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Kocuria rhizophila As the Etiological Agent Infecting Rainbow Trout (*Oncorhynchus mykiss Walbaum, 1792*) in Iran

Nazila Yeganeh[a], Amir Hossein Smiley[a], Mohammad Reza Kalbassi[a], Margaret Crumlish[b]

[a] Department of Fisheries Engineering and sciences, Faculty of Natural Resources and Marine Sciences, University of Tarbiat Modares, Tehran, Iran.
[b] Institute of Aquaculture, Faculty of Natural Sciences, University of Stirling, Stirling, UK.

ABSTRACT

An natural acute disease outbreak occurred in two rainbow trout farms located in northern Iran. Affected fish exhibited clinical symptoms, including ocular opacity, scale desquamation, skin necrosis, superficial to deep lesions on the dorsal and lateral body surfaces, loss of caudal peduncle tissue, mild exophthalmia, skin melanization, hepatic hemorrhage and paleness, excessive mucus accumulation in the abdominal cavity, renal paleness, and intestinal infection. Following biochemical analysis of the bacterium, gene sequencing was conducted, leading to the identification and registration of a strain known as *K. rhizophila* TMU97910 in the NCBI gene bank. After the elucidation of the bacterium's identity, salinity tolerance, and antibiogram tests were conducted. The results demonstrated that the strain exhibited complete resistance to salinity, allowing it to thrive in a wide range of salinity conditions. In antimicrobial assays, the isolate showed resistance to Fosfomycin, while remaining sensitive to Florfenicol, Doxycycline, Oxytetracycline, Enrofloxacin, Sulfamethoxazole/Trimethoprim, Erythromycin, Gentamycin, and Kanamycin. To investigate the behavior of this bacterium in rainbow trout, pathogenicity, LD_{50}, and histopathology analyses were conducted in three replicates. Pathogenic symptoms in trout remained consistent. Histopathological examination of the liver, kidney, spleen, and gills revealed extensive tissue damage caused by this strain, including necrosis, hemorrhage, degeneration, and tissue wounds. Subsequently, bacterial recovery was achieved, and negative results for the detection of other pathogens confirmed that Kocuria rhizophila was responsible for the disease outbreak.

Keywords

Kocuria rhizophila, Rainbow trout, Pathogenicity, histopathology, antibiogram test, Susceptibility

Abbreviations

No abbreviations

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Corresponding author:
Amir Hossein Smiley
Email: amirh.smiley@modares.ac.ir
Tel:+98(91)1678-5090

Introduction

One of the prominent species cultured in the aquaculture industry is Rainbow trout (*Oncorhynchus mykiss*). Despite the increasing investment in its cultivation to produce and supply aquatic food products, rainbow trout is susceptible to a variety of bacterial infections, leading to significant production losses due to morbidity and mortality. Prompt identification and reporting of emerging infectious diseases are crucial to ensure optimal biosecurity practices that support the development of health and welfare in farmed fish, as well as the promotion of global product placement.

Understanding the inhibiting factors that impact the progression of aquaculture is crucial. Therefore, this essay delves into the outbreak of a disease in rainbow trout farms situated in northern Iran, which was documented in November 2018. Affected fish exhibited symptoms such as hemorrhagic septicemia, extensive wounds on the dorsal and lateral areas, and lesions on the caudal peduncle, r in some cases leading to the complete exposure of the vertebral column. Additionally, some parts of the skin were detached from the underlying tissue. Reports from the affected sites also indicated occurrences of exophthalmia, skin melanization, ocular opacity, and elevated mortality rates.

Preliminary diagnosis based on both clinical observations and biochemical analyses suggested a bacterial etiology, indicating the emerging pathogen Kocuria spp. Members of the Kocuria belong to the family Micrococcaceae, within the order Actinomycetes [1]. Based on the phylogenetic reclassification of the genus Micrococcus using 16S rDNA sequencing and chemotaxonomic data, the genus was found to be heterogeneous, with its species distributed across five distinct clusters. Consequently, only *M. luteus* and *M. lylae* were retained within the emended genus Micrococcus, while three species *M. roseus*, *M. varians,* and *M kristina* were transferred to the novel genus Kocuria [2]. These Gram-positive coccoid bacteria whose main habitat is soil were first isolated and reported from the rhizoplane of Typha angustifolia [3].

Overall, The aforementioned information serves as the basis for asserting that a recent discovery has identified a specific species within the Kocuria genus, Kocuria rhizophila, as an "emerging pathogen" responsible for causing illness in brown trout and farmed rainbow trout in Poland [4]. It is clear that there is limited available data regarding the impact of this microorganism on natural disease outbreaks in aquaculture settings. Therefore, the present study

aims to investigate the prevalence, virulence factors, histopathological features, and control measures of an emerging disease caused by *Kocuria rhizophila* in rainbow trout farms in Iran, through the isolation and identification of the bacterium from regional aquaculture facilities.

Results

Fish Sampling During a Natural Disease Outbreak

Moribund rainbow trout exhibited a range of external clinical signs consistent with systemic bacterial infection. These included hemorrhagic septicemia, deep ulcerative lesions on the dorsal and lateral body surfaces, and lesions localized to the caudal peduncle. Additional clinical manifestations included exophthalmia, skin melanization, and ocular opacity. Internally, the fish displayed renal opacity, liver congestion, and intestinal inflammation.

Bacteria Isolation, Identification, and Morphological Characteristics

Regardless of the agar type used, colony growth was completed at 22°C after approximately 48-72 hours and at 37°C within 24 hours. The bacterial colonies were characterized as non-mucoid, yellow pigmented, convex circular, and umbonate with entire margin. No hemolytic activity was observed on blood agar. The cultures were identified as Gram-positive cocci, exhibiting positive oxidase and catalase reactions. The isolates were non-motile, and exhibited a non-fermentative metabolic profile, as indicated by the TSI test, which showed no gas or H2S production. Additionally, the bacterium did not metabolize tryptophan, mannitol, or citrate, and no glucose fermentation was detected.

Bacterial DNA extraction and identification using PCR and 16SrRNA sequencing

A distinct 1500 bp amplicon was observed in the eubacterial PCR assay. However, 16S rRNA sequencing yielded a partial gene sequence of 1394 bp, which was subsequently submitted to the GenBank database. Sequence analysis of the 16S ribosomal RNA confirmed the isolate as Kocuria rhizophila strain TMU97910. The strain name was officially registered in the National Center for Biotechnology Information (NCBI) database. The nucleotide sequence has been assigned the accession number MW947584, and the complete genome sequence, and interpretation of *K. rhizophila* TMU97910 can be accessed via the following NCBI link: https://www.ncbi.nlm.nih.gov/nuccore/MW947584. The phylogenetic tree is presented in Fig 1.

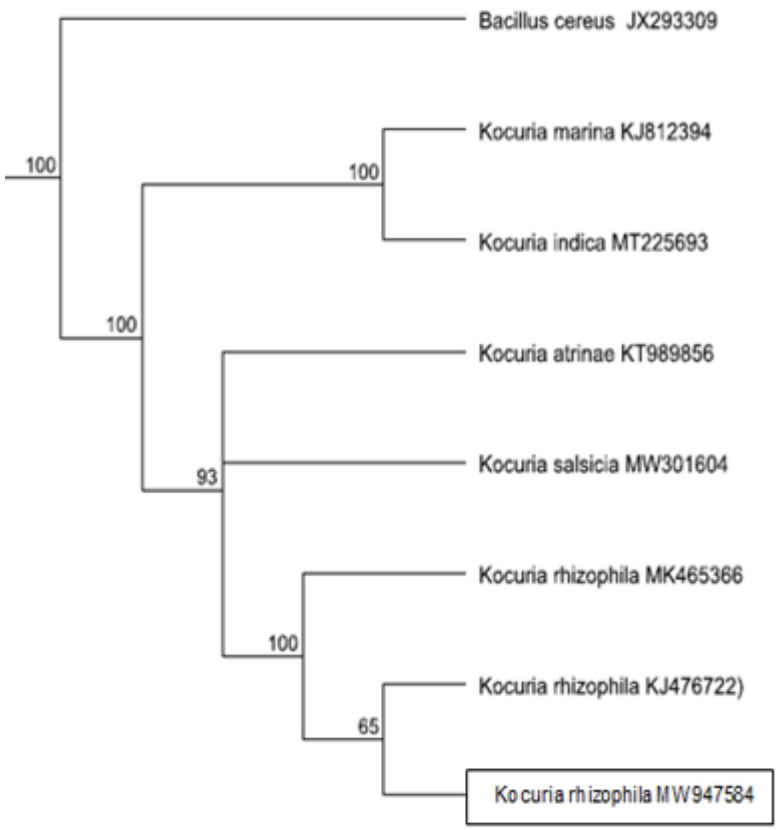


Figure 1.
The Kocuria rhizophila phylogenetic tree based on 16SrRNA sequence

Salinity tolerance test

The strain *K. rhizophila* TMU97910 exhibited robust growth across all NaCl concentrations, as indicated by viable colony counts. Conversely, no growth was detected in the negative control.

Antibiogram Testing

The antibiotic susceptibility profile of *K. rhizophila* strain TMU97910 is presented in Table 1. The strain demonstrated sensitivity to several antibiotics, including Florfenicol, Enrofloxacin, Oxytetracycline, Doxycycline, Sulfamethoxazole/Trimethoprim, Erythromycin, Gentamycin, and Kanamycin. However, the strain exhibited resistance to Fosfomycin.

Table 1.
Antibiogram test of Kocuria rhizophila TMU97910

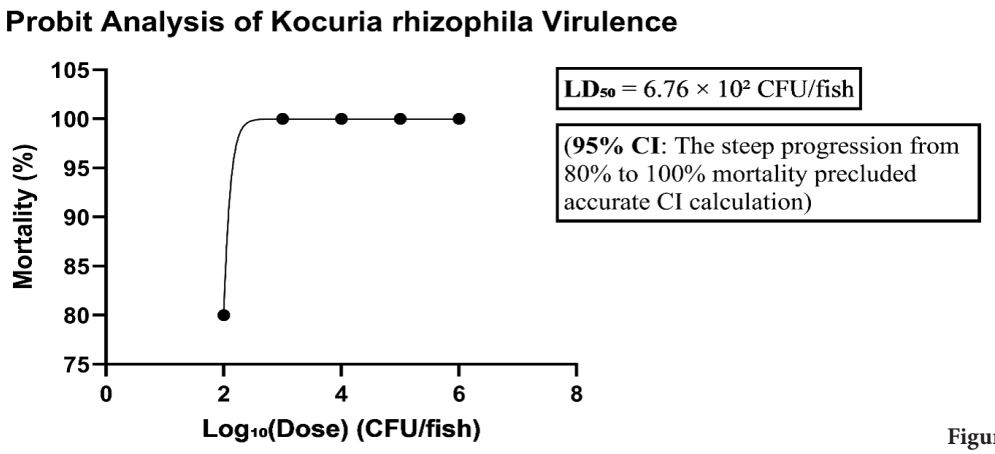
Kocuria rhizophila	Disc content	Antimicrobial agent
40 mm- S	30 µg	Florfenicol
37 mm- S	1.25/23.75 µg	Trimethoprime/sulfamethoxazole
40 mm- S	10 µg	Gentamicine
21 mm- S	30 µg	Kanamycine
35 mm-S	30 µg	Oxytetracycline
40 mm-S	30 µg	Doxycycline
0 mm-R	20 µg	Fosfomycin
35 mm-S	5 µg	Enrofloxacin

Pathogenicity and LC_{50}

The mortality of rainbow trout began in all fish groups exposed to the bacterium within 48 hours post-injection. The mortality patterns were consistent across all concentrations during the initial four days. At the lowest concentration (102 CFU/fish), mortality reached 60%. Subsequently, after seven days of stability, mortality increased rapidly to 80% by day 11. In the 10^3 CFU/fish group, mortality also reached 60%, remained stable for six days, and then increased sharply to 100% by day 11 post-infection. Higher concentrations (10^4, 10^5, and 10^6 CFU/fish), resulted in rapid mortality within the first three days, reaching 60%, 80%, and 40% respectively. After one day of stability from day 4, there was a rapid increase in mortality exceeding 100%. The results of the probit analysis and the survival plot are presented in Figures 2A and 2B, respectivel.

The experimental challenge study revealed typical clinical signs observed in moribund rainbow trout exposed to the bacterium. Affected fish exhibited skin damage, necrotic areas leading to tissue damage of the caudal peduncle, focal ulcers on the dorsal and lateral body surfaces, ocular opacity, and pale liver and kidney.

A



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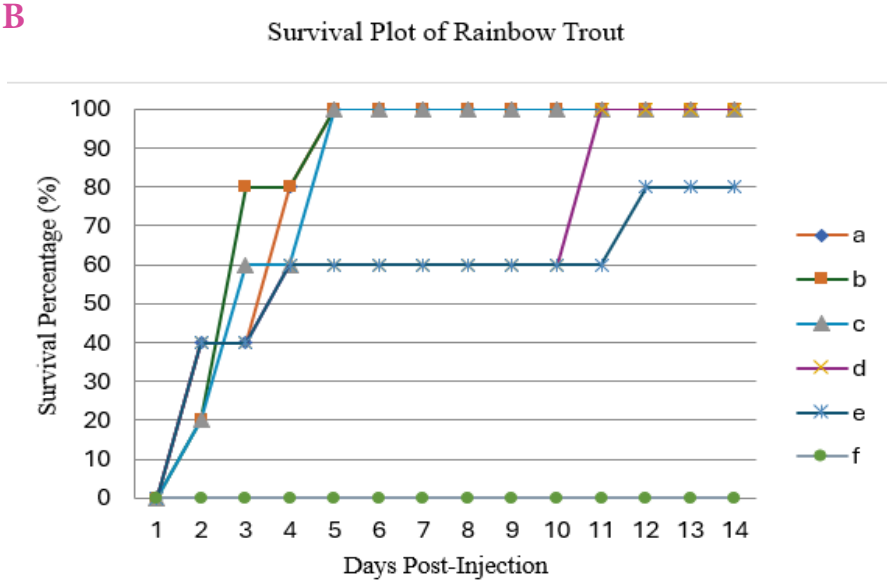

Figure 2.
2A. Probit analysis for determination of LD_{50} in rainbow trout challenged with Kocuria rhizophila TMU97910.
2B. Kaplan-Meier survival plot of rainbow trout challenged with Kocuria rhizophila. Statistical significance was determined using Log-rank test ($p < 0.0001$).

Histopathology

Histopathological results are presented in Fig 3. Re-isolation of the bacterium from the liver, kidney, spleen, and gills, followed by biochemical tests, in accordance with Koch's hypothesis, verified the presence of *K. rhizophila* in these organs. The histopathological changes in the rainbow trout (B, E, H, K) injected with *K. rhizophila* were examined. The histological alterations in the kidney demonstrated tubular degeneration, severe lesions, and necrosis (B). The liver illustrated cellular necrosis, hypertrophy, congestion, hemorrhage, sinusoidal dilation, and the presence of pyknotic nuclei. The gills showed necrosis, edema, hypertrophy of mucous cells, curvature, clubbing of the tips, shortening of secondary lamellae, and epithelial individuation (H). The spleen tissue of the rainbow trout illustrated hemosiderin, necrosis, white pulp, and red pulp (K).

Discussion

To address the emerging threat of *Kocuria rhizophila* in rainbow trout aquaculture, this investigation focused on determining the occurrence, virulence attributes, histopathological damage, and practical control strategies, drawing upon bacterial strains successfully isolated from affected farms across Iran. The present study successfully fulfilled Koch's postulates, confirming that the strain identified as Kocuria rhizophila TMU97910 is capable of inducing disease under experimental conditions, similar to the natural disease observed in rainbow trout. Although this bacterium was not considered a pathogen to farmed fish species before the first report [4], the results from the current study, similar to Pekala's report, support the emergence of *K. rhizophila* as an emerging fish pathogen. In the current study, the disease emerged in an acute form in the investigated farms. The naturally infected fish presented a broad range of clinical signs, including exophthalmia, melanization, skin lesions,

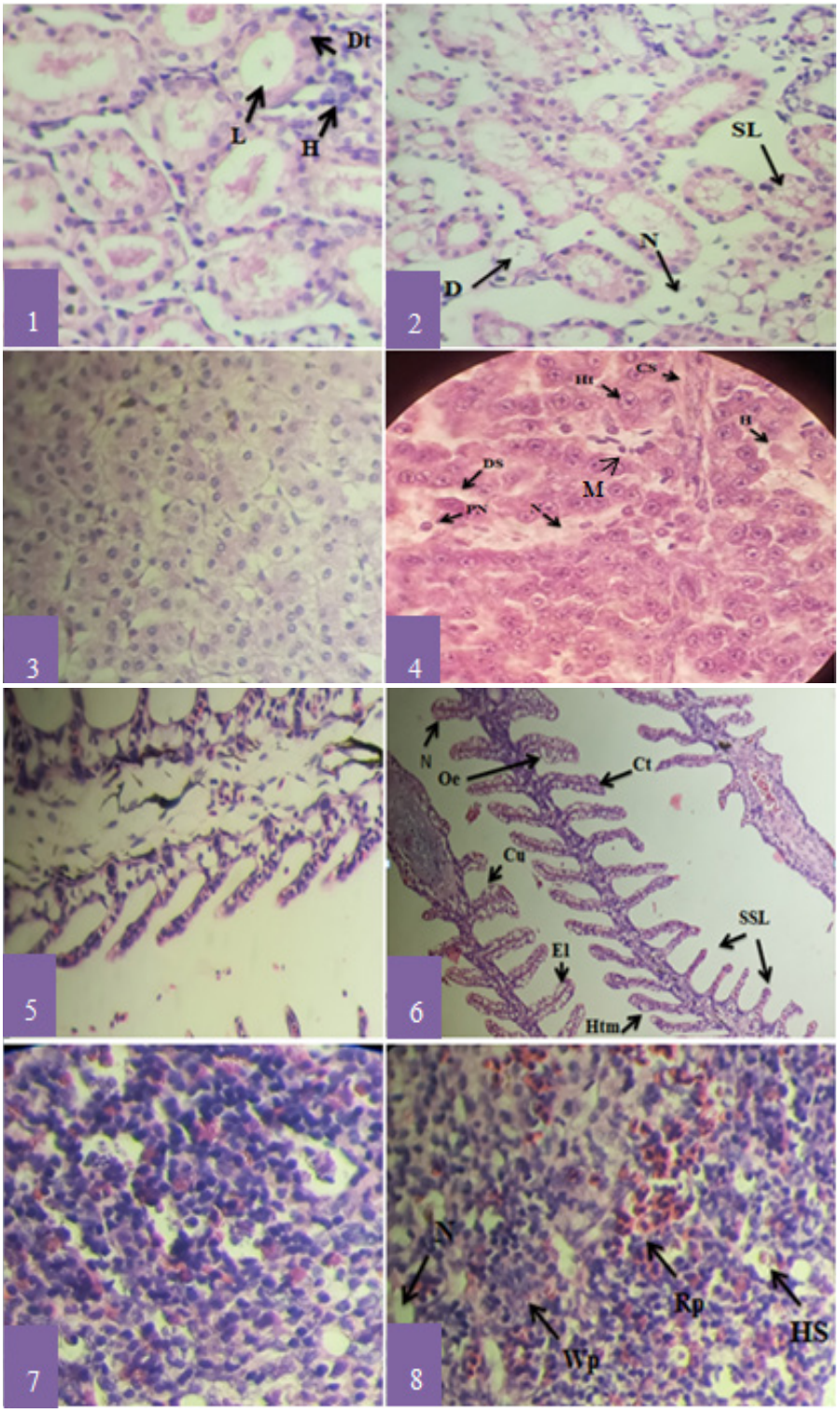

Figure 3.
Histopathological changes in rainbow trout due to Kocuria rhizophila infection were observed in various tissues (2, 4, 6, 8). The histological examination of the kidney from the infected group revealed tubular degeneration, severe lesions, and necrosis (2), whereas the liver exhibited melanomacrophages, necrosis, hypertrophy, congestion, hemorrhage, sinusoidal dilatation, and pyknotic nuclei (4). Similarly, the gills showed necrosis, edema, hypertrophy of the mucous glands, curvature, clubbing of the tips, shortening of secondary lamellae, and epithelial individuation (6). The spleen tissue of the infected rainbow trout displayed hemosiderin, necrosis, white pulp, and red pulp (8). Abbreviations: (M) Melanoma macrophages; (H) hematopoietic tissue; (L) tube lumen; (Dt) distal tubules; (D) degeneration of tubules; (SL) Severe lesions; (N) necrosis; (Ht) Hypertrophy; (Cs) Congestion; (H) Hemorrhage; (Ds) Dilatation Sinusoidal; (PN) Pyknotic Nuclei; (HS) Hemosiderin; (Oe) Oedema; (Htm) Hypertrophy of the mucous glands; (Cu) Curvature; (Ct) Club tip; (SSL) shortening of secondary lamellae; (El) Epithelial individuation; (Wp) White pulp; (Rp) Red pulp; Fibroblast. Stained with hematoxylin and eosin (x400)

necrotic tissue erosion, ocular opacity, superficial to deep wounds on the body tissue, pale liver and gills, along with intestinal inflammation. Notably, a similar spectrum of clinical symptoms was observed in rainbow trout infected by *K. rhizophila* naturally and artificially (experimental challenge).

Prior to 2016, data in the NCBI Gene Bank database did not document the isolation of *K. rhizophila* from diseased fish. The first report of its pathogenicity in aquaculture industry was recorded in 2018 in Poland [4].

In the current study, *K. rhizophila* strain TMU97910 required an incubation period between 48 to 72 h when grown on artificial media incubated at 22 or 37°C. This was in agreement with previous studies by Savini [5] and Pekala [4] on strains of *K. rhizophila*.

Another remarkable feature of the genus Kocuria is its halotolerant nature. Several species, including K. kristinae, *K. rhizophila*, and K. marina, have been reported to tolerate sodium chloride concentrations exceeding 10% [3; 6]. In the present study, the strain *K. rhizophila* TMU97910 could grow at all salinity ranges tested under in vitro conditions, aligning with previous studies for members of this genus. This feature demonstrates that the strain has the potential to cause disease not only in brackish and freshwater fish but also in saltwater fish. In other words, the strain showed to survive in saline environments from zero to saturation effortlessly.

The antibiotic susceptibility profile of Kocuria species remains incompletely understood. Although there are case reports of antimicrobial susceptibility test results, they are insufficient to determine the exact susceptibility of Kocuria species [7]. Limited data is available on the antibiotic sensitivity of emerging fish pathogen *K. rhizophila*. For example, Pakala et al. [4] observed susceptibility of their *K. rhizophila* strains to doxycycline and amoxicillin/clavulanate, whereas resistance to norfloxacin was observed in a study where *K. rhizophila* was isolated from the blood of a child with sepsis [8]. Another study reported resistance of *K. rhizophila* isolated from a sick child's blood to ciprofloxacin and erythromycin [9]. Meletis [10] found that Kocuria isolated from a continuous ambulatory peritoneal dialysis (CAPD) patient was resistant to levofloxacin. Lee [11] highlighted the importance of Kocuria in nosocomial infections, suggesting that Kocuria species should be considered potential pathogens in immuno-compromised patients, particularly in intensive care and neonatal units. They reported that over 50% of patients suffered invasive infections with Kocuria as a secondary disease. Studies on drug resistance genes have indicated that DnaE2, a component of replicating DNA polymerases, may contribute to drug resistance in Mycobacterium tuberculosis [12]. The genome of *K. rhizophila* DC2201 also contains a dinB homolog (KRH_14930) that may encode the Y family-susceptible DNA polymerase IV, potentially leading to drug resistance [13]. In the present study, *K. rhizophila* exhibited resistance only to fosfomycin while remaining sensitive to a broad range of antibiotics, including florfenicol, doxycycline, oxytetracycline, enrofloxacin, sulfamethoxazole/trimethoprim, erythromycin, gentamicin, and kanamycin, which could aid in controlling and treating diseases caused by this strain.

It is important to note that the identification and confirmation of pathogenic agents require pathogenicity studies. In other words, in the case of potential emerging or novel diseases, pathogenicity assessment are essential to confirm whether the potential pathogen is the etiological agent responsible for the observed disease outbreak. In the presented study, the bacterium was introduced via injection, which is a common method for determining etiology but is also the most unnatural, as it bypasses the host's immune response, providing a better chance for the bacteria to spread and cause disease. Therefore, studies on the natural exposure of fish to bacteria are necessary to determine disease risk. However, the bacterial strain recovered from natural outbreaks in the farms not only caused high mortality in rainbow trout but also induced clinical signs of the disease.

As no accessible results were available to determine the LD_{50} of this bacterium in rainbow trout, we investigated the LD_{50} in *O. mykiss*. According to the linear graph, the mortality trend increased rapidly in the first three days and reached 100% by the 14 day in all groups except at a concentration of 10^2 CFU/fish. The LD_{50} at 72 hours was 5×10^9 CFU/fish. Therefore, these results serve as a warning to the aquaculture industry regarding the significance of this bacterium in causing disease in these fish.

Histopathological analysis remains the primary diagnostic tool available for fish disease, while other methods are used for laboratory confirmation of pathogen identification. In the present study, the histological changes in the kidney demonstrated tubular degeneration, severe lesions, and cellular necrosis. The liver showed accumulation of melanomacrophages, necrosis, hypertrophy, congestion, hemorrhage, sinusoidal dilatation, and pyknotic nuclei [14]. Haaparanta [15] reported that pigments within melanomacrophage centers possess bactericidal properties, which may explain the accumulation of melanomacrophages during infection. Additionally, as a result of the entry of foreign agents into the fish body, phagocytic cells accumulate at the site and continue to cleanse the dead agents and cells after their elimination [16].

In the gills, necrosis, edema, mucous glands hypertrophy, club tip, shortening of secondary lamellae, epithelial individuation, and curvature were observed. The shortening of secondary lamellae in gills may lead to reduced respiratory gas exchange, as reported in the study by Ostaszewska [17]. Edema and epithelial individuation in rainbow trout are likely associated with osmotic imbalance, as previously described by Al-Bairuty [14]. Moreover, hypertrophy is a compensatory reaction to decrease the absorption of environmental factors. Overall, these mechanisms increase the distance between the external environment and the blood, serving as an obstacle to the entrance of foreign agents. The decrease in surface area causes a reduction in respiration, as recorded in the study by Camargo [18].

In the spleen, the presence of necrosis, hemosiderin deposits, and white and red pulp were observed. The presence of hemosiderin in the rainbow trout infected by *K. rhizophila* at a concentration of 106 CFU/fish is due to b-hemolysin, which performs hemolysis in the fish's body and causes the deposition of hemosiderin, as reported in the study by Abdelhamed [19].

The areas of necrosis are the most significant lesions created in all four tissues. Histological changes might be toxin-mediated bacterial infections, evidenced by the level of hemorrhages and cellular necrosis, similar to descriptions reported by Abdelhamed [19]. Other studies have indicated that when fish are exposed to toxic substances, the gills present lesions such as necrosis, hypertrophy, and hypertrophy of the mucous glands [20; 21; 22].

In a study by Dong [23], it was stated that histopathological changes induced by gram-negative and positive bacteria are often similar, including necrosis, infiltration of inflammatory cells, degeneration, hydrolysis, fat degeneration, and hemorrhage. According to them, kidney damage in fish was observed with almost all isolates of gram-positive bacteria. It is evident that serious damage has been done to the tissues by this strain.

Despite the documentation of Kocuria species in various countries, there has been no prior documentation of their pathogenicity in the Iranian aquaculture industry. This study revealed that *K. rhizophila* is an emerging fish pathogen capable of causing disease in rainbow trout, representing the first reported rainbow trout infection by this strain in Iran. Given the significance of this disease in the aquaculture industry, this study conducted pioneering research on the histopathology, pathogenicity, and LD_{50} of this bacterium. Additionally, as it is an emerging disease without a specific designation, the disease caused by *K. rhizophila* can be termed "Kocuriasis."

Materials and Methods

Fish sampling and bacteria isolation

In November 2018, a total of 20 moribund rainbow trout exhibiting clinical manifestations, consistent with those described by Pekala [4], were collected from two aquaculture facilities in Amol, northern Iran. The fish were transported to the laboratory of the Faculty of Marine Sciences at Tarbiat Modares University (Noor), under aeration at a temperature of 17°C for 45 minutes. Upon arrival at the laboratory, the fish underwent a gross examination to assess clinical signs of disease, and samples were collected for bacterial isolation following the methodologies outlined in the study by Pekala [4].

Prior to sampling, the fish were rinsed with physiological saline (0.9% NaCl, Merck) to eliminate any external contaminants. Subsequently, aseptically, tissue samples from the kidney, liver, and spleen were cultured on Blood Agar plates (BA; 5% sheep blood, Ibresco), Trypticase Soy Agar (TSA, Ibresco), and Nutrient Agar (NA, Liofilchem). The samples were then incubated at 22°C for 24-72 hours until visible colonies appeared. Blood culturing (from the caudal vessel) was conducted following the protocol outlined by Weinstein [24]. Briefly, 0.1 mL of broth was spread-plated onto BA, TSA, and NA media and incubated at 22°C for 24-72 hours. Daily monitoring of all samples continued for a maximum of 72 hours, with detailed descriptions of colony growth and morphology. Purification was carried out as necessary. Subsequent subculturing of a single colony on BA was performed at both 22°C and 37°C for 24-48 hours. Pure cultures were then preserved at -80°C in Tryptic Soy Broth (TSB, Ibresco) supplemented with 15% glycerol (Merck) [4].

Bacteria identification and morphological characteristics

Morphological and biochemical characterizations were conducted according to the methods outlined in Buller [25]. Phenotypic and biochemical reactions were assessed through a series of tests, including Gram staining, oxidase and catalase tests, hemolysis analysis, motility examination, Indole test, iron utilization test, sodium thiosulfate test (SIM), growth on Mannitol Salt Agar (MSA), Triple Sugar Iron (TSI) test, MacConkey agar (MAC) growth, Methyl Red/Voges-Proskauer (MRVP) test, and Citrate utilization test as described by Buller [25]. Each sample was incubated at either 22°C or 37°C for a period of 48-72 hours, with positive and negative controls set at each temperature. Subsequently, the results of these tests were recorded.

Bacterial DNA extraction and identification using PCR and 16SrRNA sequencing

Genomic DNA was extracted from the isolate utilizing the methodology outlined in the commercially accessible Sinagen kit (Iran). In summary, a single colony was cultured into 100 ml of Tryptic Soy Broth (TSB) for 24 hours at 37°C, and bacterial DNA was isolated using a digestion buffer comprising Sodium Dodecyl Sulfate (SDS), sodium Chloride-Tris-EDTA (STE) buffer, and proteinase K. The purification process involved two stages of extraction with Phenol, Chloroform, and Isoamyl alcohol (25-24-1), followed by DNA precipitation with absolute ethanol. Subsequently, the DNA was eluted in 50 µL of sterile distilled water. The sample underwent 16SrRNA PCR analysis as per the techniques delineated by Sanger [26] employing the primer set: 27F (AGAGTTTGATCCTGGCTCAG) and 1492R (TACGGYTACCTTGTTACGACTT) [26]. The PCR was carried out in a PeQlab thermocycler (Germany), with under the following conditions: initial denaturation at 95°C for 15 minutes, followed by 35 cycles comprising denaturation at 95°C for 30 seconds, annealing at 72°C for 25 seconds, and extension at 72°C for 10 minutes.

Subsequently, 5 µl of the product was visualized on a 2% agarose gel in TBE 1X buffer supplemented with Greenview fluorescent dye. The PCR product underwent purification using a Column RNA isolation kit and was then sent for sequencing to Transferred (BJI Company; China). A comparison between the sequence of the isolate and existing entries in Gen Bank was conducted, and a phylogenetic tree was constructed utilizing MEGA 7.0 software.

Salinity tolerance test

Salinity tolerance was evaluated using a standard bacterial growth assay as previously described [3]. A single colony was aseptically inoculated into 10 ml of Tryptic Soy Broth (TSB) with varying NaCl concentrations (5% - 40% w/v). The cultures were incubated in a rotary shaker (150 rpm) at 22°C for 24 hours. Bacterial growth (turbidity) was quantified using a spectrophotometer (JENWAY 6305 UV/VIS) at OD_{600} [27]. After 24 hours, 100 µl of the culture was aseptically spread on Tryptic Soy Agar (TSA) plates to confirm cell viability and then incubated at 22°C for another 24 hours.

Antibiogram test

Antibiogram tests were conducted using the Kirby-Bauer disc diffusion assay, following the procedures outlined in CLSI (2006). In summary, a bacterial suspension was prepared in sterile physiological saline and adjusted to match the 0.5 McFarland standards. Lawn cultures were established by aseptically applying swabs saturated with the bacterial suspension onto clean Muller Hinton agar (Ibresco) plates. Subsequently, the plates were allowed to air dry at room temperature for 5 minutes before antibiotic-impregnated paper discs (procured from Padtan Teb) were carefully positioned on the agar and then incubated at 22°C for 24 hours. Each agar plate accommodated a maximum of 5 discs. The zones of inhibition were measured, and the findings were categorized as sensitive (≥16 mm), intermediate (12–15 mm), or resistant (≤11 mm) based on the diameter of the inhibition zones [28]. The antibiogram tests included: Florfenicol (FF-30 µg), Enrofloxacin (NFX-5 µg), Oxytetracycline (T-30 µg), Doxycycline (D-30 µg), Sulfamethoxazole/Trimethoprim (SXT-1.25 / 23.75 µg), Erythromycin (E-15 µg), Gentamycin (GM-10 µg), Kanamycin (K-30 µg), and Fosfomycin (Fos-20 µg).

In-vivo Fish Challenge

To assess the pathogenicity, an in vivo challenge was conducted on rainbow trout. The fish (130-145 g) were obtained from a local farm in Mazandaran province, transported, and placed in 100-liter aerated tanks under a 12-hour light/dark cycle, with a water temperature of 17°C, oxygen levels at 8 mg/l, and a pH of 7.2. Throughout the 14-day acclimation period, the fish were fed twice daily with a standard commercially extruded feed, and fifty percent of the water was exchanged daily.

To enhance the bacterial virulence, individual fish (n=3) were injected with 1.2×10^8 CFU/fish. The bacterium was then isolated from the moribund fish and subsequently identified. This passaged strain was selected for the challenge and subsequent LD_{50} studies.

For LD_{50} determination, fish were randomly distributed into experimental groups (n = 5 per tank). There were six treatment groups where each fish were exposed to the bacterium through intraperitoneal injection of a 0.1 ml volume of the bacterial suspension at concentrations of $10^2/10^3/10^4/10^5/10^6$ CFU/fish. The final treatment group served as a control, where fish were treated in the same manner but did not receive the bacteria; instead, the control fish were injected with 0.85% (w/v) saline. The LC_{50} (96h) was calculated using Probit Analysis with a 95% confidence interval [29].

For the bacterial challenge, the bacterium was cultured in TSB for 24 hours at 22°C. The cells were then harvested by centrifugation and resuspended with sterile saline to reach a specific optical density (OD_{600}). The concentration of the bacterium resulting in more than 50% mortality was confirmed during the LD_{50} stage using viable colony counts, following the method described by Miles and Misra [30].

Each fish in treatment group one was exposed to the bacterium through intraperitoneal injection of a 0.1 ml volume of the bacterial suspension. Fish in treatment group two received a similar volume of sterile 0.85% (w/v) saline via intraperitoneal injection, as outlined by Alghabshi [31]. The fish were monitored daily for 14 days, with mortality and morbidity recorded every 24 hours. Any moribund fish was removed and sampled for histopathology (limited to moribund fish only), with bacterial recovery and identification conducted as previously described.

The fish were observed daily for any clinical signs of disease, including reduced feeding and behavioral changes. Samples were collected for bacterial recovery and histopathology as outlined. These trials were conducted to fulfill Koch's postulates.

Histopathology

Tissue samples from the gills, liver, spleen, and anterior kidney were collected from three fish per treatment group (when they exhibited immobility and weakened operculum performance). Samples were were aseptically extracted and processed for histopathological analysis, following the procedures outlined in the study by Chen [13]. Initially, all tissues were immersed in 10% formalin (Sigma-Aldrich) for 24 hours for fixation followed by processing using a Tissue Processor (DS2080/H, Iran) and Tissue Float Machines (Labtron, Iran), followed by embedding in wax. The tissue sections were sliced to a thickness of 5 micrometers using a Microtome Accu-Cut (Accu-Cut® SRM™ 200 Rotary Microtome) and subsequently stained with hematoxylin and eosin (HE) as described by Chen [32]. The tissue samples affixed to microscope slides were examined under a light microscope at magnifications of up to 100×, equipped with a camera (Optika, B-293, Italy) for image capture, in accordance with the methodology detailed by Legario [33].

Authors' Contributions

Amir Hossein Smiley , Mohammad Reza Kalbassi conceived and planned the experiments. Nazila Yeganeh , Amir Hossein Smiley carried out the experiments. Nazila Yeganeh, Amir Hossein Smiley, Mohammad Reza Kalbassi, Margaret Crumlish contributed to the interpretation of the results. Nazila Yegane, Amir Hossein Smiley took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

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Competing Interests

The authors declare that there is no conflict of interest.

Reference

1. Kocur M. The genus Micrococcus. In: Balows A, Trüper HG, Dworkin M, Harder W, Schleifer KH, editors. The Prokaryotes. 2nd ed. New York: Springer; 1991. p. 1300-11. Doi:10.1007/0-387-30743-5_37.

2. Stackebrandt E, Koch C, Gvozdiak O, Schumann P. Taxonomic dissection of the genus Micrococcus: Kocuria gen. nov., Nesterenkonia gen. nov., Kytococcus gen. nov., Dermacoccus gen. nov., and Micrococcus Cohn 1872 gen. emend. International Journal of Systematic Bacteriology. 1995;45(4):682-92. Doi:10.1099/00207713-45-4-682.

3. Kovács G, Burghardt J, Pradella S, Schumann P, Stackebrandt E, Màrialigeti K. Kocuria palustris sp. nov. and Kocuria rhizophila sp. nov., isolated from the rhizoplane of the narrow-leaved cattail (Typha angustifolia). International Journal of Systematic and Evolutionary Microbiology. 1999;49(1):167-73. Doi:10.1099/00207713-49-1-167.

4. Pękala A, Paździor E, Antychowicz J, Bernad A, Głowacka H, Więcek B, et al. Kocuria rhizophila and Micrococcus luteus as emerging opportunist pathogens in brown trout (Salmo trutta Linnaeus, 1758) and rainbow trout (Oncorhynchus mykiss Walbaum, 1792). Aquaculture. 2018;486:285-89. Doi:10.1016/j.aquaculture.2017.12.028.

5. Savini V, Catavitello C, Masciarelli G, Astolfi D, Balbinot A, Bianco A, et al. Drug sensitivity and clinical impact of members of the genus Kocuria. Journal of Medical Microbiology. 2010;59(12):1395-402. Doi:10.1099/jmm.0.021709-0.

6. Kim SB, Nedashkovskaya OI, Mikhailov VV, Han SK, Kim KO, Rhee MS, et al. Kocuria marina sp. nov., a novel actinobacterium isolated from marine sediment. International Journal of Systematic and Evolutionary Microbiology. 2004;54(5):1617-20. Doi:10.1099/ijs.0.02742-0.

7. Kandi V, Palange P, Vaish R, Bhatti AB, Kale V, Kandi MR, et al. Emerging bacterial infection: identification and clinical significance of Kocuria species. Cureus. 2016;8(8). Doi:10.7759/cureus.731.

8. Becker K, Rutsch F, Uekötter A, Kipp F, König J, Marquardt T, et al. Kocuria rhizophila adds to the emerging spectrum of micrococcal species involved in human infections. Journal of Clinical Microbiology. 2008;46(10):3537-39. Doi:10.1128/JCM.00823-08.

9. Moissenet D, Becker K, Mérens A, Ferroni A, Dubern B, Vu-Thien H. Persistent bloodstream infection with Kocuria rhizophila related to a damaged central catheter. Journal of Clinical Microbiology. 2012;50(4):1495-98. Doi:10.1128/JCM.06038-11.

10. Meletis G, Gogou V, Palamouti M, Spiropoulos P, Xanthopoulou K, Tantou P, et al. Catheter-related relapsing peritonitis due to Kocuria varians in a patient undergoing continuous ambulatory peritoneal dialysis. Nefrología. 2012;32(4):541-42. Doi:10.3265/Nefrologia.pre2012.Apr.11471.

11. Lee MK, Choi SH, Ryu DW. Descending necrotizing mediastinitis caused by Kocuria rosea: a case report. BMC Infectious Diseases. 2013;13:475. Doi:10.1186/1471-2334-13-475.

12. Boshoff HI, Reed MB, Barry CE, Mizrahi V. DnaE2 polymerase contributes to in vivo survival and the emergence of drug resistance in Mycobacterium tuberculosis. Cell. 2003;113(2):183-93.Doi:10.1016/S0092-8674(03)00270-8.

13. Takarada H, Sekine M, Kosugi H, Matsuo Y, Fujisawa T, Omata S, et al. Complete genome sequence of the soil actinomycete Kocuria rhizophila. Journal of Bacteriology. 2008;190(12):4139-46. Doi:10.1128/JB.01853-07.

14. Al-Bairuty GA, Shaw BJ, Handy RD, Henry TB. Histopathological effects of water-borne copper nanoparticles and copper sulphate on the organs of rainbow trout (Oncorhynchus mykiss). Aquatic Toxicology. 2013;126:104-15. Doi:10.1016/j.aquatox.2012.10.005.

15. Haaparanta A, Valtonen ET, Hoffmann R, Holmes J. Do macrophage centres in freshwater fishes reflect the differences in water quality? Aquatic Toxicology. 1996;34(3):253-72. Doi:10.1016/0166-445X(95)00042-3.

16. Grundy MM, Ratcliffe NA, Moore MN. Immune inhibition in marine mussels by polycyclic aromatic hydrocarbons. Marine Environmental Research. 1996;42(1-4):187-90. Doi:10.1016/0141-1136(95)00033-X.

17. Ostaszewska T, Chojnacki M, Kamaszewski M, Sawosz-Chwalibóg E. Histopathological effects of silver and copper nanoparticles on the epidermis, gills, and liver of Siberian sturgeon. Environmental Science and Pollution Research. 2016;23:1621-33. Doi:10.1007/s11356-015-5391-9.

18. Camargo MM, Martinez CB. Histopathology of gills, kidney and liver of a Neotropical fish caged in an urban stream. Neotropical Ichthyology. 2007;5:327-36. Doi:10.1590/S1679-62252007000300013.

19. Abdelhamed H, Ibrahim I, Baumgartner W, Lawrence ML, Karsi A. Characterization of histopathological and ultrastructural changes in channel catfish experimentally infected with virulent Aeromonas hydrophila. Frontiers in Microbiology. 2017;8:1519. Doi:10.3389/fmicb.2017.01519.

20. Mallatt J. Fish gill structural changes induced by toxicants and other irritants: a statistical review. Canadian Journal of Fisheries and Aquatic Sciences. 1985;42(4):630-48. Doi:10.1139/F85-083.

21. Winkaler EU, das Graças Silva A, Galindo HC, dos Reis Martinez CB. Biomarcadores histológicos e fisiológicos para o monitoramento da saúde de peixes de ribeirões de Londrina, Estado do Paraná. Acta Scientiarum Biological Sciences. 2001;23:507-14. Doi:10.4025/actascibiolsci.v23i0.2708.

22. Fontaínhas-Fernandes A, Luzio A, Garcia-Santos S, Carrola J, Monteiro S. Gill histopathological alterations in Nile tilapia, Oreochromis niloticus exposed to treated sewage water. Brazilian Archives of Biology and Technology. 2008;51:1057-63. Doi:10.1590/S1516-89132008000500023.

23. Dong HT, Senapin S, Phiwsaiya K, Techatanakitarnan C, Dokladda K, Ruenwongsa P, et al. Histopathology and culturable bacteria associated with "big belly" and "skin nodule" syndromes in ornamental Siamese fighting fish, Betta splendens. Microbial Pathogenesis. 2018;122:46-52. Doi:10.1016/j.micpath.2018.06.005.

24. Weinstein MP. Current blood culture methods and systems: clinical concepts, technology, and interpretation of results. Clinical Infectious Diseases. 1996;23(1):40-6. Doi:10.1093/clinids/23.1.40.

25. Buller NB. Biochemical identification tables. In: Bacteria from Fish and Other Aquatic Animals. Wallingford: CABI Publishing; 2004. p. 137-206. Doi:10.1079/9780851997384.0137.

26. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. Proceedings of the National Academy of Sciences of the United States of America. 1977;74(12):5463-67. Doi:10.1073/pnas.74.12.5463.

27. Damodaran T, Mishra VK, Jha SK, Pankaj U, Gupta G, Gopal R. Identification of rhizosphere bacterial diversity with promising salt tolerance, PGP traits and their exploitation for seed germination enhancement in sodic soil. Agricultural Research. 2019;8:36-43. Doi:10.1007/s40003-018-0343-5.

28. Crumlish M, Dung TT, Turnbull JF, Ngoc NT, Ferguson HW. Identification of Edwardsiella ictaluri from diseased freshwater catfish, Pangasius hypophthalmus (Sauvage), cultured in the Mekong Delta, Vietnam. Journal of Fish Diseases. 2002;25(12):733-36. Doi:10.1046/j.1365-2761.2002.00412.x.

29. Udagani C, Yallappa S. Synthesis and characterization of colloidal solution silver nanoparticles effect on behavioural changes and LC50 value of fish Cyprinus carpio (Linnaeus). International Journal of Pharma and Bio Sciences. 2019;10(2):68-74. Doi:10.22376/ijpbs.2019.10.2.b68-74.

30. Miles AA, Misra SS, Irwin JO. The estimation of the bactericidal power of the blood. Journal of Hygiene. 1938;38(6):732-49. Doi:10.1017/S002217240001158X.

31. Alghabshi A, Austin B, Crumlish M. Aeromonas salmonicida isolated from wild and farmed fish and invertebrates in Oman. International Aquatic Research. 2018;10:145-52. Doi:10.1007/s40071-018-0195-4.

32. Chen N, Jiang J, Gao X, Li X, Zhang Y, Liu X, et al. Histopathological analysis and the immune related gene expression profiles of mandarin fish (Siniperca chuatsi) infected with Aeromonas hydrophila. Fish and Shellfish Immunology. 2018;83:410-15. Doi:10.1016/j.fsi.2018.09.023.

33. Legario FS, Choresca CH Jr, Turnbull JF, Crumlish M. Isolation and molecular characterization of streptococcal species recovered from clinical infections in farmed Nile tilapia (Oreochromis niloticus) in the Philippines. Journal of Fish Diseases. 2020;43(11):1431-42. Doi:10.1111/jfd.13247.

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