Wang C, Xie Y, Deng Z, et al. Identification of *Acinetobacter schindleri* isolated from Chinese giant salamanders (*Andrias davidianus*). *Israeli Journal of Aquaculture - Bamidgeh*. 2024;76(2):91-101. doi:10.46989/001c.116476

Original Research Articles

Identification of *Acinetobacter schindleri* isolated from Chinese giant salamanders (*Andrias davidianus*)

Cheng Wang[1], Yixing Xie[2], Zhiyong Deng[2], Huanyan Yuan[2], Mingzhu Tian[2], Pan Mao[2], Yong Zhou[3a], Ying Wei[2b]

[1] Hunan Zhangjiajie Giant Salamander National Nature Reserve Affairs Center, Zhangjiajie, 427400, China, [2] Hunan Zhangjiajie Giant Salamander National Nature Reserve Affairs Center, [3] Yangtze River Fisheries Research Institute, Chinese Academy of Fishery Sciences

Keywords: Andrias davidianus, Acinetobacter schindleri, 16S rRNA gene sequencing, histopathology

https://doi.org/10.46989/001c.116476

Israeli Journal of Aquaculture - Bamidgeh

Vol. 76, Issue 2, 2024

At a particular aquaculture facility in Zhangjiajie in China, the Chinese giant salamander (*Andrias davidianus*) exhibited analogous clinical manifestations, culminating in sequential mortalities. This study used rigorous aseptic sampling methods to isolate bacteria from the affected liver of salamanders to determine the causative agent behind the decline in amphibians. A bacterial pathogen was isolated from diseased *A. davidianus*, and the strain was named DN-3. This isolate was subjected to bacterial identification, antibiotic susceptibility assays, reinfection experiments, and biochemical profiling. The isolated bacterial strain was definitively identified as *Acinetobacter schindleri* using 16S rRNA sequence analysis and biochemical identification. Antibiotic susceptibility testing revealed that this isolate was susceptible to neomycin, macrolides, doxycycline, piperacillin, nitrofurantoin, and carbenicillin. Subsequent reinfection assays, in which varying concentrations of the bacterial inoculum were administered to healthy salamanders, confirmed that the pathogen elicited varying degrees of morbidity or mortality within an eight-day observation period. The median lethal dose (LD50) of *A. schindleri* DN-3 for *A. davidianus* was calculated to be 6.25×104 CFU/mL. This result supports the significant pathogenicity of the strain for *A. davidianus*. The findings of this study provide empirical insights into the clinical management and epidemiological control of diseases affecting *A. davidianus*.

INTRODUCTION

The giant Chinese salamander (*Andrias davidianus*) is a relict animal endemic to China that retained morphological features from 165 million years ago and is the largest living amphibian worldwide.[1] It has historically enjoyed widespread distribution across the Yangtze, Pearl, and Yellow River basins.[2,3] However, anthropogenic activities, such as habitat degradation and overexploitation, have rapidly caused declines in wild populations of this species.[3,4] As a result, the International Union for Conservation of Nature (IUCN) has designated this species "critically endangered",[5] making it a top priority for global amphibian conservation efforts.[6-8] Despite its precarious conservation status in natural ecosystems, *A. davidianus* has been subject to more than 50 years of aquaculture and product development in numerous provinces across China owing to its considerable biological, medicinal, and culinary value.[9-11] The increased intensity of artificial breeding practices and frequent trade activities have ushered in bacterial infections as an inescapable malady, thereby constituting a significant barrier to the sustainable development of the *A. davidianus* aquaculture industry, with substantial economic ramifications requiring focused mitigation.[12,13]

Acinetobacter species are ubiquitously distributed in various environmental matrices, such as water and soil, and are capable of thriving on biotic and abiotic surfaces.[14,15] Notably, it is a primary carrier for hospital-acquired infections, including respiratory viral infections, septicemia, urinary tract infections, and wound infections.[16,17] Its high mortality rate and multidrug resistance represent considerable threats to public health.[18] Furthermore, as zoonotic pathogens, *Acinetobacter* species exhibit extensive transmission across many animal taxa, including mammals, avians, amphibians, and piscine species.[19] This spread endangers global wildlife and domesticated animal populations, culminating in mass morbidity and mortality.[20]

Zhangjiajie City is renowned as the "Home of the Chinese Giant Salamander," serving as a critical hub for the distribution and production of these wild creatures in

a Corresponding author. Yong Zhou, Email: zhouy@yfi.ac.cn

b Corresponding author. Ying Wei, Email: 18685100701@163.com

China.[21] Numerous farmers engage in the artificial rearing and trading of this species.[22] However, as salamander farming has continued to grow in size and intensity, a number of diseases have subsequently surfaced, especially recently discovered illnesses that have severely harmed the industry's bottom line.[21,23] The revenue and viability of *A. davidianus* farming have been directly threatened by the epidemics that have resulted from this.[7] Identification of the diseases and their causative agents will help to develop strategies to control and prevent the incidence of diseases in farmed *A. davidianus*, thus reducing loss to farmers.

Due to the higher number of sick patients and deaths among *A. davidianus* at the research site, this project has performed pathogen diagnostics, antibiotic sensitivity tests, reinfection experiment on diseased samples. The present study aimed to understand the characteristics and pathogenicity of this pathogen, it can provide scientific basic data for the healthy breeding of giant salamanders in the future.

MATERIALS AND METHODS

2.1. EXPERIMENTAL MATERIALS

In February 2023, specimens(n=10) exhibiting typical symptoms and characterized by overt health were collected from a designated *A. davidianus* aquaculture facility in Zhangjiajie. Subsequently, the specimens were meticulously partitioned into thermal containers and shipped to the Yangtze River Fisheries Research Institute under the Chinese Academy of Fishery Sciences for isolation, identification, and further experimental evaluation of etiological agents.

2.2. PATHOGEN ISOLATION

Ailing *A. davidianus* was subjected to topical disinfection using 75% ethanol, followed by aseptic dissection within a biosafety cabinet to expose the abdominal cavity. Freshly dissected kidney, spleen, and intestine organ surfaces were inoculated with sterile inoculating loops before the sample was transferred to Brain-Heart Infusion (BHI; HopeBio, Qingdao, China) agar plates.[24] For a full day, the plates were incubated at 28°C. To obtain pure colonies, dominant individual bacterial colonies were chosen after incubation and streaked onto fresh BHI agar plates for 24 hours under the same culture conditions. After being inoculated into 5 mL of BHI liquid media, a few selected individual colonies were grown for approximately 24 hours at 28°C with shaking at 200 rpm. After the bacterial culture was complete, aliquots were placed in 1.5 mL Eppendorf tubes and kept in an ultralow temperature freezer at −80°C. An equivalent volume of glycerol was then added. DN-3 is the designation given to the isolated strain of bacteria.

2.3. MORPHOLOGICAL OBSERVATION

Purified bacterial colonies were streaked onto fresh BHI agar medium and incubated at 28°C for approximately 24 h. After incubation, the colony morphology was assessed. Bacterial suspensions were prepared using phosphate-buffered saline (PBS; Procell, Wuhan, China) as the solvent, followed by Gram staining procedures.[24] To examine the ultrastructure of each colony under a scanning electron microscope, we fixed, dehydrated, dried, and coated the cells with gold sputtering agent (Hitachi, Tokyo, Japan).

2.4. 16S RRNA SEQUENCE ANALYSIS

Single bacterial colonies were selected and transferred to 1.5 mL Eppendorf tubes inside a biosafety cabinet. The Bacterial DNA Kit (Tiangen, Beijing, China) procedure was used to extract genomic DNA. Amplification of the 16S rRNA region of the isolated bacterial strain was conducted via polymerase chain reaction (PCR) utilizing universal 16S rRNA primers (27F: AGAGTTTGATCATGGCTCAG; 1492R: TACGGTTACCTTGTTACGACTT). A 25 μL reaction mixture comprising 12.5 μL of PCR mix, 1 μL of upstream and downstream primers at a concentration of 10 μmol/L, 1 μL of template DNA, and 9.5 μL of nuclease-free water was used for PCR. The settings for thermocycling were as follows: a five-minute initial denaturation at 94°C; thirty cycles of denaturation at 94°C for thirty seconds, one minute of annealing at 55°C, thirty seconds of extension at 72°C, and five minutes of final elongation at 72°C.

The amplified products were electrophoresed on a 1% agarose gel for verification. Sequence homology analysis was performed on the resulting sequences using the National Center for Biotechnology Information Basic Local Alignment Search Tool (BLAST, www.ncbi.nlm.nih.gov). MEGA 11.0 created a phylogenetic tree via the neighbor-joining (NJ) technique. Next, 1000 bootstrap replications were used to confirm the confidence level of the trees.

2.5. ANTIMICROBIAL SUSCEPTIBILITY ASSAY

After the experimental bacterial strains were cultured on an orbital shaker, the bacterial concentration was standardized with aseptic PBS. A 100 μL aliquot of the bacterial suspension was uniformly spread across the surface of a Mueller–Hinton (MH) agar plate within the confines of a biosafety cabinet. Following thorough absorption of the bacterial inoculum into the agar medium, prearranged antibiotic disks were meticulously placed on the surface of the agar plate. The plate was incubated at a consistent 28°C for 24 h in an inverted orientation. After incubation, the diameters of the inhibition zones were accurately measured in mm and interpreted for antimicrobial susceptibility, as delineated in the guidelines provided by the antibiotic sensitivity test kit.

2.6. REINFECTION EXPERIMENTS

After being separated and refined, the DN-3 bacterial strain was cultivated at 30°C with agitation at 200 rpm in liquid BHI medium. After the bacterial culture reached an optical density (OD) of 0.5, the supernatant was discarded, and the culture was centrifuged for two minutes at 4,000 rpm. The process was carried out three times, aseptically washing the bacterial pellet with PBS. Subsequently, the bacterial con-

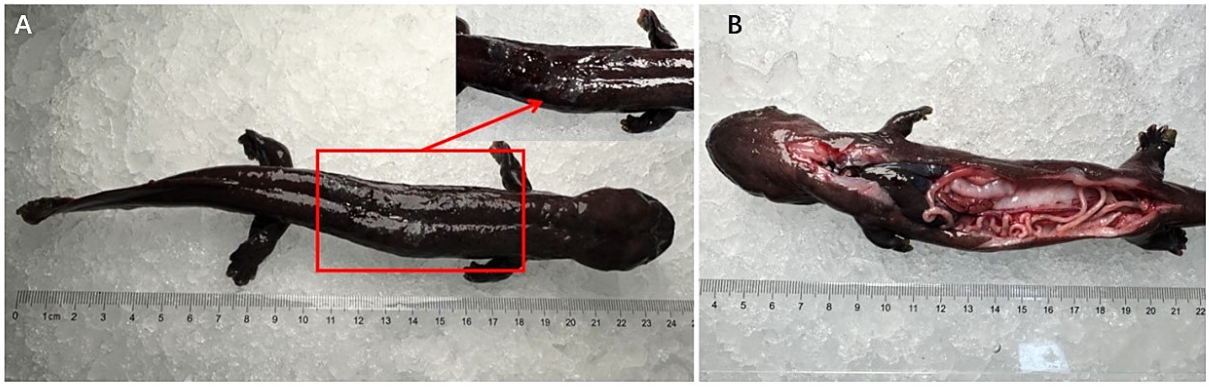

Figure 1. Diseased *A. davidianus*. The left panel shows the dissected internal structures, and the right panel shows the clinical symptoms of internal organs. A shows the individual's length, and the foci are indicated by arrows. B shows the dissected internal structures.

centration was adjusted to 1×10^4, 1×10^5, 1×10^6, or 1×10^7 CFU/mL using aseptic PBS. The specimens of *A. davidianus* temporarily housed in a laboratory setting were arbitrarily segregated into four groups, each consisting of 30 individuals. Intramuscular injections of various concentrations of *A. schindleri* bacterial suspensions were administered to *A. davidianus*, and all procedural steps were meticulously documented.

RESULTS

3.1. CLINICAL MANIFESTATIONS

The affected *A. davidianus* exhibited an average body length of approximately 24 cm, pronounced lethargy and reduced surface mucus secretion (Figure 1). Postmortem anatomical inspection revealed conspicuous pathological alterations, including hepatic tissues exhibiting a darkened hue, mild darkening of the spleen, and nominal intestinal hemorrhage. discernible swelling of the internal organs was also observed.

3.2. HISTOPATHOLOGICAL ANALYSIS

Tissue sections of renal, splenic, and intestinal organs were procured from afflicted and healthy *A. davidianus* for microscopic evaluation. The healthy subjects' renal tissue architecture was intact, exhibiting normal intercellular spacing and the absence of discernible tissue edema (Figure 2A). Conversely, the afflicted *A. davidianus* renal tissues exhibited prominent vacuolization, accompanied by widened interstitial spaces between the glomerular and tubular components (Figure 2D). In the context of splenic tissues, the healthy specimens exhibited closely packed lymphocytes and a comparatively well-organized structural morphology (Figure 2B). In contrast, the splenic tissue of the afflicted specimens demonstrated a notable decrease in lymphocyte density, widespread cellular necrosis, and a filamentous, loosely arranged structure (Figure 2E). For intestinal tissues, the mucosal, submucosal, and serosal layers and epithelial cells of healthy *A. davidianus* plants were cultured under optimal conditions and characterized by closely arranged cells (Figure 2C). However, histological sections of intestinal tissues from afflicted *A. davidianus* showed signs of inflammatory infiltration in the mucosal and submucosal layers, laxity in the serosal layer, and extensive necrosis and disruption of the mucosal epithelial cells (Figure 2F).

3.3. COLONY MORPHOLOGY AND BACTERIAL CHARACTERISTICS

After 24 h of incubation on BHI agar plates, the DN-3 strain yielded colonies with a pale-yellow coloration, a diameter ranging from approximately 0.8–1.4 mm, and a shiny surface. Gram-staining assays confirmed that the organism was gram-positive. Scanning electron microscopy revealed that the DN-3 bacterial cells possessed a smooth surface devoid of any protuberances or indentations, conforming to a bacillus-shaped morphology and growing as individual cells (**Figure 3**).

3.4. 16S RRNA IDENTIFICATION

PCR was used to amplify the 16S rRNA fragment of the isolated DN-3 strain. The resulting sequences were subjected

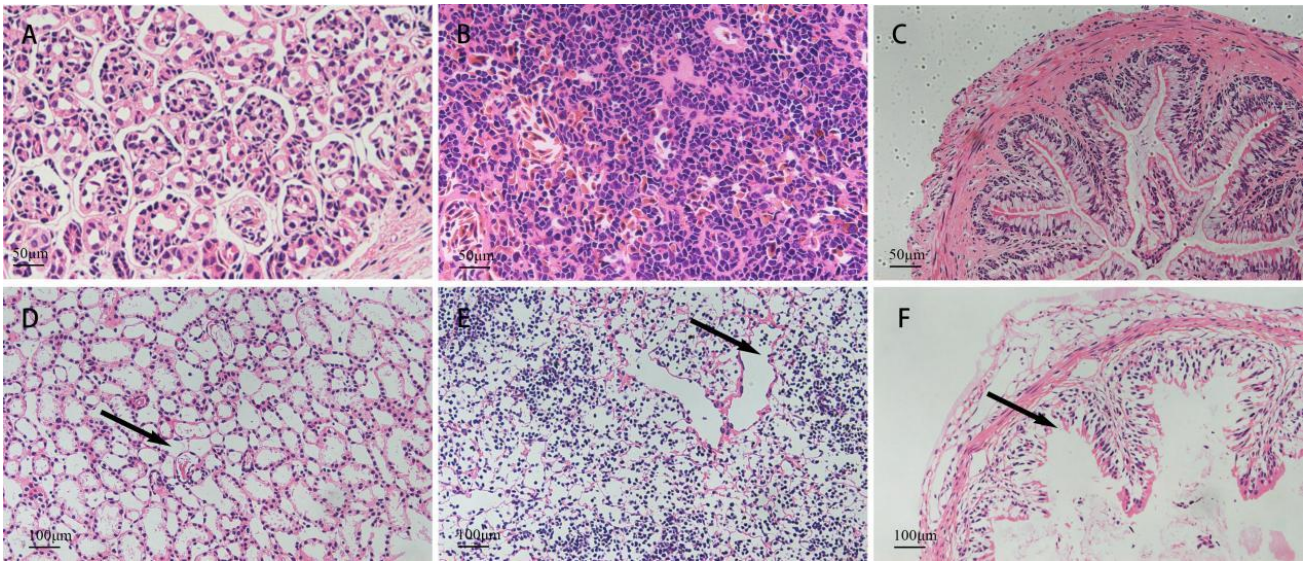

Figure 2. Pathological observation of *A. davidianus* tissue (healthy scale bar: 50 μm; diseased scale bar: 100 μm). A: Kidney tissue of healthy *A. davidianus*. B: Spleen tissue of healthy *A. davidianus*. C: Intestinal tissue of healthy *A. davidianus*. D: Kidney tissue of diseased *A. davidianus*. E: Spleen tissue of diseased *A. davidianus*. F: Intestine tissue of diseased *A. davidianus*.

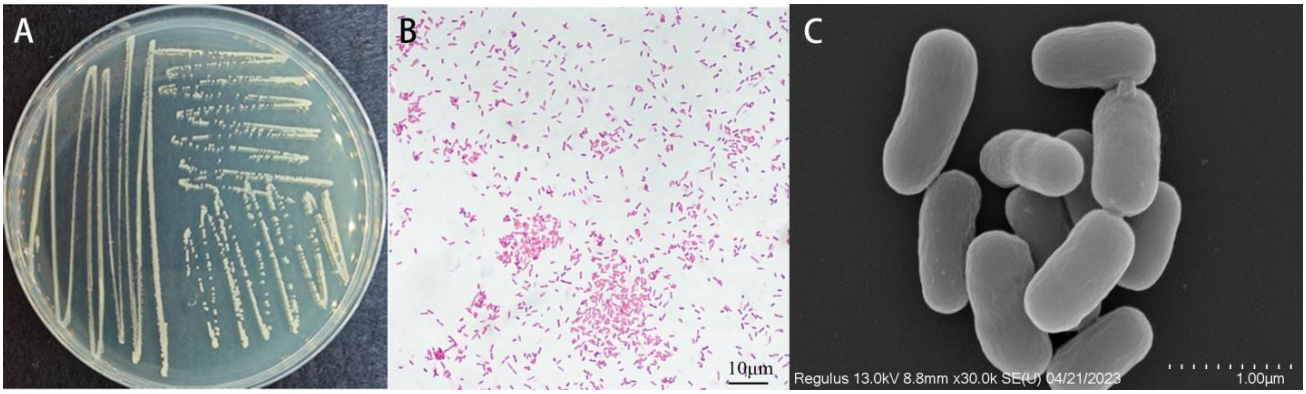

Figure 3. Morphological characteristics of DN-3 cells. (B) Gram staining of DN-3 cells (scale bar: 10 μm). (C) Scanning electron microscope image of DN-3 cells (scale bar: 1 μm).

to BLAST comparison against the NCBI database, which revealed 99% sequence similarity between the strains isolated from the liver of the afflicted salamander and *A. schindleri*. Phylogenetic trees were constructed using MEGA 11.0 software, which included nine other *Acinetobacter* species with high homology to the DN-3 strain, as indicated in Figure 4. The results demonstrated that the DN-3 strain (PP478637) exhibited 96% sequence similarity to *A. schindleri* with the GenBank accession number NR 025412.1, indicating close phylogenetic affinity (Figure 4).

3.5. BIOCHEMICAL IDENTIFICATION OF BACTERIA

The DN-3 strain was subjected to identification utilizing the Biolog Automated Microbial Identification System. The resultant data confirmed that DN-3 belongs to the *A. schindleri* family, and its physiological and biochemical markers are presented in **Table 1**.

3.6. ANTIMICROBIAL SUSCEPTIBILITY TESTING

Fourteen antimicrobial agents were selected for susceptibility testing against the DN-3 strain, as directed by the instructions provided by the antimicrobial susceptibility testing kit (**Table 2**). The assay results indicated that the DN-3 strain exhibited high sensitivity to six drugs: neomycin, midecamycin, doxycycline, piperacillin, nitrofurantoin, and carbenicillin. Additionally, the fungus exhibited moderate sensitivity to vancomycin and resistance to seven drugs, namely, erythromycin, gentamicin, flurbiprofen, streptomycin, norfloxacin, ciprofloxacin, and sulfonamides.

R, resistant; S, sensitive; I, intermediate sensitivity.

3.7. REINFECTION EXPERIMENT

Healthy *A. davidianus* were inoculated with varying concentrations of the DN-3 bacterial suspension. No fatalities were observed in the control group (Figure 5). The fish in-

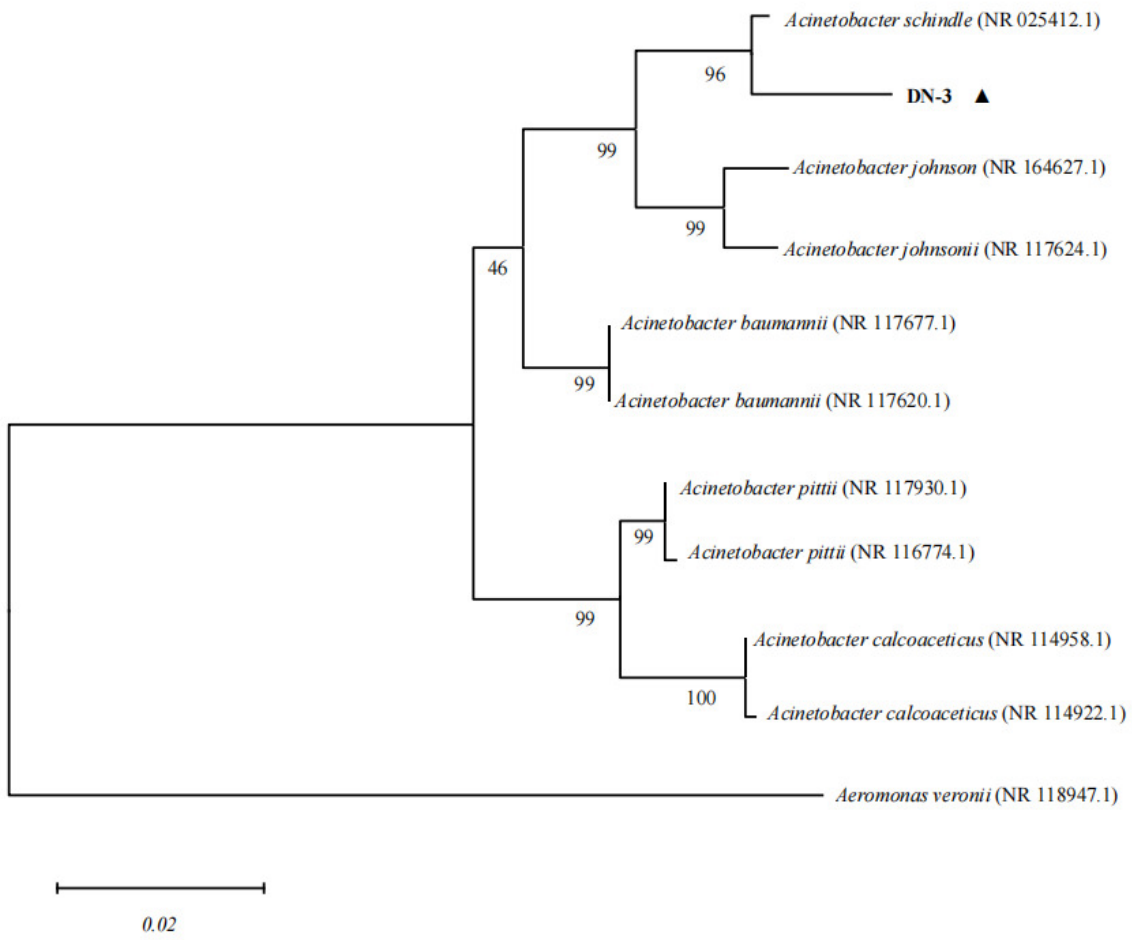

Figure 4. Phylogenetic analysis of the 16S rRNA gene sequence of isolate DN-3. The 16S rRNA gene sequences of nine members of the genus *Acinetobacter* and the isolate DN-3 were compared (the sequences were taken from the NCBI database). MEGA 11.0 was used to create the phylogenetic tree via the neighbor-joining technique. The number of branches represents the percentage of bootstrap values for a thousand replicates. The number of substitutions per site is displayed on the scale bar.

jected with DN-3 died on the third day post-injection. A 100% mortality rate occurred under the 1×10^7 CFU/mL treatment with DN-3. The median lethal dose (LD_{50})[16] of *A. schindleri* DN-3 for *A. davidianus* was calculated to be 6.25×10^4 CFU/mL. Bacterial isolates obtained from the infected group were subsequently identified, confirming the pathogenicity of the bacteria.

DISCUSSION

Acinetobacter species have been detected more frequently in animals due to the significant increase in pathogenic bacteria infecting them. Nevertheless, less focus has been placed on the infection status and prevalence of resistance in *Acinetobacter* species from animals.[16] In this study, diseased *A. davidianus* from a farm in Zhangjiajie were examined, and the isolated strain DN-3 was identified as *A. schindleri* through morphology staining, 16S rRNA sequencing, biochemical identification, and regression infection experiments. This is the first report of *A. schindleri* infection in *A. davidianus*, and this strain has caused many deaths. The LD_{50} of *A. avidianus* DN-03 was confirmed to be similar to that of *A. baumannii*,[25] *A. johnsonii*,[26] *A. lwoffii*,[27] etc., causing pathologic lesions in several organs and tissues and exhibiting strong pathogenicity. *Acinetobacter* species can be transmitted between humans and animals.[17,28] *A. schindleri,* which poses a zoonotic threat of pathogenicity, may be transmitted among breeders, transporters, and giant salamanders. This risk is particularly pronounced in Zhangjiajie city, a well-developed hub for *A. davidianus* aquaculture and trade. Consequently, it is crucial to reinforce the surveillance, prevention, and management of *A. schindleri* and related pathogens to curtail the mortality risk posed to the giant salamander population.

A. schindleri has recently been isolated and found in ungulates,[29] fish,[30] and poultry,[31] revealing that the bacterium is widespread in the animal kingdom and that financial losses in the aquaculture sector frequently accompany its discovery. This bacterium causes red eye infection in the affected *Pangasius sutchi*, leading to symptoms such as gill damage, dermatitis, swollen and reddened eyes, or internal bleeding.[30] This showed a phenomenon of group outbreak and demonstrated strong pathogenicity. Like other *Acinetobacter* infections in this study,[25,26] *A. schindleri* in-

Table 1. Results of the biochemical identification of strain DN-3

	Reaction item	*Result*[1]		*Reaction item*	*Result*[1]
A1	1% NaCl	P	E1	L-Alanine	P
A2	1% Sodium Lactate	P	E2	L-Arginine	N
A3	3-Methyl Glucose	N	E3	L-Aspartic Acid	N
A4	4% NaCl	N	E4	L-Fucose	B
A5	8% NaCl	N	E5	L-Galactonic Acid Lactone	B
A6	Acetic Acid	P	E6	L-Glutamic Acid	B
A7	Acetoacetic Acid	P	E7	L-Histidine	N
A8	Acidic PH PH5	N	E8	Lincomycin	P
A9	Acidic PH PH6	P	E9	Lithium Chloride	B
A10	Aztreonam	P	E10	L-Malic Acid	P
A11	Bromo-Succine-Acid	P	E11	L-Pyroglutamic Acid	N
A12	Citric Acid	N	E12	L-Rhamnose	N
B1	D-Arabitol	N	F1	L-Serine	N
B2	D-Aspartic acid	N	F2	Methyl Pyruvate	P
B3	D-Cellobiose	N	F3	Minocycline	N
B4	D-Ducose	B	F4	Mucic Acid	N
B5	Dextrin	B	F5	Myo-inositol	N
B6	D-Fructose	N	F6	N-Acetyl Neuraminic	N
B7	D-Fructose-6-po4	B	F7	N-Acetyl-D-Galactosamine	N
B8	D-Galactonic Acid	N	F8	N-Acetyl-D-Glucosamine	N
B9	D-Galactose	B	F9	N-Acetyl-β-Maanosamine	N
B10	D-Galacturonic Acid	B	F10	Nalidixic Acid	P
B11	D-Glucose-6-po4	N	F11	Negative control	N
B12	D-Glucuronic Acid	B	F12	Niaproof 4	B
C1	D-Lactic Acid Methyl Ester	N	G1	Pectin	B
C2	D-Malic Acid	N	G2	P-Hydroxy-Phenylacetic Acid	N
C3	D-Maltose	N	G3	Positive control	P
C4	D-Mannitol	N	G4	Potassium Tellurite	P
C5	D-Mannose	N	G5	Propionic Acid	P
C6	D-Melibiose	N	G6	Quinic Acid	N
C7	D-Raffinose	N	G7	Rifamycin SV	P
C8	D-Saccharic Acid	N	G8	Sodium Bromate	N
C9	D-Salicin	N	G9	Sodium Butyrate	P
C10	D-Serine	B	G10	Stachyose	N
C11	D-Serine	N	G11	Sucrose	N
C12	D-Sorbitol	N	G12	Tetrazolium Blue	P
D1	D-Trehalose	N	H1	Tetrazolium Violet	P
D2	D-Turanose	N	H2	Troleandomycin	P
D3	Formic Acid	N	H3	Tween 40	P
D4	Fusidic Acid	N	H4	Vancomycin	B
D5	Gelatin	N	H5	α-D-Glucose	B
D6	Gentiobiose	B	H6	α-D-Lactose	N
D7	Glucuronamide	B	H7	α-Hydroxy- Butyric-Acid	B
D8	Glycerol	N	H8	α-Keto-Butyric acid	B
D9	Glycyl-L-Proline	N	H9	α-Keto-Glutaric acid	N
D10	Guanidine HCl	B	H10	β--Hydroxy-D, LButyric-Acid	B

	Reaction item	*Result*[1]		*Reaction item*	*Result*[1]
D11	Inosine	N	H11	β-Methyl-D-Glucoside	N
D12	Lactic Acid	P	H12	γ-Amino-Butyric-Acid	B

[1] Abbreviations: P, positive; B, borderline; N, negative.

Table 2. Drug sensitivity tests of the DN-3 strain.

Medicine Name	*Inhibition Zone (mm)*	*Sensitivity*	*Medicine Name*	*Inhibition Zone (mm)*	*Sensitivity*
erythromycin	6	R	Vancomycin	14	I
gentamycin (antibiotic)	6	R	Neomycin	16	S
florfenicol	6	R	Medithromycin	19	S
Streptomycin	8	R	Doxycycline	24	S
Norfloxacin	6	R	Piperacillin	25	S
Ciprofloxacin	6	R	Furazolidone	17	S
Sulfanilamide	6	R	Carbenicillin	23	S

R, resistant; S, sensitive; I, intermediate sensitivity.

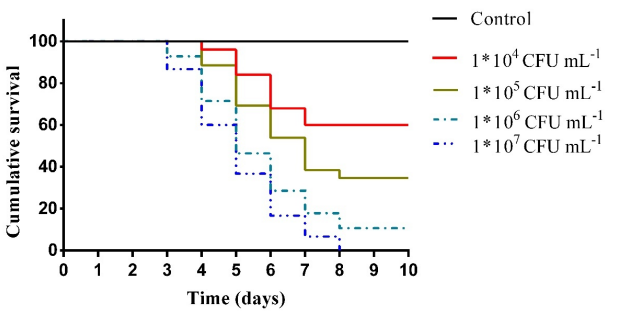

Figure 5. *A. davidianus* regression infection analysis with isolate DN-3. Control group: Intraperitoneal injection of 0.5 mL of sterile PBS. Test group: 0.5 mL of solution of various concentrations of bacteria was intraperitoneally injected (1×10^4, 1×10^5, 1×10^6, or 1×10^7 CFU/mL). Ten days were spent monitoring both groups.

duced salamander skin inflammation, internal organ enlargement, intestinal hemorrhage, secondary infections, and even death. These factors have made it challenging to prevent and control diseases in the breeding of *A. davidianus* because they are all drug-resistant.

One of the most severe public health issues in the 21st century is antimicrobial resistance, which threatens the efficient prevention and treatment of many diseases caused by pathogenic organisms.[32] *Acinetobacter*, clinically induces a multiorgan inflammatory response in humans, triggering other diseases, and is often drug-resistant.[33] *Acinetobacter* species of animal pathogens typically cause severe tissue damage to key organs, such as the kidney, liver, spleen, heart, and muscle.[34] For instance, renal tubular epithelial cells of pathogenic *A. davidianus* exhibit vacuolar degeneration in the liver and macrophage infiltration (Figure 2D). This lesion adversely affects the health of *A. davidianus*, leading to weakened immunity, skin inflammation, cell necrosis, intestinal hemorrhage, and subsequent infection, ultimately resulting in death. *A. schindleri* has been shown to infect humans and animals, but this organism has been poorly characterized in antibiotic susceptibility studies of aquatic animals.[24,35] Clinical trials have shown that *A. schindleri* is sensitive to antibiotics such as beta-lactams, aminoglycosides, chloramphenicol, and tetracyclines, among others.[35] In the present study, the *A. davidianus* DN-3 strain was sensitive to neomycin, mesomycin, doxycycline, piperacillin, nitrofurantoin, and carbenicillin, similar to clinically sensitive drugs. Such consequences pose significant challenges to successfully breeding and maintaining healthy *A. davidianus* populations.

Zhangjiajie City, situated in Hunan Province, is renowned as the "Home of the Chinese Giant Salamander," serving as a critical hub for distributing and producing these wild creatures in China.[36-38] Numerous farmers engage in the artificial rearing and trading of this species. According to incomplete statistics, there are over a hundred aquaculture enterprises of *A. davidianus* in Zhangjiajie, with a cultivation volume exceeding one million[22]. This makes it a critical trading hub and production base for *A. davidianus* in China.[21,39] However, as *A. davidianus* farming scales up and becomes more intensive, it faces a surge of various diseases, particularly newly emerging ones, inflicting substantial economic losses on the industry.[40,41] The research into salamander diseases lags, with the primary approach being the application of fish disease treatments, which have proven largely ineffective.[21,40] Consequently, it is crucial to delve deeper into the pathogenic testing and pathological analysis of newly discovered, highly pathogenic bacteria in salamanders, such as *A. schindleri*.[42] This will enable the development of scientific medication and the formulation of prevention and control measures. By doing so, we can minimize the occurrence of diseases to the greatest extent and effectively control their spread.

CONCLUSIONS

Animal-origin *A. schindleri* strains are poorly understood, but they pose a serious risk to human and animal health.[17, 25] The bacterium *A. schindleri* DN-3 was isolated in this study from *A. davidianus* via physiological and biochemical identification, 16S rRNA gene sequencing, and scanning electron microscopy. The LD_{50} for *A. davidianus* was determined to be 6.25×10^4 CFU/mL. This pathogen exhibited a high level of pathogenicity, leading to internal bleeding in *A. davidianus* individuals. The findings of this investigation will provide valuable insights into identifying and managing infections caused by *A. schindleri* in *A. davidianus*. This will pave the way for developing scientific medications and formulating effective prevention and control measures.

ACKNOWLEDGMENTS

We are very grateful to the Yangtze River Fisheries Research Institute, Chinese Academy of Fishery Sciences, for helping us with the experiments in this paper.

AUTHORS' CONTRIBUTION

Conceptualization: Cheng Wang (Equal), Yong Zhou (Equal), Ying Wei (Equal). Formal Analysis: Cheng Wang (Equal), Yong Zhou (Equal). Investigation: Cheng Wang (Equal), Yixing Xie (Equal), Zhiyong Deng (Equal). Methodology: Cheng Wang (Equal), Yixing Xie (Equal), Yong Zhou (Equal). Resources: Cheng Wang (Equal), Yixing Xie (Equal). Software: Cheng Wang (Equal), Yixing Xie (Equal). Validation: Cheng Wang (Equal), Yong Zhou (Equal), Ying Wei (Equal). Writing – original draft: Cheng Wang (Lead). Writing – review & editing: Cheng Wang (Equal), Yong Zhou (Equal), Ying Wei (Equal). Supervision: Huanyan Yuan (Equal), Mingzhu Tian (Equal), Pan Mao (Equal). Funding acquisition: Ying Wei (Lead). Project administration: Ying Wei (Lead).

COMPETING OF INTEREST – COPE

No competing interests were disclosed.

ETHICAL CONDUCT APPROVAL – IACUC

The article adheres to the Convention on Biological Diversity and the Convention on Trade in Endangered Species of Wild Fauna and Flora Research.

INFORMED CONSENT STATEMENT

All authors and institutions have confirmed this manuscript for publication.

DATA AVAILABILITY STATEMENT

All are available upon reasonable request.

Submitted: January 20, 2024 CDT, Accepted: March 18, 2024 CDT

REFERENCES

1. Fei L, Hu S, Ye S, Huang Y. *Fauna Sinica (Amphibia I)*. Science Press; 2006.

2. Wang J, Zhang H, Xie F, Wei G, Jiang JP. Genetic bottlenecks of the wild Chinese giant salamander in karst caves. *Asian Herpetological Research*. 2017;8:174-183. doi:10.16373/j.cnki.ahr.170038

3. Wang X, Zhang K, Wang Z, Ding Y, Wu W, Huang S. The decline of the Chinese giant salamander *Andrias davidianus* and implications for its conservation. *Oryx*. 2004;38(2):197-202. doi:10.1017/s0030605304000341

4. Lu C, Chai J, Murphy RW, Che J. Giant salamanders: farmed yet endangered. *Science*. 2020;367(6481):989-989. doi:10.1126/science.abb2375

5. Liang G, Geng B, Zhao E. *Andrias Davidianus. The IUCN Red List of Threatened Species*. IUCN; 2004. doi:10.2305/iucn.uk.2004.rlts.t1272a3375181.en

6. Isaac NJB, Redding DW, Meredith HM, Safi K. Phylogenetically-informed priorities for amphibian conservation. *PLoS ONE*. 2012;7(8):e43912. doi:10.1371/journal.pone.0043912

7. Cunningham AA, Turvey ST, Zhou F, et al. Development of the Chinese giant salamander *Andrias davidianus* farming industry in Shaanxi Province, China: conservation threats and opportunities. *Oryx*. 2016;50(2):265-273. doi:10.1017/s0030605314000842

8. Chen S, Cunningham AA, Wei G, et al. Determining threatened species distributions in the face of limited data: Spatial conservation prioritization for the Chinese giant salamander (*Andrias davidianus*). *Ecology and Evolution*. 2018;8(6):3098-3108. doi:10.1002/ece3.3862

9. He D, Zhu W, Zeng W, et al. Nutritional and medicinal characteristics of Chinese giant salamander (Andrias davidianus) for applications in healthcare industry by artificial cultivation: A review. *Food Science and Human Wellness*. 2018;7(1):1-10. doi:10.1016/j.fshw.2018.03.001

10. Zhang K, Wang X, Wu W, Wang Z, Huang S. Advances in conservation biology of Chinese giant salamander. *Biodiversity Science*. 2002;10(3):291-297. doi:10.17520/biods.2002040

11. Wu YA, Liang ZQ, Wang DW. *The Ecological Breeding of Chinese Giant Salamander*. Hunan Science and Technology Press; 2015:1-145.

12. Wang WB, Liu P, Dou LL, et al. A survey of Chinese giant salamander (*Andrias davidianus*). *Acta Hydrobiologica Sinica*. 2021;45:464-472. doi:10.7541/2021.2020.184

13. Yu Z, Jiang H, Zhong L, Xiao K, Tan Q, Mao P. Isolation and identification of pathogenic bacteria from giant salamander with bacterial infection syndrome and drug sensitivity analysis. *Journal of Natural Science of Hunan Normal University*. 2012;35:74-79.

14. Baumann P. Isolation of *Acinetobacter* from soil and water. *J Bacteriol*. 1968;96(1):39-42. doi:10.1128/jb.96.1.39-42.1968

15. Al Atrouni A, Joly-Guillou ML, Hamze M, Kempf M. Reservoirs of non-baumannii Acinetobacter species. *Front Microbiol*. 2016;7. doi:10.3389/fmicb.2016.00049

16. Wong D, Nielsen TB, Bonomo RA, Pantapalangkoor P, Luna B, Spellberg B. Clinical and pathophysiological overview of Acinetobacter infections: a century of challenges. *Clin Microbiol Rev*. 2017;30(1):409-447. doi:10.1128/cmr.00058-16

17. Visca P, Seifert H, Towner KJ. *Acinetobacter* infection – an emerging threat to human health. *IUBMB Life*. 2011;63(12):1048-1054. doi:10.1002/iub.534

18. Kalidas R, Rajdeep S. Multidrug-resistant acinetobacter infection and their susceptibility patterns in a tertiary care hospital. *Niger Med J*. 2012;53(3):126-128. doi:10.4103/0300-1652.104379

19. Müller S, Janssen T, Wieler LH. Multidrug resistant Acinetobacter baumannii in veterinary medicine—emergence of an underestimated pathogen. *Berl Münch Tierärztl Wochenschr*. 2014;127:435-446. doi:10.2376/0005-9366-127-43

20. Worthylake KM, Hovingh P. Mass mortality of salamanders (Ambystoma tigrinum) by bacteria (Acinetobacter) in an oligotrophic seepage mountain lake. *The Great Basin naturalist*. 1989;49:364-372. doi:10.5962/bhl.part.22646

21. Jiang W, Lan X, Wang J, Xiang, Tian H, Luo Q. Recent progress in the germplasm resources conservation and utilization of the Chinese giant salamander (*Andrias davidianus*). *Journal of fisheries of China*. 2022;46(4):683-705. doi:10.11964/jfc.20210812993

22. Lan X, Luo Q, Wang J, et al. Impacts of COVID-19 and countermeasures onto the captive-breeding industry of Chinese giant salamander in Zhangjiajie. *China Academic Journal Electronic*. 2022;40(3):76-83.

23. Geng Y, Wang KY, Zhou ZY, et al. First report of a ranavirus associated with morbidity and mortality in farmed Chinese giant salamanders (*Andrias davidianus*). *Journal of Comparative Pathology*. 2011;145(1):95-102. doi:10.1016/j.jcpa.2010.11.012

24. Reed LJ, Muench H. A simple method of estimating fifty per cent endpoints. *Am JHyg*. 1938;27(3):493-497. doi:10.1093/oxfordjournals.aje.a118408

25. Behera BK, Paria P, Das A, Bhowmick S, Sahoo AK, Das BK. Molecular characterization and pathogenicity of a virulent Acinetobacter baumannii associated with mortality of farmed Indian Major Carp Labeo rohita (Hamilton 1822). *Aquaculture*. 2017;471:157-162. doi:10.1016/j.aquaculture.2017.01.018

26. Bi B, Yuan Y, Jia D, et al. Identification and pathogenicity of emerging fish pathogen acinetobacter johnsonii from a disease outbreak in Rainbow Trout (*Oncorhynchus mykiss*). *Aquaculture Research*. Published online February 4, 2023:1995494. doi:10.1155/2023/1995494

27. Cao S, Geng Y, Yu Z, et al. *Acinetobacter lwoffii*, an emerging pathogen for fish in*Schizothorax*genus in China. *Transbound Emerg Dis*. 2018;65(6):1816-1822. doi:10.1111/tbed.12957

28. Wilharm G, Skiebe E, Higgins PG, et al. Relatedness of wildlife and livestock avian isolates of the nosocomial pathogen *Acinetobacter baumannii* to lineages spread in hospitals worldwide. *Environmental Microbiology*. 2017;19(10):4349-4364. doi:10.1111/1462-2920.13931

29. Mlynarcik P, Bardon J, Sedlakova MH, Prochazkova P, Kolar M. Identification of novel OXA-134-like β-lactamases in Acinetobacter lwoffii and Acinetobacter schindleri isolated from chicken litter. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub*. 2019;163(2):141-146. doi:10.5507/bp.2018.037

30. Reddy MRK, Mastan SA. Emerging Acinetobacter schindleri in red eye infection of Pangasius sutchi. *Afr J Biotechnol*. 2013;12:6992-6996. doi:10.5897/AJB2013.12342

31. Smet A, Cools P, Krizova L, et al. Acinetobacter gandensis sp. nov. isolated from horse and cattle. *International journal of systematic and evolutionary microbiology*. 2014;64(Pt_12):4007-4015. doi:10.1099/ijs.0.068791-0

32. Dadgostar P. Antimicrobial resistance: implications and costs. Infection and Drug Resistance. *IDR*. 2019;12:3903-3910. doi:10.2147/idr.s234610

33. Montaña S, Palombarani S, Carulla M, et al. First case of bacteraemia due to Acinetobacter schindleri harbouring bla NDM-1 in an immunocompromised patient. *New Microbes and New Infections*. 2018;21:28-30. doi:10.1016/j.nmni.2017.10.004

34. Cao S, Geng Y, Yu Z, et al. *Acinetobacter lwoffii*, an emerging pathogen for fish in*Schizothorax*genus in China. *Transbound Emerg Dis*. 2018;65(6):1816-1822. doi:10.1111/tbed.12957

35. Dortet L, Legrand P, Soussy CJ, Cattoir V. Bacterial identification, clinical significance, and antimicrobial susceptibilities of *Acinetobacter ursingii* and *Acinetobacter schindleri*, two frequently misidentified opportunistic pathogens. *J Clin Microbiol*. 2006;44(12):4471-4478. doi:10.1128/jcm.01535-06

36. Zhang KJ, Wang XM, Wu W, Wang ZH, Huang S. Advances in conservation biology of Chinese giant salamander. *Biodiversity Science*. 2002;10(3):291-297. doi:10.17520/biods.2002040

37. Wang X, Zhang K, Wang Z, Ding Y, Wu W, Huang S. The decline of the Chinese giant salamander *Andrias davidianus* and implications for its conservation. *Oryx*. 2004;38(2):197-202. doi:10.1017/s0030605304000341

38. Luo Q, Liu Y, Zhang L, Chen G, Kang L. Investigaton on resources of Chinese Giant Salmander in Zhangjiajie City. *Sichuan Joumal of Zoology*. 2009;28:422-267.

39. Luo Q, Liu Y, Zhang L. Effectiveness of releasing artificially-bred Chinese giant salamander (Andrias davidianus) into the wild in Zhangjiajie, Hunan. *Biodiversity Science*. 2009;17(3):310-317. doi:10.3724/SP.J.1003.2009.09041

40. Wang W, Liu P, Dou L, et al. A Survey of Chinese Giant Salamander (*Andrias davidianus*). *Acta Hydribiologica Sinica*. 2021;45(2):464-472. doi:10.7541/2021.2020.184

41. Qi Z, Zhang Q, Wang Z, et al. Transcriptome analysis of the endangered Chinese giant salamander (Andrias davidianus): immune modulation in response to Aeromonas hydrophila infection. *Veterinary Immunology and Immunopathology*. 2016;169:85-95. doi:10.1016/j.vetimm.2015.11.004

42. Li W, Luo L, Li H, et al. Advance on the artificial breeding technologies of Chinese giant salamander (*Andrias davidianus*). *Chin Fish Qual Stand*. 2018;8(5):18-24. doi:10.3969/j.Issn.2095-1833.2018.05.003