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First insights into *Weissella tructae* infections in rainbow trout (*Oncorhynchus mykiss*) in Vietnam: Bacterial characterization, pathogenicity and antimicrobial susceptibility

Doan Thi Nhinh [a], Dang Thi Hoa [a], Tran Thi Trinh [a], Mai Van Tung [a], Nguyen Thi Huong Giang [b], Kim Van Van [a], Truong Dinh Hoai [a,*,1]

[a] Faculty of Fisheries, Vietnam National University of Agriculture, Hanoi 131004, Viet Nam
[b] Faculty of Veterinary Medicine, Vietnam National University of Agriculture, Hanoi 131004, Viet Nam

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ABSTRACT

Weissellosis has emerged as a significant disease impacting farmed rainbow trout (*Oncorhynchus mykiss*), resulting in high global mortality rates. In the summer of 2023, an outbreak in Northern Vietnam's rainbow trout farms was linked to Weissellosis, with mortality rates ranging from 20 % to 60 %, prompting an investigation into the causative agent. A total of 106 diseased fish exhibiting panophthalmitis and hemorrhagic eyes were collected from 25 affected farms. Twenty-five representative isolates, each sourced from a different farm, were identified as *Weissella tructae* through phenotypic and biochemical characterisation, PCR, and 16S rRNA sequencing. Challenge experiments indicated a relatively high virulence of *W. tructae* in rainbow trout weighing 35–40 g, with LD_{50} values estimated at 1.2×10^4 CFU/fish. Histopathological examination revealed significant tissue damage, including hemorrhages in the brain, spleen, and kidney. Antimicrobial susceptibility testing of eight antibiotics authorized for use in Vietnam showed the lowest minimum inhibitory concentration (MIC) values for Amoxicillin, Amoxicillin-Clavulanic Acid, and Erythromycin (0.06–1 mg/L). In contrast, most isolates exhibited MIC values of $\geq$ 512 mg/L for Sulfamethoxazole/Trimethoprim. This study represents the first report of Weissellosis in Vietnamese rainbow trout, highlighting the geographic expansion of the disease and underscoring the urgent need for effective strategies to prevent and control this pathogen.

1. Introduction

The rainbow trout (*Oncorhynchus mykiss*) is probably the most widely introduced fish species globally. Since its initial translocation beyond its native range, this species has been introduced to at least 99 countries, establishing self-sustaining populations in various regions (Crawford and Muir, 2008). The remarkable global success of the rainbow trout can be attributed to several key factors such as its status as a highly valued game fish within Western culture, its resilience to relatively high temperatures, the ability to manipulate life histories through selective breeding, its rapid growth rate, and its suitability for hatchery cultivation. These attributes collectively enhance its economic significance in food production (Crawford and Muir, 2008; Halverson, 2008; Woynarovich et al., 2011). However, the translocation of rainbow trout across national borders presents several risks related to its status as an invasive species. Furthermore, it is essential to consider the associated risks arising from the introduction and transfer of pathogens, which have the potential to result in transboundary diseases (Castrejón-Nájera et al., 2018; Kim et al., 2015; Shahi et al., 2018).

Weissellosis has recently emerged as a significant threat as a new emerging disease to rainbow trout (*Oncorhynchus mykiss)* (Welch and Good, 2013; Castrejón-Nájera et al., 2018; Medina et al., 2020). Initially identified as being caused by the bacterium *Weissella ceti*, this pathogen belongs to the genus *Weissella* within the family *Lactobacillaceae*. It is a Gram-positive, catalase-negative, α-hemolytic bacterium characterised by non-endospore-forming cells that exhibit a coccoid or irregularly rod-shaped morphology (Collins et al., 1989; Welch and Good, 2013). This pathogen was first isolated from a beaked whale (*Mesoplodon bidens*) (Vela et al., 2011) but has been identified as a causative agent in outbreaks affecting rainbow trout farms across several countries,

* Corresponding author.
E-mail address: tdhoai@vnua.edu.vn (T.D. Hoai).
[1] ORCID: 0000–0002-2271–849X

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including Brazil, the USA, Japan, and Mexico (Figueiredo et al., 2012; Welch and Good, 2013; Costa et al., 2015; Mitomi et al., 2018; Castrejón-Nájera et al., 2018). More recently, cases have been reported in Peru (Pereira et al., 2022), highlighting the rapid and transboundary spread of this emerging disease among rainbow trout populations worldwide. The pathogen primarily manifests clinical signs such as unilateral or bilateral exophthalmia and hemorrhages in various external and internal organs, leading to significant economic losses and fish mortality rates ranging from 20 % to 80 % during the summer months (Figueiredo et al., 2012; Castrejón-Nájera et al., 2018; Pereira et al., 2022). Notably, a recent study has classified the *Weissella* strain isolated from rainbow trout as a novel species, designated *Weissella tructae*, based on distinct phenotypic and phylogenetic differences from the type strain *W. ceti* recovered from the beaked whale (Pereira et al., 2022). Despite the growing prevalence of this bacterial pathogen worldwide, details regarding its pathogenicity and antibiotic susceptibility remain inadequately documented.

This study presents the inaugural research findings on *W. tructae* in relation to outbreaks of weissellosis in rainbow trout farming in northern Vietnam. It specifically focuses on the pathogenicity of the bacterium and its susceptibility to commonly used antibiotics, with the objective of enriching and advancing the understanding of this transboundary pathogen. The results of this research will provide a critical foundation for the development of effective strategies aimed at disease prevention and management.

2. Materials and methods

2.1. Disease outbreak and fish samples

During the summer of 2023, rainbow trout farms in Lao Cai, a mountainous province in northern Vietnam and the country's largest producer of rainbow trout, reported mortality rates of cultured fish ranging from 20 % to 60 % with the symptom of Weissellosis. In the affected regions, these farms are primarily situated in four main culture areas including Thac Bac (TB), Ngu Chi Son (NCS), Den Sang (DS), and Y Ty (YT). All farms employ a flow-through system, sharing water sources that flow from upstream to downstream along the streams. A total of 106 diseased fish exhibiting symptoms of panophthalmitis and hemorrhagic eyes were sampled from 25 farms for further investigation (see Table 1). The sampled fish were transported to the laboratory in sealed plastic bags, partially filled with oxygenated water, to ensure their integrity during transit.

2.2. Bacterial isolation

A clinical examination was immediately conducted upon the arrival of the collected samples at the laboratory. The fish were weighed and inspected for external and internal gross clinical signs. Tissue smears were prepared from each fish, including the brain, kidney, spleen, and liver, and Gram-stained to detect the presence of bacteria in the organs. The stained tissues showed a distinct morphology of bacteria, which were Gram-positive, irregular-rod shapes arranged in short chains. Other bacterial forms were absent or negligible. The initial RT-PCR diagnosis of the sampled fish revealed negative results for the three major viral pathogens in rainbow trout, including Viral hemorrhagic septicemia virus (VHSV), Infectious hematopoietic necrosis virus (IHNV), and Infectious pancreatic necrosis virus (IPNV), as previously described (Chico et al., 2006; Dhar et al., 2008; Lopez-Lastra et al., 1994), and no parasites were detected in wet mount examinations of the affected fish (data not shown). Consequently, the study was prioritised in diagnosing bacterial infection retrieved from internal organs. Simultaneously, the samples were aseptically streaked onto TSA and TSA supplemented with 5 % sheep blood (TSA blood; Merck, Darmstadt, Germany) and incubated at 25°C for 48 h. The predominant colony types were subsequently sub-cultured, and pure isolates were preserved in TSA broth containing 15 % glycerol and stored at −80°C.

2.3. Morphological and biochemical identification

The morphology of Gram-stained bacterial cells and colonies on TSA and TSA-blood agar plates was examined under a microscope. Oxidase and catalase activities were assessed using 3 % H_2O_2 (Merck, Darmstadt, Germany) for catalase testing and BBL™ Oxidase Reagent Droppers

Table 1
Information on disease outbreaks occurring in rainbow trout recorded in the study.

Farm code[β]	Fish size (g)[γ]	Water temperature (°C)[σ]	Cumulative mortality (%)*	No. of fish used for bacterial isolation (n = 106)	Representative isolates
TB.01	210–290	17.2	45–50	4	RB.TB01
TB.02	30–40	18.6	40–50	5	**RB.TB02****
TB.03	550–610	17.5	55–60	5	RB.TB03
TB.04	630–680	18.8	50–55	3	RB.TB04
TB.05	280–360	18.2	50–55	3	RB.TB05
TB.06	60–85	19.2	50–60	5	RB.TB06
TB.07	55–135	18.6	45–50	3	RB.TB07
TB.08	510–580	18.7	20–25	5	RB.TB08
TB.09	250–310	19.2	20–25	5	RB.TB09
TB.10	670–720	19.5	45–50	5	RB.TB10
NCS.01	870–930	19.3	40–45	4	RB.NCS01
NCS.02	410–430	17.8	30–35	5	RB.NCS02
NCS.02	810–850	17.2	25–30	3	RB.NCS02
NCS.03	170–230	17.6	30–35	4	RB.NCS03
NCS.04	55–70	19.3	50–60	4	RB.NCS04
NCS.05	480–510	18.3	30–35	4	RB.NCS05
NCS.06	25–35	19.5	40–50	4	**RB.NCS06**
NCS.07	630–680	18.6	25–30	3	RB.NCS07
DS.01	610–670	18.4	25–30	5	**RB.DS01**
DS.02	360–410	19.1	20–25	5	RB.DS02
DS.03	310–390	19.5	45–50	3	RB.DS03
YT.01	630–675	17.4	20–25	5	RB.YT01
YT.02	450–510	17.7	35–40	4	RB.YT02
YT.03	765–835	17.4	30–35	5	RB.YT03
YT.04	40–45	19.5	25–30	5	**RB.YT04**

TB, Thac Bac; NCS, Ngu Chi Son; DS, Den Sang; YT, Y Ty; RB, Rainbow trout
[β]Each code indicates geographical location and farm number; [γ]Weigh of sampled fish; [σ]Measure at the time of sampling; *Estimated by farmer;
The isolates in bold were selected for 16S rRNA sequencing; ** isolate used for challenge test

(Merck, Darmstadt, Germany) for oxidase testing. Additional biochemical characterizations were performed using the commercial API 20 STREP kit (BioMérieux, Marcy-l'Étoile, France). API analysis was performed according to the manufacturer's instructions at 25°C. *W. ceti* reference strain, 1119–1A-09, isolated from the beaked whale, purchased from the Spanish type culture collection, was tested simultaneously with the isolates in the study. The biochemical test results were compared with those of strain NC36 (from rainbow trout in the USA), as reported by Welch and Good (2013), and the pathogenic strain found in rainbow trout in Japan, as described by Mitomi et al. (2018).

2.4. DNA extraction

Genomic DNA of 25 representative isolates each from an affected farm was extracted using the InstaGene Matrix kit (Bio-Rad, California, USA) following the manufacturer's protocol. The extracted DNA was preserved at −20°C for molecular identification.

2.5. Sequence, phylogenetic analysis of the 16S rRNA gene and PCR confirmation

The amplification of the 16S rRNA gene sequence was conducted on four representative isolates, each from a different stream system, using universal primers 27 F (AGAGTTTGATCMTGGCTCAG) and 1525 R (AAAGGAGGTGATCCAGCC) (Lane, 1991). The PCR products of the 16S rRNA gene were purified using the QIAquick PCR extraction kit (Qiagen) and subsequently sequenced by Macrogen (Seoul, Korea). The obtained sequences were assembled using BioEdit version 7.0 (Hall et al., 2011) and deposited in the GenBank database to obtain accession numbers. The sequences were then aligned with related sequences in GenBank using the Basic Local Alignment Search Tool (BLAST) nucleotide search. Phylogenetic analysis was performed to compare the 16S rRNA sequences of the four representative isolates with the sequences of other species of the genus *Weissella*, retrieved from GenBank using the ClustalW program (Thompson et al., 2003). Phylogenetic trees were constructed using MEGA 11 software with the neighbor-joining method (Saitou and Nei, 1987). A bootstrap value of 1000 replicates was applied to assess the robustness of the phylogenetic analysis. Duplex PCR assays were conducted using genus-specific and species-specific primers as described by Snyder et al. (2015). The genus-specific primer (Weig) amplified a 725-bp 16S rDNA sequence with F:CGTGGGAAACCTACCTCTTA; R: CCCTCAAACATCTAGCAC (Jang et al., 2002). The species-specific primer with sequences F: TCTAGGAGCGAATAAGAACG and R:CTGTTGATGCAGAAATAGCA (Snyder et al., 2015) amplifed a 500-bp. The DNA of the *Weissella ceti* 1119–1A-09 strain was used as the positive control.

2.6. Challenge experiment

Apparently, healthy rainbow trout (35–40 g) were obtained from a commercial fish farm in Lao Cai, Vietnam, for the challenge experiments. The fish were acclimated to the experimental conditions for one week before the experiments began. Five fish were randomly selected and subjected to examination of their external and internal appearances. Following this, bacterial isolation was conducted to confirm that the fish were healthy and free of *W. tructae*. A representative bacterial isolate (RB.TB02) was selected for experimental infection. The bacteria were cultured in TSB (Merck, Darmstadt, Germany) at 25°C for 24 h. The viable bacterial density of the stock suspension was determined using the plate count method and then adjusted to approximately 1×10^8 CFU/ml by adding an equivalent volume of PBS buffer. Ten-fold serial dilutions of the bacteria, ranging from 10^3 to 10^8 CFU/ml, were prepared for the virulence test.

A total of 210 fish were divided into seven groups, each placed in 100 L tanks (10 fish per tank, three replicates per group). Fish from six groups were intraperitoneally (i.p.) injected with 0.1 ml of the bacterial suspensions, resulting in final bacterial concentrations ranging from 10^2 to 10^7 CFU per fish. The control group was injected with PBS without bacteria. Water parameters of the experiment were dissolved oxygen > 5 mg/L, pH 7, and temperature 18 ± 0.5°C using a chiller water system (F5000, Fogeen, China). The mortality of fish was monitored daily for 14 days.

The medium lethal doses (LD50) of *W. tructae* on rainbow trout were calculated as described by Reed and Muench (1938): $LD50 = 10^{a+x}$, in which, $x = (50\text{-}PL)/(PH–PL)$, a: the power number of the bacterial density at the level of mortality just below 50 %, PH: the cumulative proportion of deaths at the upper dose (the lowest dose at which the cumulative mortality is above 50 %), and PL: the cumulative proportion of deaths at the lower dose (the lowest dose at which the cumulative mortality is below 50 %). Representative moribund and freshly dead fish ($n = 3$) from each challenge group, as well as apparently healthy fish from the control group at the end of the experiment ($n = 3$), were subjected to bacterial re-isolation and histopathological analysis.

2.7. Histopathological examination

Representative moribund rainbow trout ($n = 3$) from each challenge group, as well as naturally infected fish, were examined for histopathological changes. Tissue samples from affected fish, including the spleen, kidney, liver, brain, and intestine, were collected and preserved in 10 % buffered formalin. After 24 h of fixation, the tissues were dehydrated in a graded ethanol series, embedded in paraffin, and sectioned at a thickness of 5 μm. The sections were then stained with hematoxylin and eosin following standard histological procedures. Histopathological changes were observed under a light microscope equipped with a digital camera (Olympus, Tokyo, Japan).

2.8. Minimum inhibitory concentration (MIC) assays

Eight antimicrobial agents (Merck, Darmstadt, Germany) were used to assess the susceptibility of *W. tructae* isolates. These agents included amoxicillin (AMO), amoxicillin-clavulanic acid (AMC), erythromycin (ERY), florfenicol (FLO), tetracycline (TC), oxytetracycline (OTC), trimethoprim-sulfamethoxazole (TRS, 1/19), and doxycycline (DOX). All of these antimicrobial agents are currently approved for use in aquaculture in Vietnam.

To date, there are no established guidelines for antimicrobial susceptibility testing of *Weissella* species. The Clinical and Laboratory Standards Institute (CLSI) guideline for aquatic animal pathogens (CLSI VET04, 2020) remains limited, addressing only two groups of Gram-negative bacteria, including *Aeromonas* spp. and *Flavobacterium* spp. Therefore, MIC assays in this study were conducted using the broth microdilution method described by CLSI M45 (2015) for the *Lactobacillaceae* family, to which *Weissella* species belong. Each microdilution assay included a positive control (bacteria without antimicrobial agents), a negative control (broth only), and serial two-fold dilutions of each antimicrobial agent at 14 concentrations ranging from 0.06 to 512 mg/L. *Streptococcus pneumoniae* ATCC 49619 (ATCC, USA) was used as the quality control (QC) strain, and the acceptable MIC ranges provided in CLSI M45 (CLSI, 2015) were applied. The assays were performed in cation-adjusted Mueller-Hinton broth supplemented with lysed horse blood (CAMHB-LHB, 2.5 % v/v; ThermoFisher, Massachusetts, USA). The MIC was defined as the lowest concentration of the antimicrobial agent that completely inhibited visible bacterial growth (CLSI, 2015, 2020). Currently, there are no officially established breakpoint values for the *Weissella* genus. Therefore, the MIC values obtained in this study were compared, where relevant, with those reported for *Lactobacillaceae* (CLSI, 2015) and other *Weissella* species by Jeong and Lee (2015) and Vay et al. (2007).

2.9. Data analysis

The data on fish mortality rates between injection doses of bacteria in the challenge tests were logarithm (y + 1) transformed and then compared by one-way ANOVA with Tukey posthoc analysis using the SPSS program 20.0, and the significance was identified as $p \leq 0.05$.

3. Results

3.1. Disease outbreak characterization

The outbreak of Weissellosis in rainbow trout was suspected in 25 fish farms, all farms were strategically located adjacent to streams that draw from a shared source of cold water, where both the inflow and outflow of water to and from the farms originate from the same stream. Mortality occurred for rainbow trout cultured in flow-through systems during the summer months (August to September). Water temperatures during the course of the outbreak varied from 17.2°C to 19.5°C. Affected fish initially exhibited signs of anorexia, followed by lethargy, melanosis, and other indications of illness within the culture systems. According to farmer records, mortality rates ranged from 20 % to 60 % within 4–5 days of detection (Table 1). The disease impacted fingerlings, juveniles, and marketable fish, with infected fish sizes ranging from 25 to 930 g. External examinations revealed that the affected fish exhibited panophthalmitis and hemorrhages around the eyes, mouth, fins, and anal region. Gross inspections of the sampled fish indicated the presence of ascites, hemorrhage in the brain, swim bladder, intestines, and abdominal wall, as well as spleen enlargement and congestion, gut congestion, and petechiae in the liver (Fig. 1A-C).

3.2. Bacterial isolation and phenotypic characterisation

In total, 106 infected fish, which were sampled from 25 farms, were subjected to bacterial isolation (Table 1). Typical small opaque colonies on TSA media were predominantly recovered from the brain, spleen, and anterior kidney of all sampled fish (Fig. 2C). Since the disease symptoms observed in fish collected from the farms were similar, and the pathogenic bacteria present in the tissue samples and retrieved isolates were morphologically identical, one representative isolate from each farm was randomly selected for further identification (Table 1). All 25 isolates exhibited phenotypic homogeneity. The bacterial cells spread in infected fish tissues or in pure cultures were irregularly shaped rods, occurring in pairs or chains, measuring 0.2–0.3 μm in width and 1–1.5 μm in length (Fig. 2A, D). On TSA blood agar, colonies displayed α-hemolytic activity, opaque, circular, and slightly convex, with diameters ranging from 0.5 to 1.0 mm (Fig. 2B). Using the API 20 STREP kit, the isolates showed complete similarity to strains responsible for disease in Japanese rainbow trout (Mitomi et al., 2018) in all 20 biochemical tests (Table 2). Additionally, they matched the reference strains NC36 and 1119–1A-09 in 19 of 20 tests, which were reported by Welch and Good (2013).

3.3. Molecular identification, phylogenetic and phylogenomic analysis

Sequence analysis revealed that all four representative isolates had identical partial 16S rRNA gene sequences, producing a 1497 bp product. The nucleotide sequences obtained in this study were deposited in the GenBank database under the accession numbers PV203440-PV203443. BLAST analysis of the 16S rRNA sequence showed a 99.45 % similarity to the *W. ceti* 1119–1A-09T strain from a beaked whale and a 99.86 % similarity to a *W. ceti* strain from Japanese rainbow trout, with 8 and 2 SNP mismatches detected, respectively. Additionally, the sequences exhibited a 100 % match with the 16S rRNA of *Weissella tructae*. In contrast, their percentage identity to other *Weissella* species ranged from 92 % to 96 %. Phylogenetic analysis of the 16S rRNA sequences further demonstrated that the four isolates in this study clustered together with *W. ceti* isolates from infected rainbow trout in Japan and with the *W. tructae* strain from rainbow trout in Brazil, as recently proposed by Pereira et al. (2022). Conversely, the four isolates were distinct from other *Weissella* species within the same genus (Fig. 3). Therefore, the biochemical characteristics and 16S gene sequencing analysis indicated that the causative agent was *W. tructae*. The duplex PCR test confirmed that all 25 isolates produced both a 725-bp amplification product specific to the 16S rRNA gene of the genus and a 500-bp amplification product targeting a unique virulence factor locus of *W. tructae* (Figure S1). Overall, the findings confirmed that the mortality of rainbow trout from 25 rainbow trout farms located in Laocai province, Vietnam, was caused by *W. tructae*.

3.4. Experimental infection

The cumulative fish mortality (%) over 14 days following exposure to different bacterial concentrations (CFU/fish) presented a clear dose-dependent effect. The highest doses (1×10^7 and 1×10^6 CFU/fish) resulted in 90–100 % mortality (Fig. 4). In contrast, lower doses significantly reduced mortality ($p < 0.05$), with the lowest dose (10^2 CFU/fish) causing only 13.3 % mortality, while no mortality was

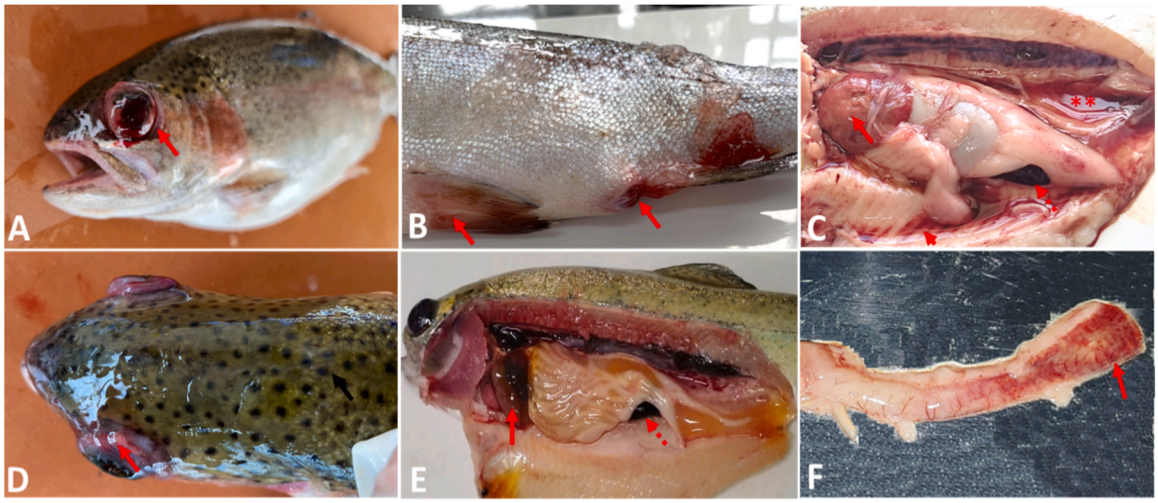

Fig. 1. Clinical and gross signs of naturally infected (A, C) and experimentally infected (D-F) rainbow trout with *W. tructae*. Panophthalmitis and hemorrhages around the eyes (A, D; arrow); hemorrhages on the fins and anal region (B; arrow); the presence of ascitic fluid (C; **); hemorrhage in the abdominal wall (C; short arrow); splenomegaly and congestion (C, E; dashed arrows); petechiae in the liver (C, E, long arrows), and hemorrhage gut (F, arrow).

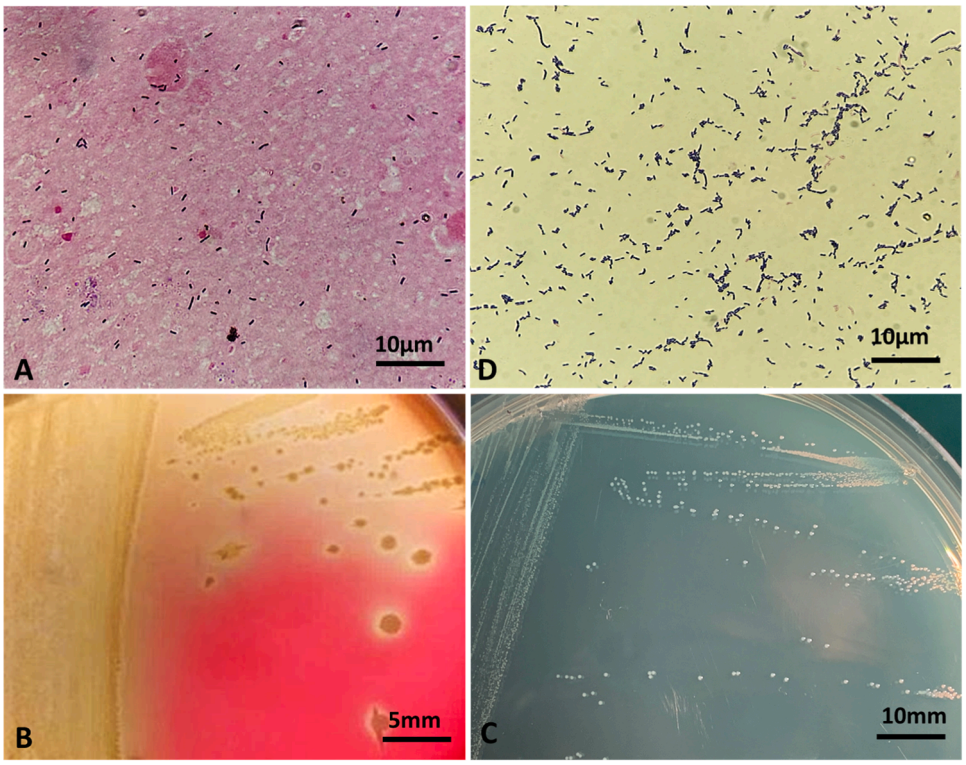

Fig. 2. Bacteria spread in the brain of diseased fish (A); pure culture of bacteria on TSA blood agar (B) and on TSA (C); and morphology of the bacteria (D).

observed in the control group. At higher doses, mortality increased rapidly within the first 5–7 days post-challenge, whereas lower doses led to a slower progression. The LD50 was at 1.2×10^4 CFU/fish. Affected fish in the challenge experiment exhibited clinical signs similar to those observed in naturally infected fish, including unilateral or bilateral exophthalmia, eye hemorrhages, splenomegaly, and congestion of the liver and intestine (Fig. 1D-F). Bacterial isolates recovered from the internal organs of infected fish displayed morphology identical to the challenge isolate and tested positive for *W. tructae* using a genus- and species-specific PCR assay (data not shown). No bacteria were recovered from clinically healthy fish in the control group.

3.5. Histopathological characterization

Histopathological analysis showed severe tissue damage in both naturally infected fish and those experimentally challenged with *W. tructae*. The kidneys appeared cell degradation, vacuolar degeneration of tubules, necrosis of renal glomeruli, structural loss, congestion, and hemorrhage (Fig. 5A). In the liver, blood-filled dilated sinusoids, central vein congestion, hepatic lipidosis, and aggregations of inflammatory cells were observed (Fig. 5B). The spleen exhibited congestion near the arterioles, splenitis, accumulation of proteinaceous substances around the arterioles, hemosiderosis, and aggregation of inflammatory cells (Fig. 5C). The main signs in the brain included congestion and the presence of rod-shaped bacterial cells (Fig. 5D). In the intestine, there was infiltration of blood and debris between the villi, villi clumping, hemorrhage, and the destruction of the villi (Fig. 5E, F).

3.6. Antimicrobial susceptibility

The MIC values of the quality control strain, *S. pneumoniae ATCC 49619,* all fell within the acceptable range suggested by the CLSI guidelines (CLSI, 2015). The susceptibility testing of 25 isolates against various antibiotics showed that three antibiotics, including Amoxicillin, Amoxicillin-Clavulanic Acid, and Erythromycin, exhibited the highest susceptibility, with MIC values ranging from 0.06 to 1 mg/L (Table 3). In contrast, Sulfamethoxazole/Trimethoprim showed high resistance, as all isolates had MICs $\geq$ 512 mg/L, while a relatively high MIC range was also observed for Florfenicol, extending from 2 to 32 mg/L.

Within the tetracycline class, Doxycycline and Tetracycline displayed identical MIC ranges of 1–4 mg/L. However, Oxytetracycline exhibited a broader MIC distribution, ranging from 0.5 to 4 mg/L, with most isolates (18/25) having MICs $\leq$ 1 mg/L.

4. Discussion

Since its introduction to Vietnam in 2004, rainbow trout (*Oncorhynchus mykiss*) has seen a notable increase in production, primarily within the Northern and Central Highlands. This species is now successfully farmed across 25 mountainous provinces where environmental conditions are conducive to its growth (Flerova, 2018; Trung et al., 2022; Chi et al., 2023). Among these regions, Lao Cai province has emerged as the leading producer, benefiting from abundant cold water resources and favourable natural conditions. As a translocated species, rainbow trout embryos have been imported from various international sources, including Finland and the United States (Pavlov et al., 2010). Typically, rainbow trout farms are situated along large streams that utilize a flow-through farming system, which relies on continuous water flow for raising the fish. However, this approach increases the risk of disease transmission, particularly if pathogens are present upstream, as the outlet of the water system often draws from the same stream without any treatment to mitigate potential contaminants.

Weissellosis outbreaks with high mortality rates in rainbow trout have been reported in various regions across the globe, including China, Japan, Peru, Brazil, Mexico, and the United States (Costa et al., 2015; Mitomi et al., 2018; Castrejón-Nájera et al., 2018; Medina et al., 2020), highlighting the urgent need for comprehensive research to develop effective protective measures. This study confirms the presence of Weissellosis in farmed rainbow trout in Vietnam, marking the first recorded occurrence of the disease in Southeast Asia and significantly

Table 2
Biochemical description of *Weissella tructae* isolates (n = 25) isolated from farmed rainbow trout outbreaks in Vietnam.

TEST	This study	Reference strains		
	25 isolates Rainbow trout	NC36[a]	Rainbow trout strains from Japan[b]	1119–1A-09[c]
Oxidase	-	-	-	-
Catalase	-	-	-	-
Pyruvate (Acetoin production)	+	+	+	+
Hippurate (Hydrolysis)	+	+	+	+
Esculin (b-glucosidase)	-	-	-	+
pyrrolidonyl−2-naphthylamide (Pyrrolidonylarylamidase)	-	-	-	-
6-Br−2-naphthvl-a-D-galactopyranoside (a-galactosidase)	-	-	-	-
naphthol AS-BI b-D-glucuronate (b-glucuronidase)	-	-	-	-
2-naphthyl-b-D-galactopyranoside (b-galactosidase)	-	-	-	-
2-naphthylphosphate (Alkaline phosphatase)	+	+	+	+
L-leucyl−2-naphthylamide (Leucinearylamidase)	+	+	+	+
Arginine (Arginine dihydrolase)	-	+	-	-
Ribose (acidification)	+	+	+	+
L-arabinose (acidification)	-	-	-	-
Mannitol (acidification)	-	-	-	-
Sorbitol (acidification)	-	-	-	-
Lactose (acidification)	-	-	-	-
Trehalose (acidification)	+	+	+	+
Inulin (acidification)	-	-	-	-
Raffinose (acidification)	-	-	-	-
Starch (acidification)	-	-	-	-
Glycogen (acidification)	-	-	-	-

(a) The US rainbow trout strain and the biochemical description adapted from Welch and Good, (2013)
(b) Japan rainbow trout strain and the biochemical description adapted from Mitomi et al. (2018)
(c) Strain isolated from the beaked whale in Spain (Vela et al., 2011)

expanding its known geographic distribution. Our findings also demonstrate that Weissellosis can impact rainbow trout at various growth stages, from fingerlings to marketable size, resulting in substantial economic losses within the aquaculture sector. Given the unidentifiable source of the disease outbreak, it is imperative to enforce strict quarantining protocols for imported fish embryos and juvenile fish before their distribution to farms, as well as early detection of any potential risk of *W. tructae* is crucial for the implementation of timely preventive measures, which are essential to minimizing the losses resulting from the outbreak.

In this study, distinct and well-defined bacterial colony morphology was observed when cultured on TSA blood agar, which also allowed for effective determination of hemolysis type, as recommended by Welch (2014). Additionally, the bacteria exhibited robust growth on TSA agar, producing small, flat, translucent-white colonies with regular edges. Therefore, TSA agar can be reliably employed for the cultivation of this bacterial species. Regarding bacterial identification, our findings revealed that the *W. tructae* isolates infecting rainbow trout exhibited similarities in most biochemical characteristics, as determined by the API Strep test, and over 99 % identity to the nucleotide sequence of the 16S rRNA gene from other pathogenic strains affecting both rainbow trout and beaked whales. This suggests that it is challenging to distinguish between strains of rainbow trout and those of beaked whales based on common morphology, biochemical features, 16S rRNA sequencing, and previously developed duplex PCR methods (see Figure S1; Snyder et al., 2015). To achieve differentiation among strains, a comparative polyphasic study conducted by Pereira et al. (2022) investigated the phenotypic and phylogenomic characteristics of *Weissella* strains isolated from both beaked whales and rainbow trout. They designated the name *W. tructae* for the pathogenic strains infecting rainbow trout. However, this method is currently time-consuming and not well-suited for rapid detection. Given the emergence of this new bacterial species, there is an urgent need for the development of a novel, rapid and specific molecular method for *W. tructae* determination. Which could seek the exclusively target-specific genes for PCR assay that enable the differentiation of *W. tructae* from rainbow trout to other *Weissella* species associated with various sources of infection, including beaked whales.

The present findings confirm that *W. tructae* exhibits relatively high virulence in rainbow trout, with an intraperitoneal dose of 10^6 CFU/fish resulting in over 90 % mortality in fish weighing 35–40 g under culture conditions of 18°C. Using a similar temperature and bacterial dose (1×10^6 CFU/fish), Castrejón-Nájera et al. (2018) reported a mortality rate of 87.5 % in rainbow trout weighing 12–14 g. However, Medina et al. (2020) observed a 90 % mortality rate in 9 cm-long fish when applying a lower infection dose of 10^4 CFU/fish at 16°C after 21 days of challenge experiment. Variations in fish size, duration of challenge and environmental conditions, particularly water temperature, could contribute to differences in the pathogenicity of this pathogen among the studies. Previous research has demonstrated that temperature plays a crucial role in bacterial pathogenicity and host susceptibility, influencing bacterial proliferation, immune responses, and overall disease progression (Guijarro et al., 2015; Okon et al., 2023). This is further supported by the observation in this study that disease outbreaks occurred in farms with water temperatures exceeding 17°C, consistent with those reported in previous studies (Mitomi et al., 2018; Castrejón-Nájera et al., 2018). Additionally, studies on other bacterial pathogens have indicated that variations in bacterial genotype and phenotype can significantly affect infection outcomes (Kayansamruaj et al., 2014; Kannika et al., 2017; Lian et al., 2025), highlighting the importance of characterizing the genetic and phenotypic diversity of *W. tructae* isolates. Given these findings, further research is necessary to identify the specific virulence factors associated with *W. tructae* and to assess their role in disease severity. Molecular studies investigating the expression of virulence-related genes under different environmental conditions could provide valuable insights into the pathogen's adaptability and infection mechanisms. Furthermore, controlled experimental studies evaluating host immune responses at varying temperatures may help elucidate the interaction between environmental stressors and disease susceptibility in rainbow trout.

Antibiotics are commonly used to treat bacterial diseases, particularly emerging diseases for which vaccines have not yet been developed (Alday-Sanz et al., 2012; Bondad-Reantaso et al., 2023). The main issue is that antibiotic resistance will increase rapidly when farmers misuse or overuse antibiotics (Watts et al., 2017; Hossain et al., 2022), resulting in reduced treatment effectiveness. However, to date, there are no established guidelines or standards for classifying antibiotic resistance levels in *W. tructae*. Also, previous studies on Weissellosis outbreaks have not assessed the sensitivity of the pathogen to antibiotics. This study represents the first effort to determine the minimum inhibitory concentration (MIC) ranges of *W. tructae* for common antibiotics utilized in aquaculture in Vietnam, providing a critical foundation for establishing initial treatment protocols for managing this disease. The results indicate that antibiotics with low MIC values, which are suitable for treating the disease, include amoxicillin, erythromycin, and oxytetracycline (OTC). In contrast, the MIC value for sulfamethoxazole/trimethoprim is extremely high, exceeding 512 mg/L, rendering it ineffective for disease treatment. Previously, Lee et al. (2011) reported that *W. confusa*

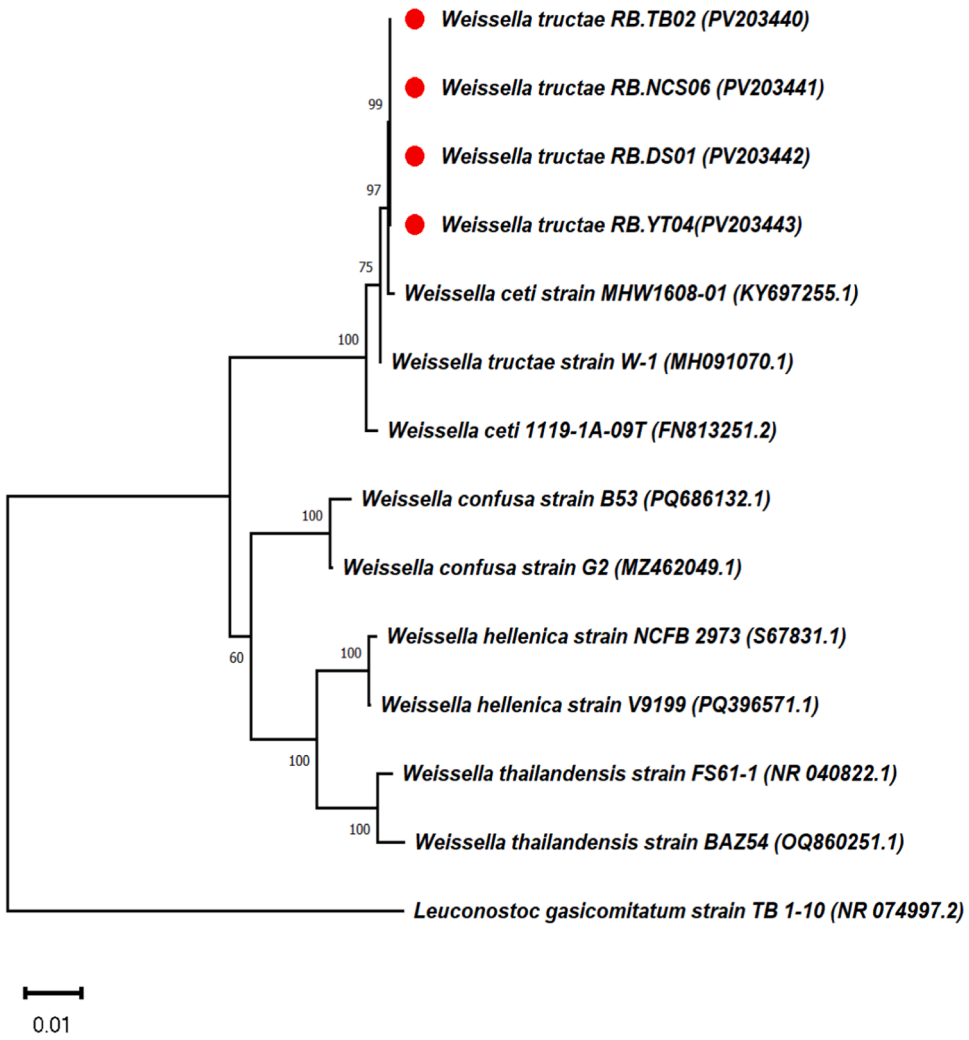

Fig. 3. The phylogenetic tree was constructed based on 16S rRNA gene sequence comparisons using the neighbor-joining method. The tree illustrates the relationships between the four isolates in this study and other *Weissella* species. *Leuconostoc gasicomitatum* TB1–10 was used as an outgroup. Bootstrap values are expressed as percentages from 1000 replications.

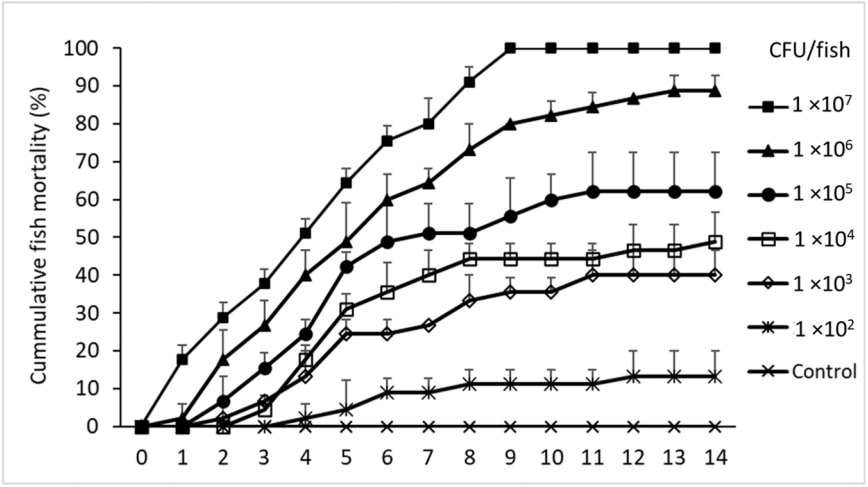

Fig. 4. Daily mean cumulative mortality of fish experimentally infected with *W. tructae* and the control group.

exhibited MIC values above 128 mg/L for sulfamethoxazole/-trimethoprim. Additionally, Muñoz-Atienza et al. (2013) noted the reduced susceptibility of *W. cibaria* to trimethoprim (>128 mg/L). These observations suggest an intrinsic resistance of *Weissella* species to this antibiotic. Further studies involving strains collected over an extended period are necessary to assess the evolution of antibiotic resistance in

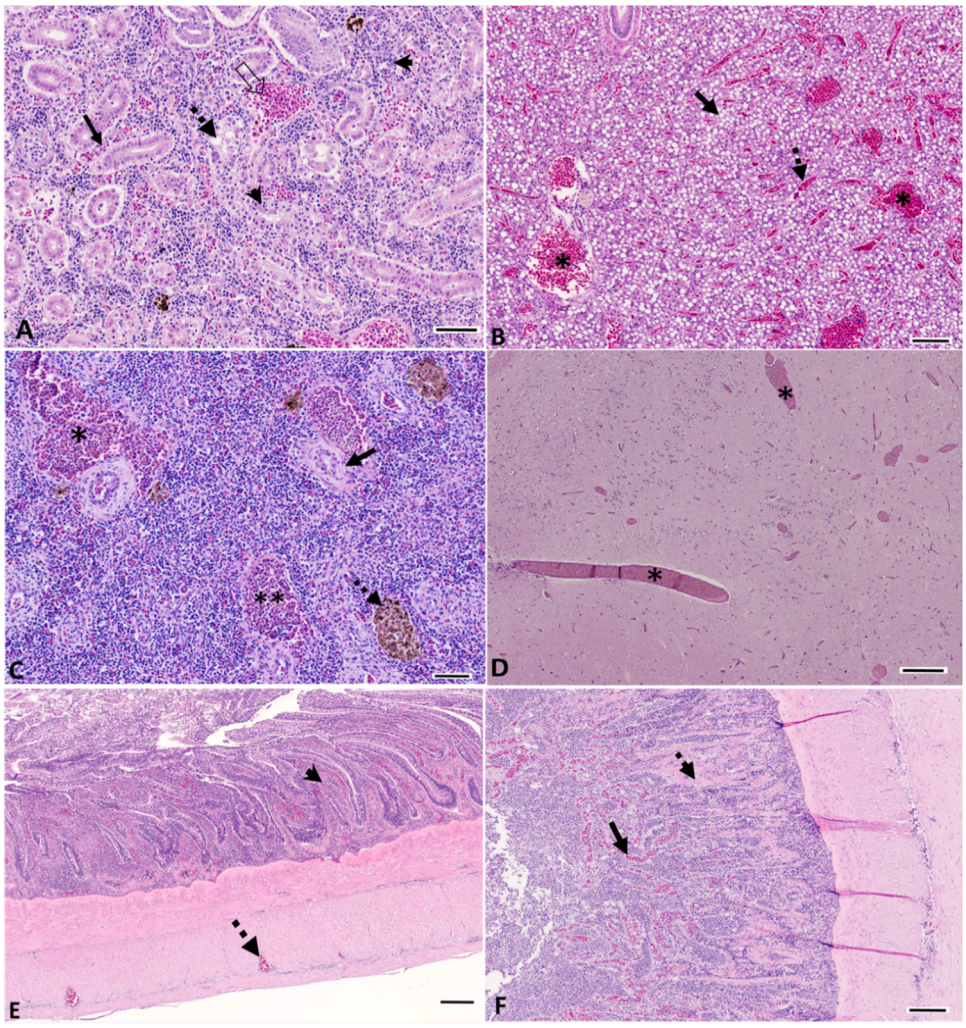

Fig. 5. Histopathological characteristics of rainbow trout affected by *W. tructae* infection. A-Kidney with cell degradation (dashed arrow), degeneration and loss structure of tubules and glomeruli (arrowheads); congestion (blanked arrow), haemorrhage (solid arrow); B-liver with blood-filled dilated sinusoids (dashed arrow), central vein congestion (*); hepatic lipidosis (solid arrow); C-spleen with congestion near arterioles (*); splenitis (**), accumulation of proteinaceous substance around arterioles (solid arrows); hemosiderosis (dashed arrow); D-brain showed congestion (*); E, F: intestine with infiltration of blood (F; solid arrows) and debris (F; dashed arrows) between the villi, villi clumping (arrowhead) and haemorrhage (dashed arrow); scale bar 10 μm.

Table 3
The distribution of MIC values of *W. tructae* isolates to eight antimicrobial agents.

Antibiotics	Ref.	Number of isolates with MIC (mg/L) (n = 25)														
		0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	> 512
Amoxicillin	-		2	5	11	6	1									
Amox-Clavulanic	-	1	5	9	8	2										
Erythromycin	0.5–1[a]		5	10	7	3										
Doxycycline	-					4	15	6								
Tetracycline	2–8[a]					5	9	11								
Oxytetracycline	-				5	13	5	2								
Florfenicol	-						3	2	14	3	3					
Sulfamethoxazole/ Trimethoprim	128–256[b]														6	19

[a] MIC values of *W.cibaria, W.confusa,* and *W.paramesenteroides* from kimchi (Jeong and Lee, 2015); [b]MIC values of *W. confusa* from hospital (Vay et al., 2007)

W. tructae. Moreover, the emergence of antibiotic-resistant genes should be investigated as resistance levels increase. More importantly, the development of preventive vaccine therapies is essential to reduce antibiotic usage, thereby mitigating the rise of antibiotic resistance in disease management, to sustainable rainbow trout development.

Ethics approval statement

The authors confirm the ethical policies of the journal, as noted on the journal's author guidelines page. Ethical approval for the challenge experiments was obtained from the Faculty of Fisheries, Vietnam National University of Agriculture Animal Care and Use Committee FFVNUA-ACUC, approval number 090123–9-KHCN-FFVNUA.

CRediT authorship contribution statement

Doan Thi Nhinh: Writing – review & editing, Writing – original draft, Validation, Methodology, Funding acquisition, Formal analysis, Data curation. **Dang Thi Hoa:** Visualization, Validation, Methodology, Formal analysis, Data curation. **Tran Thi Trinh:** Visualization, Validation, Formal analysis, Data curation. **Mai Van Tung:** Validation, Software, Formal analysis, Data curation. **Nguyen Thi Huong Giang:** Visualization, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Kim Van Van:** Writing – review & editing, Supervision, Resources. **Truong Dinh Hoai:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Data curation, Conceptualization.

Declaration of Competing Interest

None.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aqrep.2025.102955.

Data availability

The data that has been used is confidential.

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