk diffusion method, employing commercial disks with antibiotics relevant to fish pathology and therapeutic practice in aquaculture. Inhibition zone diameters were interpreted using the EUCAST Breakpoint Tables, version 14.0 (2024), whenever corresponding breakpoints were available [39]. For bacterial species or antimicrobial agents for which EUCAST breakpoints were not available, inhibition zone diameters were interpreted according to the reference criteria provided by the manufacturers of the antimicrobial disks (Oxoid and Bio-Rad), considering the current limitations in standardized susceptibility criteria for some aquatic bacterial pathogens. Quality control of antimicrobial susceptibility testing was performed using the reference strains *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 in accordance with the laboratory's standard operating procedures.

The antimicrobial panel consistently included florfenicol (FFC, 30 µg), neomycin (Ne, 30 µg), amoxicillin + clavulanic acid (AMC, 30 µg), kanamycin (K, 30 µg), doxycycline (Do,

30 µg), oxytetracycline (OT, 30 µg), lincomycin (MY, 10 µg), enrofloxacin, and trimethoprim + sulfamethoxazole (STX, 30 µg). Depending on the epidemiological characteristics of each investigated outbreak, previous antimicrobial susceptibility results, and the therapeutic requirements of the fish farm, the antimicrobial panel was expanded to include additional antimicrobial agents such as tylosin (Ty, 30 µg), flumequine (UBQ, 30 µg), spectinomycin (SPT, 30 µg), ampicillin (AMP, 30 µg), marbofloxacin (MAR, 30 µg), erythromycin (E, 30 µg), and chloramphenicol (C, 30 µg). Chloramphenicol was used exclusively for microbiological research purposes, without therapeutic relevance for aquaculture intended for food consumption.

The evaluation of antimicrobial susceptibility profiles aimed both at identifying relevant therapeutic options for the management of the investigated outbreaks and at the epidemiological characterization of susceptibility patterns associated with bacteria isolated from the case series of the analyzed farm.

3. Results

3.1. Temporal Dynamics of the Investigated Outbreaks

During the period 2023–2026, 18 episodes of recurrent clinical manifestations associated with mortality in Oncorhynchus mykiss, raised in semi-open cage culture systems located in the mountain area of Lake Bicaz, were investigated. The temporal distribution of the investigated outbreaks is presented in Table 1.

Table 1. Temporal distribution of investigated outbreaks and associated bacterial isolates.

Year	Period	Outbreak ID	Main Bacterial Isolate(s)	Mixed Infection
2023	December	F1	*Yersinia ruckeri*	No
2023	December	F2	*Yersinia ruckeri*	No
2023	December	F3	*Hafnia alvei*	No
2023	December	F4	*Aeromonas hydrophila*; *Aeromonas veronii*	Yes
2024	March	F1	*Hafnia alvei*	No
2024	March	F2	*Hafnia alvei*	No
2024	March	F3	*Yersinia ruckeri*	No
2024	March	F4	*Aeromonas hydrophila*	No
2024	October	F1	*Aeromonas hydrophila*; *Pseudomonas fluorescens*; *Aeromonas veronii*	Yes
2024	October	F2	*Providencia alcalifaciens*; *Hafnia alvei*	Yes
2025	April	F1	*Aeromonas salmonicida*	No
2025	April	F2	*Aeromonas salmonicida*	No
2025	October	F1	*Aeromonas hydrophila*	No
2025	October	F2	*Flavobacterium psychrophilum*	No
2025	October	F3	*Aeromonas hydrophila*; *Enterobacter aerogenes*	Yes
2026	April	F1	*Aeromonas hydrophila*	No
2026	April	F2	*Hafnia alvei*	No
2026	April	F3	*Hafnia alvei*	No

Abbreviation: Mixed infection indicates the simultaneous isolation of two or more bacterial species from the same individual fish within the investigated outbreak.

The bacterial outbreaks evolved in different calendar periods, namely December, March, April, and October, but without evidence of strict seasonality. The dynamics observed over time indicated the persistence of a microbiological pressure in the investigated culture system. The first outbreaks investigated in December 2023 were predominantly associated with the species *Yersinia ruckeri* and *Hafnia alvei*, whereas subsequent outbreaks revealed a random diversification of the involved bacterial spectrum. During the analyzed period, species belonging to the genus *Aeromonas* became dominant, evolving either as single infections or in bacterial coinfections. During the evolution of the pathological episodes, bacterial associations were identified between *Aeromonas hydrophila* and *Aeromonas veronii*, *Aeromonas hydrophila* and *Enterobacter aerogenes* and respectively *Providencia alcalifaciens* and *Hafnia alvei*.

In October 2024, a mixed bacterial complex was recorded when *Aeromonas hydrophila*, *Pseudomonas fluorescens*, and *Aeromonas veronii* were isolated concomitantly.

In April 2025, outbreaks were dominated by *Aeromonas salmonicida*, and in October 2025, *Flavobacterium psychrophilum* also appeared, a bacterial species important in the pathology of cold-water salmonids. During April 2026, three additional outbreaks revealed the re-emergence of the species *Aeromonas hydrophila* and *Hafnia alvei*.

3.2. Clinical and Necropsy Findings

In all specimens investigated microbiologically, the clinical and lesional aspects varied, but could be correlated with septicemic processes.

The specimens investigated presented variable clinical and lesional changes, but compatible with some bacterial septicemic processes. On external examination, congestion and hemorrhages in the oral cavity (Figure 2b,c), hemorrhages in the opercular, peripectoral, and periorbital regions (Figure 2d,f), or superficial ulcerations and changes in skin coloration (Figure 2e) were sometimes observed. In some specimens, tendencies of exophthalmia and abdominal distension were noted (Figure 2f).

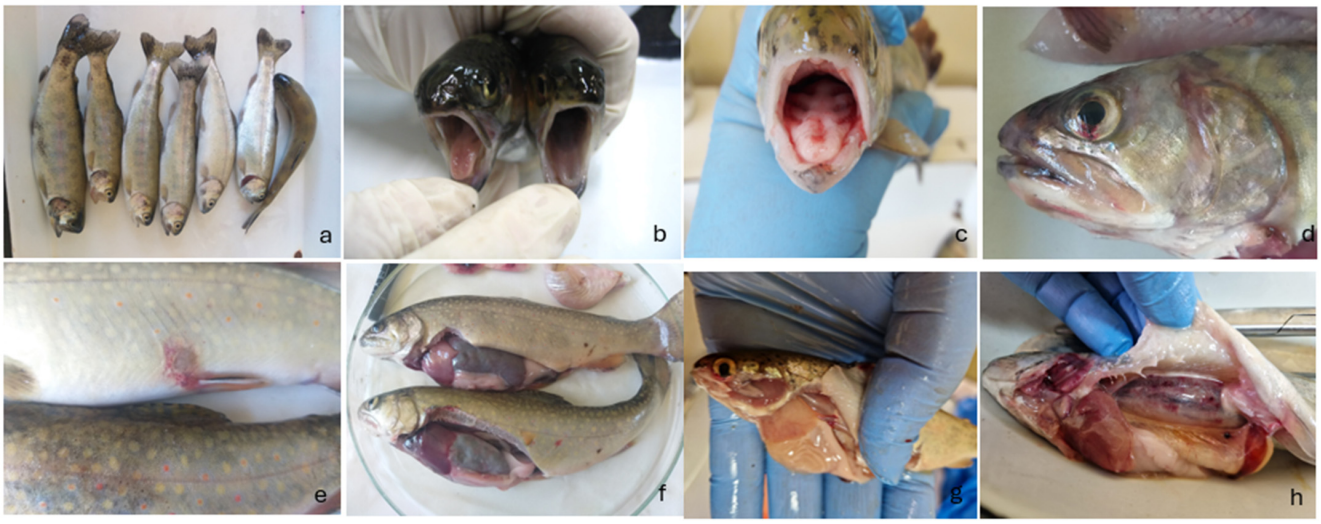


Figure 2. Clinical and lesional aspects observed in rainbow trout (*Oncorhynchus mykiss*) from different outbreaks investigated in the semi-open mountain aquaculture system: (**a**) specimens examined during microbiological investigations with no visible lesions; (**b**,**c**) congestion and hemorrhages in the oral cavity; (**d**) hemorrhages in the opercular and peripectoral region; (**e**) focal cutaneous hemorrhagic lesions; (**f**) visceral distension and hepatic congestion; (**g**) steatotic degenerative hepatopathy and periorbital hemorrhagic lesions; (**h**) hemorrhages in the swim bladder observed at necropsy.

At necropsy, internal lesions suggestive of systemic bacterial infections were observed: hepatic and splenic congestion, ascites, and inflammatory changes in the internal organs

(Figure 2f). Sometimes, steatotic degenerative hepatopathy lesions were also observed. The severity of the lesions varied between the investigated outbreaks, without a direct correlation being established between the type of isolated bacterium and the intensity of the observed macroscopic changes (Figure 2).

3.3. Distribution of Bacterial Isolates and Mixed Infections

The microbiological analyses carried out during the study period revealed an important alteration of potentially pathogenic microorganisms involved in outbreaks in the fish farm. The distribution of bacterial isolates is presented in Table 1.

Both pathogenic bacteria recognized for their affinity for salmonids were identified, as well as opportunistic microorganisms frequently encountered in natural aquatic ecosystems and intensive aquaculture systems.

Species belonging to the genus *Aeromonas* represented the predominant bacterial group within the investigated outbreaks. *Aeromonas hydrophila* was repeatedly isolated in distinct outbreaks in 2023, 2024, 2025, and 2026, being one of the most frequently identified bacteria throughout the surveillance period. Additionally, other species belonging to this genus were identified, including *Aeromonas veronii* and *Aeromonas salmonicida*.

In the outbreaks investigated in December 2023 and March 2024, episodes associated with *Yersinia ruckeri* were identified, a bacterial agent recognized for its tropism and pathogenicity towards salmonids raised in intensive and semi-intensive systems. Subsequently, the bacteriological mix in the outbreaks became progressively more heterogeneous, with opportunistic pathogenic bacteria such as *Hafnia alvei*, *Providencia alcalifaciens*, *Pseudomonas fluorescens*, and *Enterobacter aerogenes* intervening.

Bacterial coinfections were identified in several investigated outbreaks. These coinfections represented mixed bacterial cultures isolated from the same individual fish. The most complex bacterial associations were observed in October 2024 and October 2025, when two or three bacterial species were simultaneously isolated from the same pathological episode. In the October 2024 outbreak, the pathogenic species *Aeromonas hydrophila*, *Pseudomonas fluorescens*, and *Aeromonas veronii* were simultaneously isolated, while in October 2025, the triggered outbreak had a polymicrobial etiology composed of the association between *Aeromonas hydrophila* and *Enterobacter aerogenes*.

In April 2025, strains of the species *Aeromonas salmonicida* were isolated and confirmed, and in October 2025, *Aeromonas hydrophila* was isolated both as a single bacterial isolate and as part of mixed bacterial cultures with *Enterobacter aerogenes*. In the same period, an outbreak caused by *Flavobacterium psychrophilum*, a species frequently associated with the pathology of cold-water salmonids, was also activated.

In April 2026, outbreaks with *Aeromonas hydrophila* and *Hafnia alvei* reappeared, isolated in pure cultures from the examined fish samples. The temporal distribution of bacterial isolates and the recurrence of the main identified species are schematically illustrated in Figure 3.

3.4. Antimicrobial Susceptibility Profiles

The antimicrobial susceptibility profiles showed variations both between the investigated bacterial species and between the recurrent outbreaks analyzed during the investigation period. The comparative distribution of susceptibility, intermediate susceptibility, and resistance profiles, reported to the main classes of antimicrobials used, is summarized in Table 2.

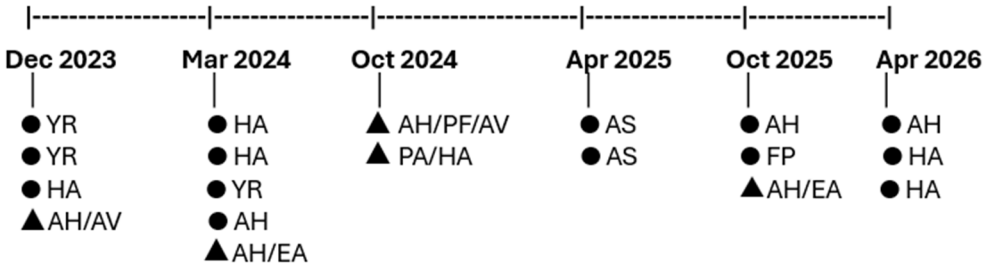


Figure 3. Temporal distribution of recurrent bacterial outbreaks investigated in rainbow trout (*Oncorhynchus mykiss*) reared in a semi-open mountain reservoir cage aquaculture system between 2023 and 2026. (●) Indicates outbreaks associated with single bacterial isolates, while (▲) indicates mixed bacterial infections involving two or more bacterial species. Abbreviations: YR = *Yersinia ruckeri*; HA = *Hafnia alvei*; AH = *Aeromonas hydrophila*; AV = *Aeromonas veronii*; AS = *Aeromonas salmonicida*; PF = *Pseudomonas fluorescens*; PA = *Providencia alcalifaciens*; EA = *Enterobacter aerogenes*; FP = *Flavobacterium psychrophilum*.

Table 2. Distribution of antimicrobial susceptibility profiles according to antibiotic classes.

Antibiotic Class	Susceptible (%)	Intermediate (%)	Resistant (%)
Aminoglycosides	26.7	60.0	13.3
Amphenicols	77.8	3.7	18.5
Beta-lactams	13.0	21.7	65.2
Fluoroquinolones	60.0	20.0	20.0
Lincosamides	10.0	30.0	60.0
Macrolides	0.0	37.5	62.5
Potentiated sulfonamides	41.2	17.6	41.2
Quinolones	10.0	40.0	50.0
Tetracyclines	43.8	31.2	25.0

The percentages were calculated based on the total number of susceptibility interpretations recorded for each antimicrobial class.

Table 2 presents a synthesis of the susceptibility profiles to different antibiotic classes, and the susceptibility details for each bacterial species and for each outbreak are available in Supplementary Table S1.

Overall, the highest sensitivity rates were identified for amphenicols and fluoroquinolones, with susceptibility values of 77.8% and 60.0%, respectively. Among these, florfenicol and enrofloxacin proved to be the most active antimicrobial molecules in the investigated outbreaks, being effectively active against *Yersinia ruckeri* and *Aeromonas salmonicida*. Moderate sensitivity rates were also observed for tetracyclines, but in general, their efficacy varied from one species to another and from one outbreak to another.

By contrast, beta-lactams had the highest proportion of resistance (65.2%), followed by macrolides (62.5%) and lincosamides (60.0%). These resistance peaks were observed particularly in isolates of the genus *Aeromonas* towards ampicillin and lincomycin. Some isolates of *Hafnia alvei* also showed reduced susceptibility or resistance to trimethoprim+sulfamethoxazole and flumequine.

For *Aeromonas salmonicida*, it was found that florfenicol, enrofloxacin, and doxycycline were effective, and for *Flavobacterium psychrophilum*, the highest sensitivity was observed for marbofloxacin and enrofloxacin. In contrast, in outbreaks with mixed bacterial flora, especially those involving *Aeromonas hydrophila*, *Aeromonas veronii* and also other opportunistic bacteria, the susceptibility profiles were more heterogeneous.

To illustrate the temporal pattern of antimicrobial susceptibility profiles, heatmaps were generated for *Aeromonas hydrophila* and *Hafnia alvei*, the main bacterial species responsible for recurrent infectious outbreaks during the investigated period (Figure 4).

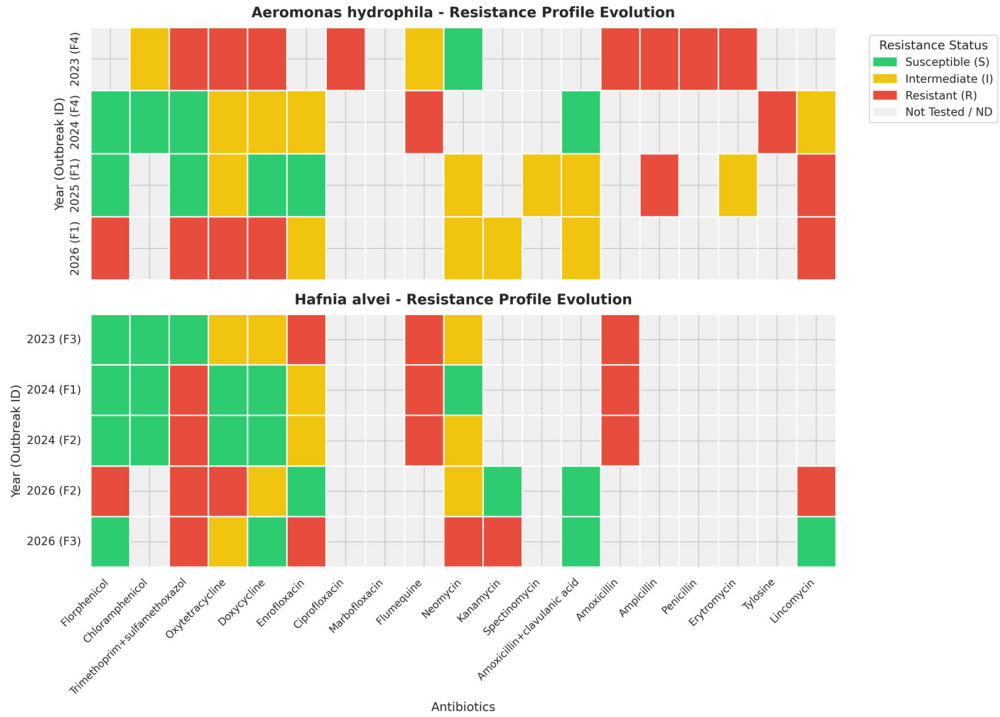


Figure 4. Heatmaps showing the antimicrobial susceptibility profiles of *Aeromonas hydrophila* and *Hafnia alvei* isolated from recurrent outbreaks during the study period (2023–2026).

The antimicrobial susceptibility profile of *Aeromonas hydrophila* varied during the investigated period. The strains isolated in 2024 and 2025 remained susceptible to some of the antimicrobial agents tested, whereas the isolate recovered in 2026 showed a broader resistance profile, with reduced susceptibility to florfenicol, tetracyclines, sulfonamides, and potentiated sulfonamides, which are commonly used for the treatment of bacterial diseases in salmonids.

For the opportunistic species *Hafnia alvei*, the susceptibility profile was different. Isolates recovered in 2023 and 2024 were more susceptible to the antimicrobial agents tested, whereas the isolates recovered in 2026 showed a slight expansion of the resistance profile to fluoroquinolones and aminoglycosides, while resistance to potentiated sulfonamides was maintained.

The results revealed heterogeneous antimicrobial susceptibility profiles, both across bacterial species and among outbreaks from different periods. Overall, no prominent temporal pattern in antimicrobial susceptibility was observed at the investigated outbreaks in salmonids raised in the semi-open system. Details of the antimicrobial susceptibility profiles for the main isolated bacteria, including the individual distribution of interpretations, are presented in Supplementary Table S1.

4. Discussion

In the present longitudinal study, recurrent bacterial outbreaks occurring over a four-year period in rainbow trout (*Oncorhynchus mykiss*) reared in a semi-open mountain aquaculture system were investigated. Our findings showed that these outbreaks involved both classical salmonid pathogens and opportunistic bacterial species, occurring as single

or mixed infections, with heterogeneous antimicrobial susceptibility profiles that varied across outbreaks and among bacterial species.

The results obtained in the present study highlight the complex and dynamic nature of bacterial health issues associated with rainbow trout farming in semi-open systems located in mountain reservoirs. The recurrence of the investigated outbreaks over several consecutive years, the diversity of the identified bacteria, and the heterogeneity of the antimicrobial susceptibility profiles suggest the existence of continuous microbiological exposure in the investigated aquatic ecosystem. At the same time, the results reflect the epidemiological particularities of cage culture systems developed in natural ecosystems, where the permanent interaction between fish, environmental microbiota, and ecological factors can significantly influence the health status dynamics of salmonid stocks.

Semi-open systems organized on mountain lakes present both advantages and vulnerabilities for salmonid farming [26]. In mountain ecosystems, relatively stable and low temperatures, good oxygenation levels, and natural dilution of organic and microbial loads can be ensured, but these are insufficient in hyperintensive or heavily stocked farms [8]. These are mandatory conditions for salmonid physiology and contribute to reducing metabolic stress and restoring chronic imbalances associated with intensive fish production systems [40,41]. At the same time, these semi-open farms in the mountain area involve continuous exposure to the microbiota of the aquatic ecosystem, facilitating permanent contact with opportunistic bacteria or with pathogens present in the surrounding environment [4]. The stability of the aquatic microbiota is closely correlated with environmental conditions, especially the ambient temperature and the specific ecological variations in mountain reservoir ecosystems.

This ecobiological manifestation appears to be reflected also by the results obtained in the present study. Although the investigated bacterial outbreaks were recurrent, their temporal distribution did not suggest the existence of massive explosive episodes, but they were sufficient to modify the dynamics of the bacterial microbiota. In this context, the longitudinal monitoring carried out over several consecutive years offers a more realistic perspective on health issues in the field compared to experimental studies conducted under strictly controlled conditions.

Another aspect signaled is the alternation between classical salmonid pathogens and opportunistic bacteria identified in the investigated outbreaks. *Yersinia ruckeri* and *Aeromonas salmonicida* remain among the most important bacterial pathogens involved in salmonid pathology internationally, being frequently associated with significant economic losses in fish farms [12,42–44]. In contrast, the repeated identification of opportunistic bacteria such as *Aeromonas hydrophila*, *Aeromonas veronii* [19], *Hafnia alvei* [45,46], *Providencia alcalifaciens*, *Pseudomonas fluorescens* [21], or *Flavobacterium psychrophilum* [47,48] suggests the existence of a dynamic microbial ecosystem in which ambient bacteria can acquire pathological relevance under certain conditions of stress or biological imbalance. The role of opportunistic bacteria is reported increasingly frequently. Their evolution is possible in all types of fish farms, favoring the occurrence of secondary infections or complex bacterial syndromes [4,29]. During the investigated outbreaks, fish farm personnel frequently reported high stocking density, rapid temperature fluctuations, and reduced dissolved oxygen levels as potential predisposing factors for disease occurrence. However, because these variables were not systematically documented during the investigation, they could not be evaluated in the present study.

Our findings are also consistent with previous reports from European rainbow trout aquaculture that include a description of how both classic salmonid pathogens as well as other opportunistic bacterial species are involved in the development of bacterial disease outbreaks in rainbow trout farms in Europe and Romania [4,21,44].

In the present study, we observe that outbreaks characterized by mixed bacterial isolates presented more heterogeneous antimicrobial susceptibility profiles than outbreaks triggered by classical pathogens. This reflects the ecological complexity of the outbreaks and a possible variability in exposure to antimicrobials that can activate resistance mechanisms.

Florfenicol and fluoroquinolones were found to be the most susceptible antimicrobial drugs tested, with other studies reporting similar results from salmonid aquaculture [49], whereas beta-lactams, macrolides, and lincosamides exhibited high rates of resistance or decreased susceptibility to a number of isolates that belonged to the genus *Aeromonas*. These observations highlight the importance of prudent use of antimicrobials and the need to perform susceptibility testing before initiating treatments in salmonid farms. Therefore, monitoring of susceptibility profiles can represent a useful tool both for health surveillance and for optimizing therapeutic strategies and reducing the empirical use of antimicrobials in aquaculture. From a practical perspective, the findings of the present study support the need for microbiological examinations and the use of antimicrobials only on the basis of antimicrobial susceptibility testing. When combined with appropriate biosecurity measures and continuous microbiological surveillance, these good practices contribute to maintaining fish health, the early detection of infectious outbreaks, and the improvement of disease management in semi-open salmonid farming systems.

The interpretation of the results must be made in the particular context of a longitudinal investigation conducted under real commercial aquaculture conditions, characterized by biological, ecological, and managerial variability between the investigated outbreaks. Complete data on mortality, water physicochemical parameters, or the detailed therapeutic history for each investigated outbreak were not available. Bacterial identification was performed by MALDI-TOF MS without molecular characterization of antimicrobial resistance genes or genomic analyses at the strain level. Moreover, the antimicrobial susceptibility data were analyzed descriptively, and no formal statistical comparisons between bacterial species or outbreaks were performed, owing to the unequal and non-independent distribution of bacterial isolates throughout the study period. Nevertheless, the main value of the study derives from the longitudinal nature of the investigation, the diversity of the identified bacteria, and the documentation of the evolution of antimicrobial susceptibility profiles in a semi-open mountain system monitored over several consecutive years. Future studies should complement these findings by including the molecular characterization of antimicrobial resistance genes, strain typing approaches such as MLST, and the evaluation of the impact of antimicrobial treatments on the environmental microbiota. Such data are relatively rarely available in the specialized literature and can contribute to a better understanding of the health issues associated with salmonid aquaculture conducted in natural reservoir ecosystems.

5. Conclusions

The results of our research are supportive of our proposed hypothesis and demonstrate the complexity and dynamism of recurrent bacterial outbreaks in semi-open mountain environments for rainbow trout production. The longitudinal monitoring conducted during successive years demonstrated both the coexistence of classical salmonid pathogens with opportunistic bacteria associated with the natural aquatic environment, as well as heterogeneous antimicrobial susceptibility profiles. Furthermore, our data indicate that semi-open mountain environments may provide suitable conditions for salmonid culture although permanent exposure to environmental microbiota seems to be conducive to recurring episodes of disease. Consequently, regular bacteriological surveillance as well as antimicrobial susceptibility assessment prior to initiating treatments is necessary for optimal therapeutic management, as well as responsible antibiotic use in aquaculture.

Lastly, due to both its longitudinal nature and data collection within an actual commercial setting, the current study provides valuable insight into the health concerns associated with salmonid aquaculture in semi-open mountain ecosystems.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/app16157561/s1: Table S1. Bacterial isolates and their antimicrobial susceptibility and resistance profiles in rainbow trout (*Oncorhynchus mykiss*) raised in a semi-open mountain system.

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