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Isolation, Characterization, and Pathogenicity of an *Aeromonas veronii* Strain Causing Disease in *Rhinogobio ventralis*

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Abstract: *Rhinogobio ventralis* is a rare fish found in the Yangtze River in China and has significant ecological and economic value. In this study, a bacterial strain (RV-JZ01) was isolated from the livers of diseased *R. ventralis*. This isolate was identified as *Aeromonas veronii* based on its morphology, biochemical features and 16S rDNA phylogenetic analysis. The artificial infection of healthy *R. ventralis* (16 ± 2 cm) with RV-JZ01 resulted in the manifestation of clinical symptoms, in accordance with those of naturally infected fish. The 50% lethal dose (LD_{50}) of RV-JZ01 for *R. ventralis* was 6.3×10^6 CFU/mL. Histopathological examination revealed various pathological changes in the diseased fish, including intestinal villus swelling and rupture, hepatocyte vacuolization, renal tubular cell nuclear enlargement and pyknosis, and myocardial fiber fracture and atrophy. RV-JZ01 infection significantly reduced the gut flora diversity of *R. ventralis*, with the relative abundances of Firmicutes and Fusobacteria increasing, and those of the Proteobacteria and Bacteroidetes decreasing. The abundance of *Lactobacillus* and *Streptococcus* dramatically increased, and the abundance of *Clostridium* and *Escherichia* reduced in the intestinal microbiota of *R. ventralis* infected with RV-JZ01. Antibiotic sensitivity testing revealed that RV-JZ01 was highly susceptible to 12 antimicrobials, including erythromycin, cefalexin, norfloxacin, furazolidone, sulfonamides, enrofloxacin, doxycycline, piperacillin, florfenicol, gentamicin, and lincomycin. These results contribute to the understanding of pathological alterations in *R. ventralis* following *A. veronii* infection, offering valuable data to support the implementation of disease treatment.

Keywords: *Rhinogobio ventralis*; *Aeromonas veronii*; 16S rRNA sequence analysis; intestinal microbiota; antibiotic sensitivity testing

Key Contribution: This study establishes *Aeromonas veronii* (RV-JZ01) as a pathogenic agent in *Rhinogobio ventralis* (*R. ventralis*), providing key insights into its clinical manifestation, histopathological changes, and alterations in the host's intestinal microbiota. Additionally, the comprehensive antibiotic sensitivity testing of RV-JZ01 contributes valuable data for targeted disease prevention and control measures in aquaculture.

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1. Introduction

Rhinogobio ventralis (Sauvage and Dabry de Thiersant, 1874), belonging to the family Cyprinidae and order Cypriniformes, is an important endemic and economic fish within the upper Yangtze River in China [1]. The *R. ventralis* prefers a torrential flow environment and produces pelagic eggs. It is widely distributed in main streams and tributaries and occupies an important position among fishery resources [1]. However, the population of *R. ventralis*

has experienced a significant decline, primarily due to environmental pollution, hydraulic projects, and overfishing [2]. Consequently, it was officially classified as endangered (EN) in 2016 and designated as a Class II National Key Protected Wild Animal in China in 2021 [3,4]. The restoration and protection of natural populations of *R. ventralis* have become urgent priorities, and stock enhancement has been identified as a necessary approach. Currently, domestication and artificial reproduction techniques for *R. ventralis* have been successfully established, and large-scale culture is being attempted [1,5].

In the year 2023, certain *R. ventralis* specimens that were being raised in a recirculating aquaculture system at the Jingzhou Experimental Station of the Yangtze River Fisheries Research Institute, Chinese Academy of Fishery Sciences (Jingzhou, Hubei, China), exhibited clinical signs such as floating in the water, slow swimming, and the presence of white spots on the abdomen. Subsequently, a pathogenic bacterial strain, designated as RV-JZ01, was isolated from the livers of the affected fish and subjected to morphological, biochemical, and 16S rRNA gene sequencing analyses for identification purposes. Furthermore, we tested the antibiotic susceptibility of RV-JZ01 through agar diffusion and examined histopathological changes and gut microbiota shifts in the diseased fish. This study aims to offer valuable support for the diagnosis, followed by the treatment of *A. veronii* infection in *R. ventralis*.

2. Materials and Methods

2.1. Fish Sampling and Materials Used

2.1.1. Fish

Diseased *R. ventralis* (n = 9) were collected from the Jingzhou Experimental Station of the Yangtze River Fisheries Research Institute, Chinese Academy of Fishery Sciences. Healthy *R. ventralis* (n = 280) used for infection experiments were provided by the same facility, with a body length of 16 ± 2 cm. No parasite was found on the skin, fins, gills, or inner organs of healthy *R. ventralis* using light microscopy, and none of them had a history of disease. Healthy fish were raised in 2 m diameter tanks with a 0.7 m water depth at 18 ± 2 °C, and the water was renewed daily at a rate of 30%. All the experimental procedures were approved by the Experimental Animal Center of the Yangtze River Fisheries Research Institute, Chinese Academy of Fishery Sciences (ID Number: YFI 2023-WXB-02).

2.1.2. Main Instruments and Reagents

Taq DNA polymerase, 10× PCR buffer, dNTPs, and a DNA ladder were purchased from Beijing Puyi Medical Technology Co., Ltd. (Beijing, China). The antibiotic susceptibility test kits were obtained from Hangzhou Tianhe Microorganism Reagent Co., Ltd. (Hangzhou, China). Biolog Universal Growth (BUG) liquid medium and BHI liquid media were acquired from Becton, Dickinson and Company (Franklin Lakes, NJ, USA). Biological bacterial identification kits and IF-A-inoculating fluid were purchased from Biolog Inc. (Biolog, Hayward, CA, USA). A fully automated microbial identification system (Biolog, Hayward, CA, USA) was obtained from Biolog Inc. The PCR thermocycler and gel imaging system (ChemiDoc XRS) were obtained from Bio-Rad (Hercules, CA, USA).

2.2. Experimental Methods

2.2.1. Pathogen Isolation

The diseased fish were anesthetized with MS-222, surface sterilized, and wiped with sterile paper. The fish were dissected and the liver tissues were sampled using disposable inoculation loops and streaked onto BHI agar plates (HopeBio, Qingdao, China) under sterile conditions. The plates were incubated at 28 °C for 24 h. Single colonies were picked, streaked onto new BHI agar plates, and cultured to obtain pure colonies. Pure colonies were inoculated in BHI broth, grown at 28 °C with shaking at 180 rpm. When the bacterial suspension reached an OD_{600} of 0.5, a portion of the culture was used for Gram staining [6]. The remaining bacterial suspension was aliquoted into Eppendorf tubes containing 50% glycerol, followed by storage at −80 °C. The strain, which was isolated from diseased *R. ventralis*, was designated as RV-JZ01.

2.2.2. Morphological Observation

The bacterial suspension of strain RV-JZ01 was centrifuged at 3500× *g* for 5 min. The pellet was resuspended in phosphate-buffered saline (PBS) and were used for Gram staining (Jiancheng, Nanjing, China) and observed with a scanning electron microscope (Hitachi, Tokyo, Japan).

RV-JZ01 cells were fixed in 2.5% glutaraldehyde for 4 h, washed with PBS, and dehydrated using an ethanol gradient (60%, 70%, 80%, 90%, and 100%). The samples were then freeze-dried for 48 h and sputter-coated with gold [7]. The prepared samples were examined using a scanning electron microscope (SEM; Olympus, Tokyo, Japan).

2.2.3. Biochemical Identification

Single colonies were streaked onto BUG agar identification plates and incubated at 28 °C for 16–24 h until colonies reached an appropriate size. Two or three single colonies were picked and placed in IF-A inoculation fluid (Biolog, Hayward, CA, USA). The mixture was transferred to GEN III identification plates at 100 µL per well using a pipette. The identification plates were loaded onto a Biolog system for automated reading and identification.

2.2.4. 16S rRNA Sequence Analysis

The 16S rRNA gene of RV-JZ01 was amplified using primers 27F (5′-AGAGTTTGATCA TGGCTCAG-3′) and 1492R (5′-TACGGTTACCTTGTTACGACTT-3′) [8]. The PCR program settings were as follows: 94 °C for 3 min, 94 °C for 30 s, 55 °C for 30 s, 72 °C for 45 s, 35 cycles; 72 °C for 10 min. The PCR products were electrophoresed on a 1% agarose gel, and positive bands were recovered for sequencing. The *16S rRNA* sequence of RV-JZ01 was analyzed for homology using the BLAST search tool on the NCBI for Biotechnology Information website (http://blast.ncbi.nlm.nih.gov, accessed on 13 March 2023). A phylogenetic tree was constructed for RV-JZ01 based on the *16S rRNA* sequences using the neighbor-joining method in MEGA7.0. A confidence test was conducted using 1000 bootstraps.

2.2.5. Histopathological Observations

Intestinal, splenic, hepatic, andrenaltissues of diseased and healthy *R. ventralis* were collected and fixed in 4% paraformaldehyde. The fixed samples were rinsed under running water for 12 h and then dehydrated using an ethanol gradient (80%, 90%, 95%, and 100%). The dehydrated samples were embedded in paraffin, sectioned at 5 µm thickness, and mounted on slides. After spreading and drying, the sections were stained with hematoxylin and eosin and observed under a light microscope.

2.2.6. Infection Trials

The frozen RV-JZ01 strain was inoculated into BHI broth and cultured at 30 °C with agitation at 200 rpm. When the OD_{600} of the bacterial culture reached 0.5, it was centrifuged at 4000× *g* for 2 min. The supernatant was discarded, and the pellet was washed three times with sterile PBS. The bacterial concentration was determined via plate counting and was diluted 10-fold in sterile PBS to 10^6, 10^7, 10^8 and 10^9 CFU/mL. The concentration of the bacterial suspension was determined using the plate colony counting method. Healthy fish were randomly divided into five groups (four infection groups and one control group) of 30 fish each. The infection groups were injected intraperitoneally with 0.2 mL of different bacterial dilutions, while the control group was injected with 0.2 mL of sterile PBS. The rearing conditions were kept consistent with the temporary rearing conditions. The disease occurrence and mortality were recorded continuously for 14 d after infection. The median lethal dose (LD_{50}) was calculated using the Reed–Muench method [9]. The bacteria were isolated from the diseased fish and identified by morphology, biochemical features and 16S rDNA phylogenetic analysis.

2.2.7. Intestinal Microbiota Analysis

The intestinal tissues from healthy (n = 9) and diseased fish (n = 9) were used to analyze the intestinal microbiota. The intestines were taken out and every three intestines were put in centrifuge tubes as samples. The intestine samples were immediately stored in a heat insulated icebox with dry ice and transported to the laboratory for Illumina sequencing. To comprehensively assess the alpha diversity of intestinal microbial communities, richness was characterized by Chao1 [10], observed species indices were characterized by Shannon [11] and Simpson indices [12], evolutionary diversity was characterized by Faith's PD index [13], evenness was characterized by Pielou's evenness index [14], and coverage was characterized by Good's coverage index [15]. Beta diversity based on genus level abundances was estimated using Bray–Curtis distances and visualized using principal coordinate analysis (PCA) and the unweighted pair-group method, with arithmetic mean (UPGMA) clustering [16]. All diversity analyses were performed using QIIME (v1.8.0).

2.2.8. Antimicrobial Susceptibility Testing

Under sterile conditions, the suspensions of strain RV-JZ01 (100 µL per plate) were spread onto BHI agar plates. Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method [17]. Sixteen antimicrobial agents were tested, including vancomycin (30 µg/piece), erythromycin (15 µg/piece), cefuroxime (30 µg/piece), norfloxacin (10 µg/piece), furazolidone (100 µg/piece), sulfadiazine (50 µg/piece), enrofloxacin (10 µg/piece), doxycycline (30 µg/piece), penicillin G (10 IU/piece), florfenicol (30 µg/piece), piperacillin (10 µg/piece), gentamicin (10 µg/piece), lincomycin (2 µg/piece), carbenicillin (100 µg/piece), neomycin (30 µg/piece), and medemycin (30 µg/piece). Antimicrobial susceptibility test disks were evenly placed on the BHI agar surface, with four disks per plate. After incubation at 28 °C for 24 h, the diameters of the inhibition zones were measured. The results were classified as sensitive (S), moderately sensitive (M) and resistant (R), according to the guidelines provided with the drug susceptibility test papers (Hangwei, Hangzhou, China).

3. Results

3.1. Clinical Signs of Diseased Fish

Diseased fish floated on the rearing water in the circular tanks of a recirculating aquaculture system, moved slowly, and showed systemic hemorrhages (Figure 1a). Some fish exhibited exophthalmos (Figure 1a). Necropsy revealed ascites fluid in the diseased fish abdominal cavity, and the hemorrhage of the spleen and kidneys (Figure 1b).

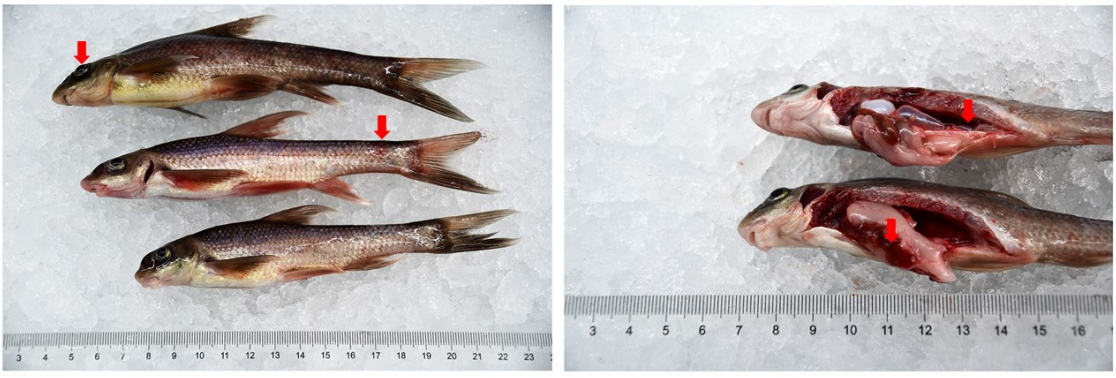

Figure 1. Clinical signs in the naturally infected *R. ventralis*. (**a**) Hemorrhage on the body surface (arrow); (**b**) visceral hyperemia, ascites (arrow).

3.2. Bacterial Morphology

In the process of bacterial isolation, the colonies on all plates were consistent in color and form. Thirty strains were selected from 10 plates for *16S rRNA* analysis, and all were *Aeromonas*. Strain RV-JZ01 was grown in BHI medium, and formed milky-white, circular, convex, and smooth colonies (Figure 2a). Gram staining revealed the bacteria were rod, and Gram-negative (Figure 2b). Scanning electron microscopy revealed that the bacteria comprised short rods with smooth, blunt ends, approximately 2–3 μm in length and 0.7–1 μm in diameter (Figure 2c).

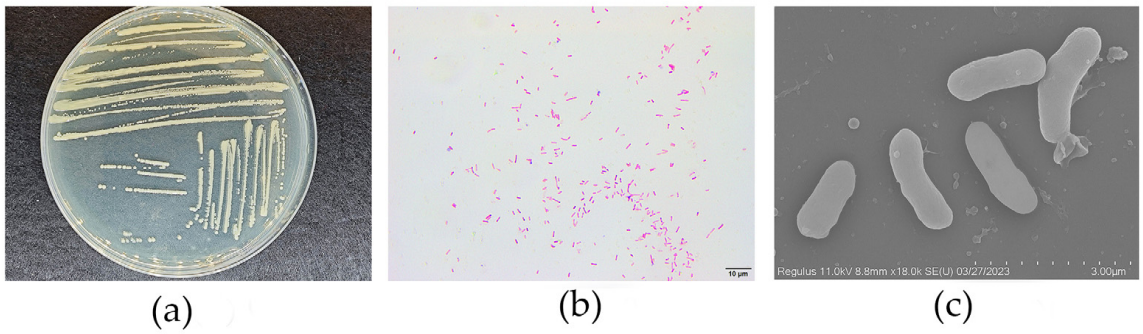

Figure 2. Morphological characteristics of strain RV-JZ01. (**a**) Colony morphology on BHI plates; (**b**) Gram staining; (**c**) microscopy via scanning electron microscope (SEM).

3.3. Analysis using 16S rRNA Gene Sequencing

The 16S rRNA gene of strain RV-JZ01 was 1407 bp in length. BLAST results show that the strain RV-JZ01 shares over 99% homology with *A. veronii* (JQ771297.1) based on the NCBI database.

A phylogenetic was constructed through the neighbor-joining method using Mega 7.0 software. The isolated strain RV-JZ01 clustered with *A. veronii* (JQ771297.1) on the same branch depended on the phylogenetic analysis (Figure 3).

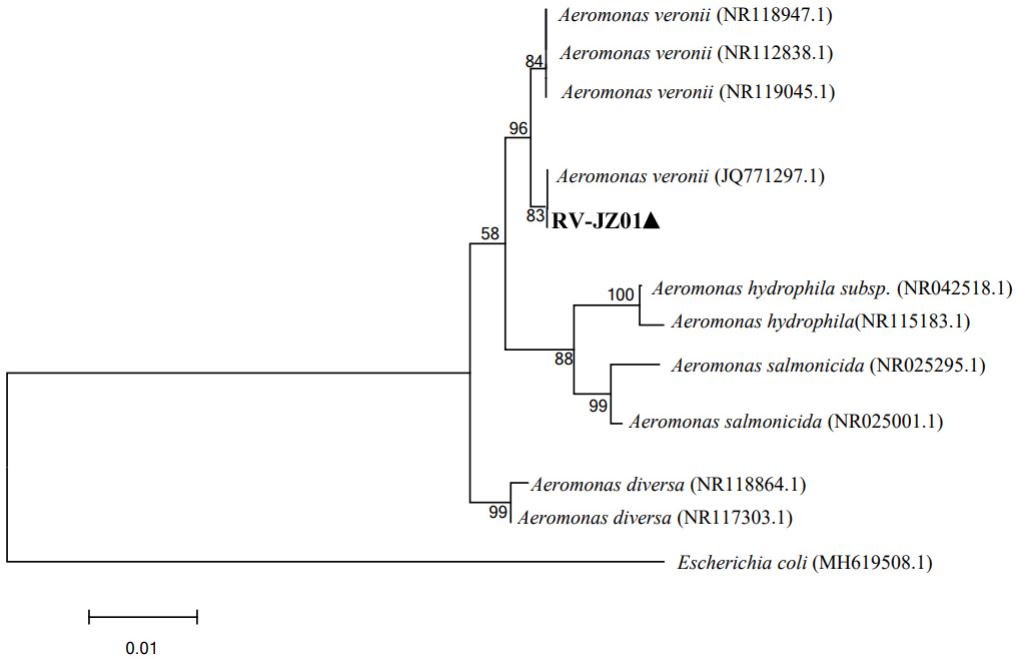

Figure 3. Phylogenetic tree of strains RV-JZ01 based on 16S rRNA gene sequences (the digits represent bootstrap values).

3.4. Biochemical Characteristics of RV-JZ01

Using the Biolog Automated Microbial Identification System, strain RV-JZ01, whose biochemical features were listed in a table below (Table 1), was identified as *A. veronii*.

Table 1. Biochemical features of strain RV-JZ01.

Reaction Item	Result	Reaction Item	Result
A1 Negative Control	N	E1 Gelatin	P
A2 Dextrin	P	E2 Glycyl-L-Proline	P
A3 D-Maltose	P	E3 L-Alanine	P
A4 D-Trehalose	P	E4 L-Arginine	P
A5 D-Cellobiose	P	E5 L-Aspartic Acid	P
A6 Gentiobiose	N	E6 L-Glutamic Acid	P
A7 Sucrose	B	E7 L-Histidine	P
A8 D-Turanose	P	E8 L-Pyroglutamic Acid	B
A9 Stachyose	N	E9 L-Serine	P
A10 Positive Control	P	E10 Lincomycin	P
A11 Acidic PH PH6	P	E11 Guanidine HCl	P
A12 Acidic PH PH5	N	E12 Niaproof 4	P
B1 D-Raffinose	B	F1 Pectin	P
B2 α-D-Lactose	B	F2 D-Galacturonic Acid	B
B3 D-Melibiose	B	F3 L-Galactonic Acid Lactone	B
B4 β-Methyl-D-Glucoside	P	F4 D-Galactonic Acid	P
B5 D-Salicin	B	F5 D-Glucuronic Acid	N
B6 N-Acetyl-D-Glucosamine	P	F6 Glucuronamide	N
B7 N-Acetyl-β-D-Mannosamine	P	F7 Mucic Acid	B
B8 N-Acetyl-D-Galactosamine	P	F8 Quinic Acid	B
B9 N-Acetyl Neuraminic Acid	P	F9 D-Saccharic Acid	B
B10 1% NaCl	N	F10 Vancomycin	B
B11 4% NaCl	N	F11 Tetrazolium Violet	B
B12 8% NaCl	P	F12 Tetrazolium Blue	P
C1 α-D-Glucose	B	G1 P-Hydroxy-Phenylacetic Acid	P
C2 D-Mannose	B	G2 Methyl Pyruvate	B
C3 D-Fructose	B	G3 D-Lactic Acid Methyl Ester	N
C4 D-Galactose	P	G4 Lactic Acid	P
C5 3-Methyl Glucose	B	G5 Citric Acid	P
C6 D-Fucose	N	G6 α-Keto-Glutaric Acid	P
C7 L-Fucose	B	G7 D-Malic Acid	P
C8 L-Rhamnose	B	G8 L-Malic Acid	P
C9 Inosine	P	G9 Bromo-Succinic Acid	N
C10 1%Sodium Lactate	B	G10 Nalidixic Acid	B
C11 Fusidic Acid	N	G11 Lithium Chloride	N
C12 D-Serine	P	G12 Potassium Tellurite	N
D1 D-Sorbitol	N	H1 Tween 40	P
D2 D-Mannitol	B	H2 γ-Amino-Butyric Acid	N
D3 D-Arabitol	N	H3 α-Hydroxy-Butyric Acid	B
D4 Myo-lnositol	B	H4 β-Hydroxy-D,L-Butyric Acid	B
D5 Glycerol	P	H5 α-Keto-Butyric Acid	B
D6 D-Glucose-6-PO4	P	H6 Acetoacetic Acid	B
D7 D-Fructose-6-PO4	P	H7 Propionic Acid	B
D8 D-Aspartic Acid	P	H8 Acetic Acid	P
D9 D-Serine	P	H9 Formic Acid	P
D10 Troleandomycin	N	H10 Aztreonam	B
D11 Rifamycin SV	B	H11 Sodium Butyrate	B
D12 Minocycline	N	H12 Sodium Bromate	N

P, Positive; N, Negative; B, Borderline.

3.5. Histopathological Changes in Diseased Fish

The visceral organs of healthy fish, including the liver, spleen, and kidneys, exhibited normal morphology, an intact structure, and no pathological changes in cells that

regularly arranged (Figure 4A,C,E,G), whereas compared with healthy fish, diseased fish displayed pronounced histopathological changes in visceral organs. Diseased fish showed intestinal villus shortening (Figure 4B). The livers of the diseased fish exhibited hepatocellular vacuolization and necrosis (Figure 4D). The diseased fish displayed extensive splenic necrosis and splenic vacuolization (Figure 4F); the diseased fish also displayed renal cell vacuolization (Figure 4H).

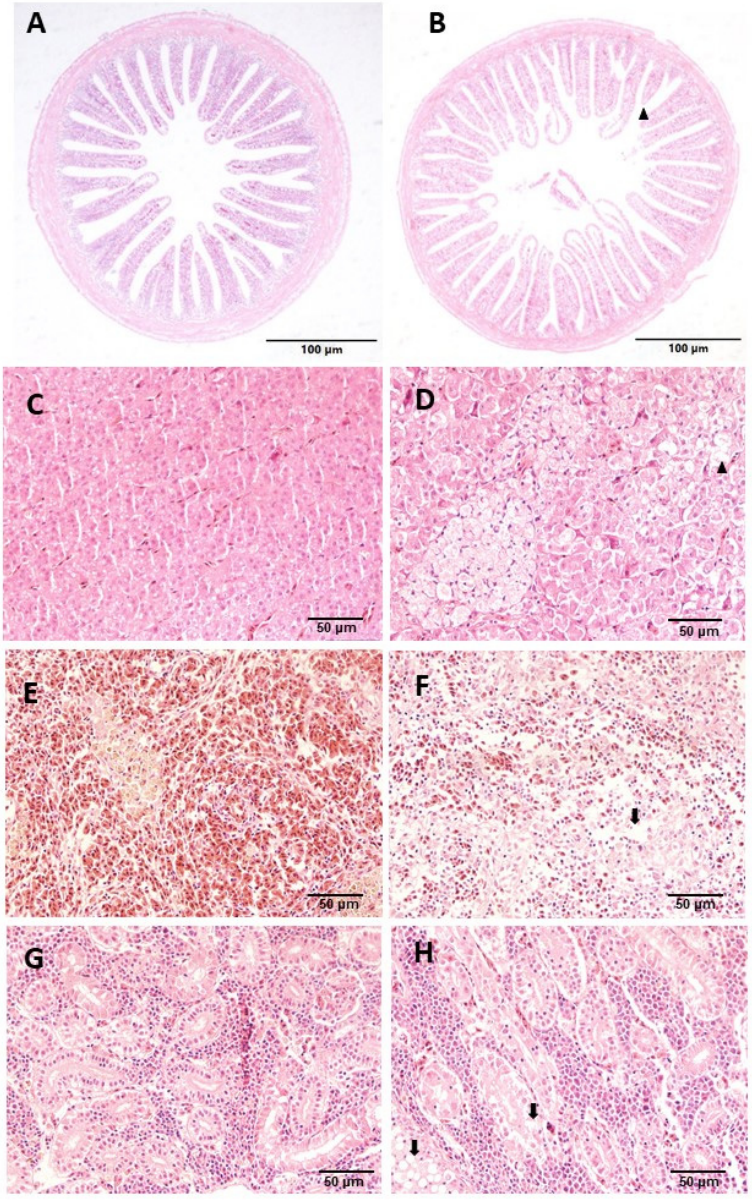

Figure 4. Histopathological changes in healthy and diseased *R. ventralis*. (**A**,**B**) The intestine of healthy fish and diseased fish, respectively. Healthy fish exhibited a normal intestinal morphology and an intact structure, whereas diseased fish displayed intestinal villus shortening (arrowhead); (**C**,**D**) the liver of healthy fish and diseased fish, respectively. Diseased fish exhibited severe hepatocellular vacuolization (arrowhead); (**E**,**F**) the spleen of healthy fish and diseased fish, respectively. The diseased fish showed extensive splenic necrosis and vacuolization (arrow); (**G**,**H**) the kidney of healthy fish and diseased fish, respectively. Diseased fish displayed renal cell vacuolization (arrow).

3.6. Infection Experiment

Healthy fish injected with different concentrations of RV-JZ01 showed varying degrees of disease signs or mortality within 14 d, whereas no mortality was observed in the control

group (Figure 5). The LD_{50} of RV-JZ01 was calculated to be 6.3×10^6 CFU/mL using the Reed–Muench method. The bacterium isolated from the livers of artificially infected fish was identified as *A. veronii*, which confirmed the pathogenicity of the isolated strain.

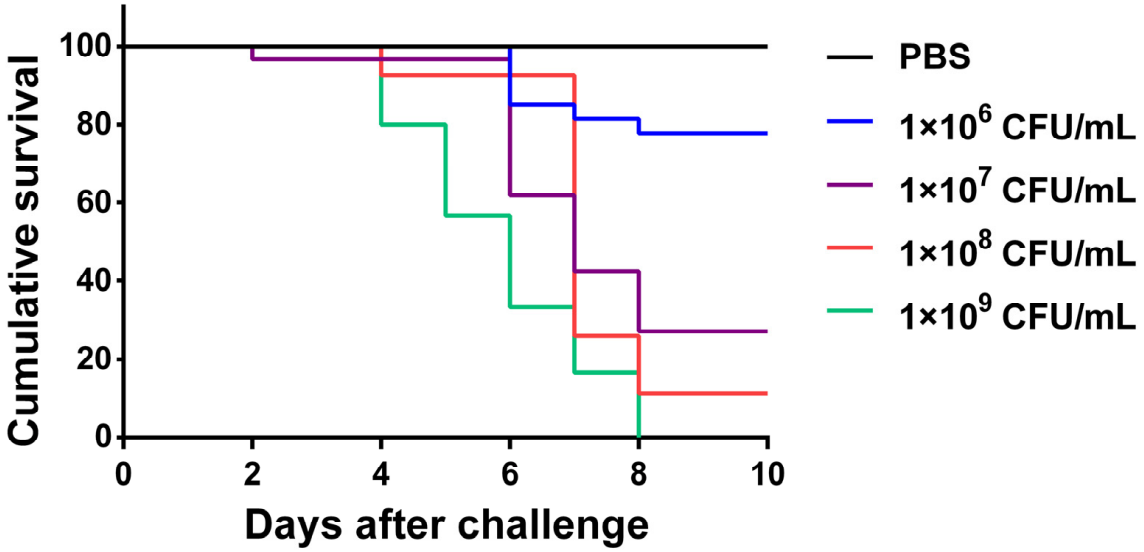

Figure 5. Survival rates of *R. ventralis* challenged with different doses of *A. veronii* RV-JZ01 for 14 days post-infection.

3.7. Intestinal Microbiota

The rarefaction and rank abundance curves showed that each sample reached sufficient sequencing depth, richness, and evenness (Figure 6a,c). These high-quality sequences were clustered into 854 ASVs/operational taxonomic units (OTUs) based on 97% nucleotide sequence homology. The Venn diagram shows that healthy fish had a higher number of OTUs than diseased fish. There were 182 shared OTUs between the healthy and diseased fish, with 365 and 307 unique OTUs, respectively. The distribution of ASVs/OTUs across groups is shown in (Figure 6b).

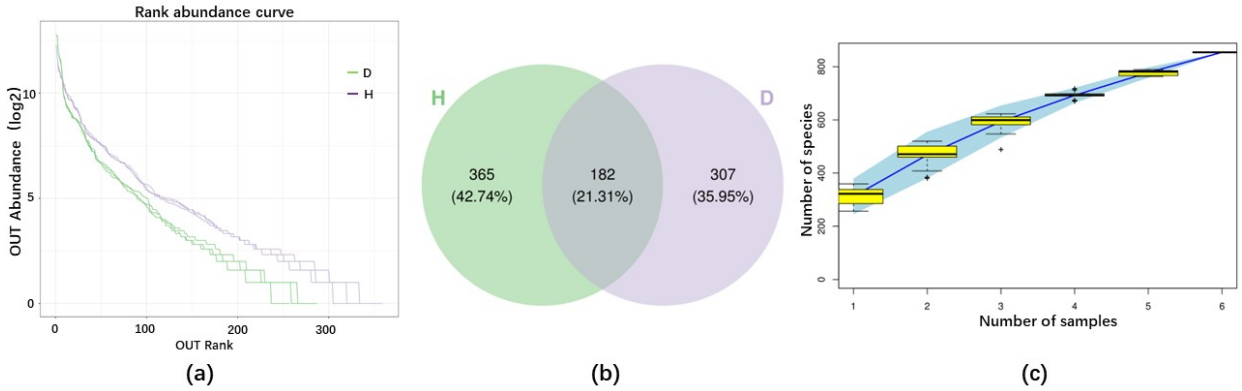

Figure 6. Rarefaction curves (**a**), Venn diagram (**b**), and rank abundance curves (**c**) of healthy and diseased *R. ventralis*. D, diseased *R. ventralis* group; H, healthy *R. ventralis* group.

The richness of the intestinal microbiota was significantly lower in the diseased group than that in the healthy group ($p < 0.05$; Figure 7a,e). The intestinal microbiota diversity, characterized using the Simpson pairwise dissimilarity index and Shannon index, was significantly lower in the diseased group than in the healthy group (Figure 6b,c). The evenness characterized by Pielou's evenness index was significantly lower in the diseased group than in the healthy group ($p < 0.05$, Figure 7d). The phylogenetic diversity, characterized

by Faith's PD index, showed no significant difference between the diseased and healthy groups ($p > 0.05$, Figure 7f). Good's coverage index indicated that the identified sequences represented the majority of the bacteria in each sample (Figure 7g).

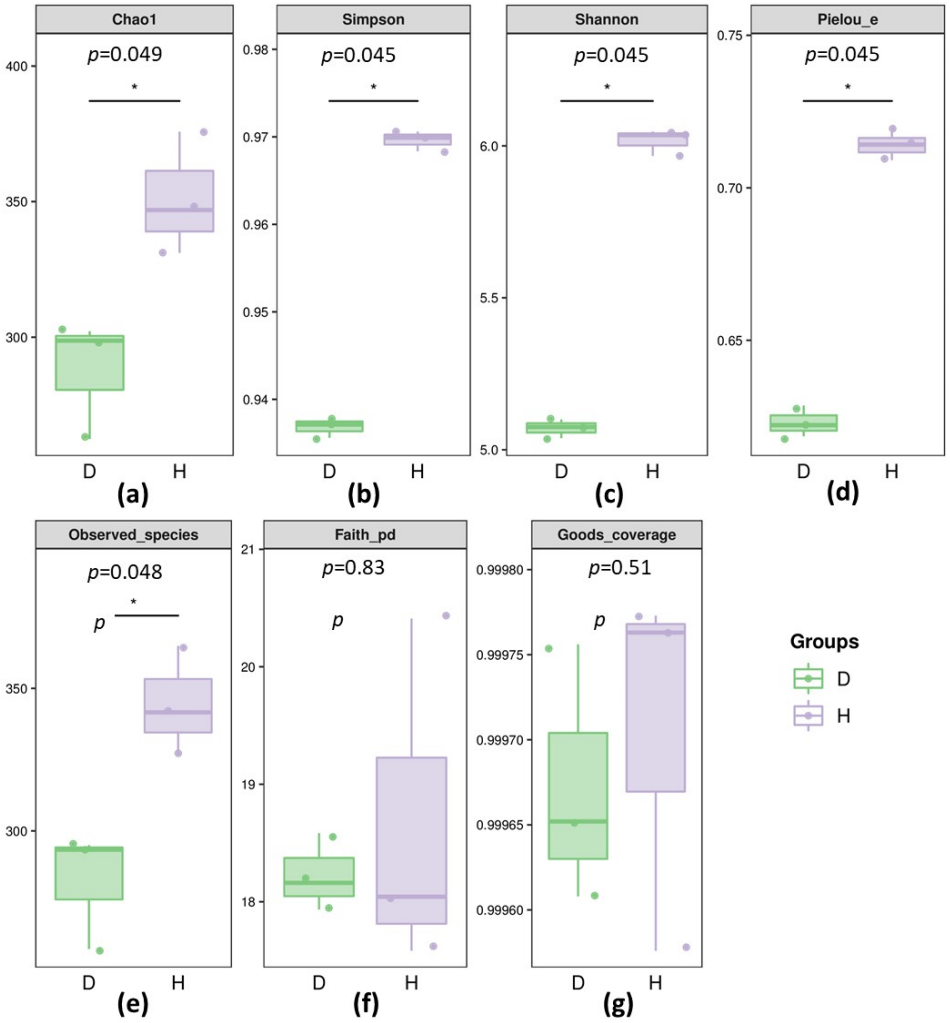

Figure 7. Boxplots of α-diversity. (**a**) Chao1 index; (**b**) Simpson index; (**c**) Shannon index; (**d**) Pielou index; (**e**) Observed species; (**f**) Faith index; (**g**) Goods coverage. D, diseased *R. ventralis*; H, healthy *R. ventralis*. Numbers under diversity indices labels are *p*-values are for Kruskal–Wallis tests. (* $p < 0.05$).

In all samples, the dominant phyla in the intestinal microbiota of healthy *R. ventralis* were Firmicutes, Proteobacteria, Fusobacteria, and Bacteroidetes, accounting for >97% (Figure 8A). The relative abundances of Firmicutes and Fusobacteria increased in diseased *R. ventralis*, whereas Proteobacteria and Bacteroidetes decreased. The dominant genera in healthy *R.ventralis* were *Peptostreptococcus*, *Clostridium*, *Streptococcus*, *Enterobacter*, *Edwardsiella*, and *Lachnospira*. After infection with *A. veronii* RV-JZ01, the relative abundances of *Lactococcus*, *Streptococcus* and *Weissella* significantly increased, whereas *Enterobacter* significantly decreased in *R. ventralis* ($p < 0.05$; Figure 8B).

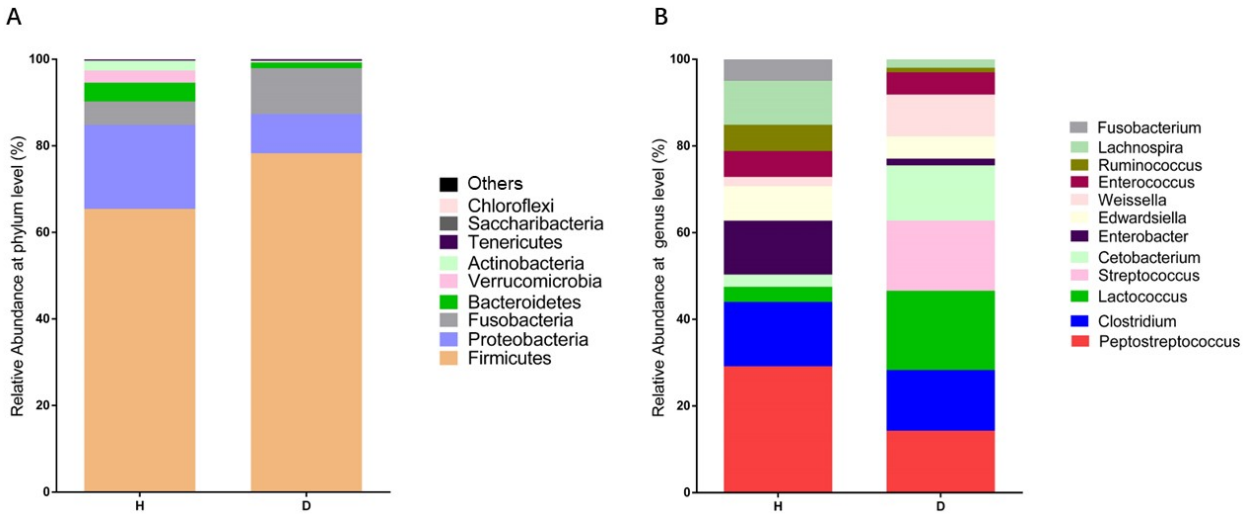

Figure 8. Dominant phyla (**A**) and genera (**B**) in intestinal microbiota of healthy (H) and diseased (D) *R. ventralis*.

Beta diversity was assessed using PCA and UPGMA clustering. The intestinal microbiota of the healthy and diseased groups clustered separately, and the inter-individual variation was smaller in the healthy group than in the diseased group (Figure 9). Overall, the PCA and UPGMA clustering results indicated that *A. veronii* infection significantly altered the intestinal microbiota structure of *R. ventralis*.

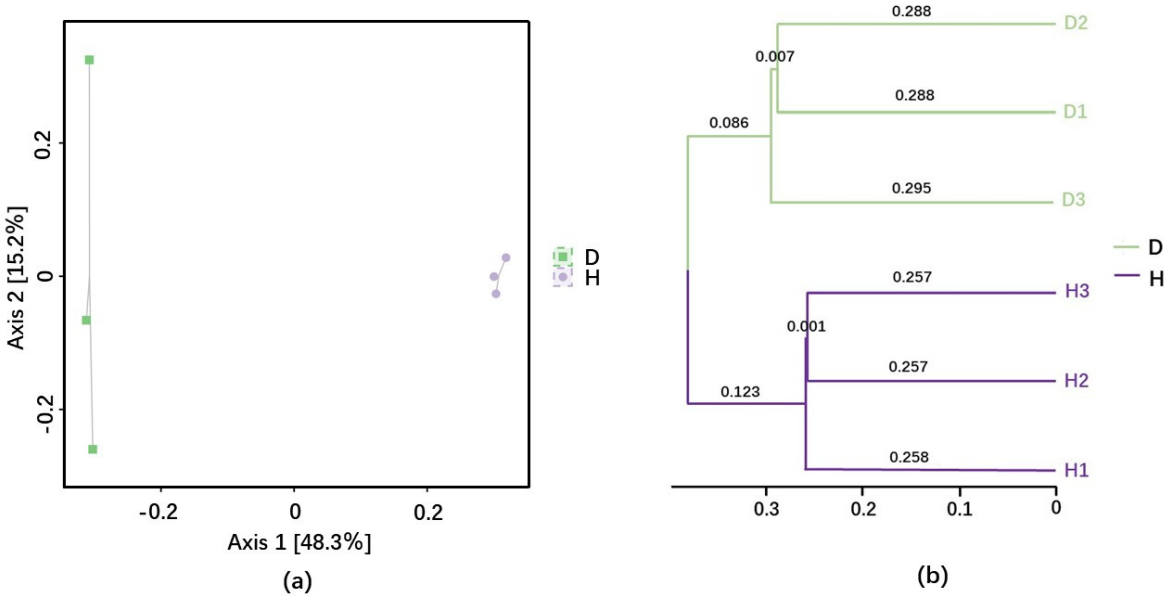

Figure 9. Beta diversity analysis of intestinal microbiota from healthy (H) and diseased (D) *R. ventralis*. (**a**) Principal coordinate analyses (PCA) of the microbiota based on the operational taxonomic unit compositions. (**b**) Unweighted pair-group method with arithmetic mean (UPGMA) hierarchical clustering tree.

3.8. Antibiotic Susceptibility Analysis

Antibiotic susceptibility testing revealed that the strain RV-JZ01 was highly sensitive to erythromycin, cefalexin, norfloxacin, furazolidone, sulfonamides, enrofloxacin, doxycycline, piperacillin, florfenicol, gentamicin, and lincomycin. It was intermediately sensitive to vancomycin, and completely resistant to penicillin G, Neomycin, and Medemycin (Table 2).

Table 2. Susceptibility profile of isolate RV-JZ01.

Medicine Name	Inhibition Zone (mm)	Sensitivity	Medicine Name	Inhibition Zone (mm)	Sensitivity
Vancomycin	11	I	Penicillin G	6	R
Erythromycin	31	S	Piperacillin	34	S
Cefuroxime	39	S	Florfenicol	25	S
Norfloxacin	31	S	Gentamicin	21	S
Furazolidone	34	S	Carbenicillin	30	S
Sulfanilamide	22	S	Lincomycin	16	S
Enrofloxacin	39	S	Neomycin	≤6	R
Doxycycline	31	S	Medemycin	≤6	R

I, intermediate; R, resistant; S, susceptible.

4. Discussion

Aeromonas species are ubiquitous in aquatic environments and can cause septicemia with high mortality rates in fish and other aquatic animals [17]. *A. veronii* is a Gram-negative bacterium similar to *A. hydrophila* and *A. caviae*. It is associated with motile aeromonadal septicemia, which is widespread in animals [18]. Increasing evidence has indicated that *A. veronii* can infect various fish species. In this study, the bacterium strain RV-JZ01 was isolated from diseased *R. ventralis*, combining molecular biology with biochemical characteristics, and the isolate was identified as *A. veronii*. Previous research has shown that fish infected with *A. veronii* generally exhibited different degrees of skin ulceration or hemorrhage, visceral lesions, and ascites, although the symptoms are not identical. For example, infected koi (*Cyprinus carpio* var. koi) [19], Chinese sucker (*Myxocyprinus asiaticus*) [20], and crucian carp (*Carassius auratus gibelio*) [21] display skin ulcers, hemorrhagic and necrotic fins, scale loss, anal swelling, and abdominal distension. In addition to the above signs, naturally infected tilapia exhibited bilateral exophthalmos and opacity [22]. In this study, *R. ventralis* infected with *A. veronii* exhibited systemic hemorrhages, with necropsy revealing ascites, splenic, and renal hemorrhages. While these clinical sign align with previous reports, some distinctions were noted. Notably, we observed no evident skin ulcers or scale loss in diseased fish, possibly due to the infection's early stage or the combined influences of this *A. veronii* strain and aquaculture conditions, resulting in modified disease presentations. Prior research has highlighted that clinical manifestations induced by different *Aeromonas* species can vary, and even identical *Aeromonas* strains may elicit diverse symptoms in different fish hosts [21].

LD_{50}, an important index in animal toxicology, reflects the toxicity of a substance [23]. Santos et al. [24] suggested that a bacterial strain is highly virulent when its LD_{50} ranges between 1.7×10^4 and 1×10^6 CFU/g body weight. The LD_{50} values of *A. veronii* infecting Chinese longsnout catfish (*Leiocassis longirostris* Günther) [25] and largemouth bass (*Micropterus salmoides*) [26] were 3.47×10^4 and 3.72×10^4 CFU/g fish weight, respectively. In this study, the LD_{50} of the isolated *A. veronii* RV-JZ01 strain on fish was 6.3×10^6 CFU/mL, which is higher than that of isolates from other fish species. This may be due to differences in the virulence of the isolates, host susceptibility, or water temperature.

Pathological diagnosis is one of the most authoritative clinical pathological diagnostic methods in modern medicine and is commonly used in aquatic animal pathology research [27]. Infected largemouth bass showed hemorrhagic and necrotic hepatocytes, abundant iron-containing pigment granules in the spleen, severe inflammatory cell infiltration in the kidneys, and necrotic glomeruli [26]. The infection of *A. veronii* with crucian carp can cause the porosity of the submucosal connective tissue, the swelling and necrosis of liver cells, the infiltration of hematopoietic cells and inflammatory cells in the kidney, and iron pigmentation in spleen cells [25]. In this study, similar to the studies mentioned above, *A. veronii*-infected *R. ventralis* showed intestinal villus rupture and severe hepatocellular vacuolization, along with common necrosis and inflammatory cell infiltration in all tissues. However, the lesions in each tissue sample of fish infected with *A. veronii* were relatively

slight. This could be attributed to the possibility that the diseased fish were in the early stages of bacterial infection, where serious tissue damage had not yet occurred.

The intestinal microbiota plays an important role in host metabolism and immune function and is vital for host health [26]. Changes in the intestinal microbiota are highly correlated with physiological, pathological, and environmental conditions [24]. Microbiota richness and diversity are closely related to its stability. Adverse environments and diseases can reduce richness and diversity [28,29]. In this study, *A. veronii* infection markedly reduced the richness and diversity of the intestinal microflora in *R. ventralis* compared with that in the healthy group. Further analyses of microbiota composition revealed the effects of *A. veronii* infection on *R. ventralis*. At the phylum level, the relative abundances of Firmicutes and Fusobacteria increased, whereas Proteobacteria and Bacteroidetes decreased following *A. veronii* infection. At the genus level, the relative abundance of *Lactococcus*, *Streptococcus* and *Weissella* significantly increased, whereas *Enterobacter* significantly decreased after infection. Environmental stress or pathogenic infections are important causes of intestinal microbial changing. In this study, the changes in the intestinal flora of diseased *R. ventralis* may be due to the damage of the immune function and intestinal barrier of *R. ventralis* by *A. veronii*.

Antibiotic susceptibility testing can be used to understand the sensitivity or tolerance of pathogenic microorganisms to various antibiotics, thereby providing a theoretical basis for the rational clinical use of antibiotics [30]. *A. veronii* isolates from different farming environments showed a variability in antibiotic resistance. For example, isolates from the Sichuan and Foshan farms were found to be sensitive to florfenicol but resistant to doxycycline [31,32], whereas the isolate in this study was resistant to doxycycline, indicating differences in drug sensitivity between the strains. An *A. veronii* strain, LB2101, isolated from infected largemouth bass (*Micropterus salmoides*), was also sensitive to florfenicol [33] which is consistent with the results of this study. The differences in antibiotic resistance may be related to factors such as the farming conditions and modes, and source of isolates [29,34].

5. Conclusions

In this study, a pathogenic *A. veronii* RV-JZ01 was isolated from diseased *R. ventralis*. The artificial infection test showed that *A. veronii* RV-JZ01 is highly pathogenic to *R. ventralis*, and this isolate is highly sensitive to erythromycin, cefalexin, norfloxacin, furazolidone, sulfonamides, enrofloxacin, doxycycline, piperacillin, florfenicol, gentamicin, and lincomycin. The results of this study provide a reference for the diagnosis and treatment of fish infection that are infected with *A. veronii*.

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Informed Consent Statement: Not applicable.

Data Availability Statement: The raw sequences of 16S rRNA gene amplicon were deposited in the GenBank with accession number OR844455.

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