

<Case Report>

Isolation of a zoonotic pathogen *Aeromonas hydrophila* from freshwater stingray (*Potamotrygon motoro*) kept in a Korean aquarium with ricefish (*Oryzias latipes*)

Saekil Yun^{1,†}, Young-Ran Lee^{1,2,†}, Sib Sankar Giri¹, Hyoun Joong Kim¹, Cheng Chi¹, Sang Guen Kim¹, Sang Wha Kim¹, Jin Woo Jun^{1,*}, Se Chang Park^{1,*}

¹Laboratory of Aquatic Biomedicine, College of Veterinary Medicine and Research Institute for Veterinary Science, Seoul National University, Seoul 08826, Korea

²Lotteworld Aquarium, Seoul 05551, Korea

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Abstract: In the present study, *Aeromonas (A.) hydrophila* was isolated from a captive-bred adult freshwater stingray (*Potamotrygon motoro*) reared at a commercial aquarium in Korea. The stingray had bites on its fins, hemorrhages on the ventral part, and congested internal organs. A bacterium was isolated from kidney and subsequently identified as *A. hydrophila*. Based on phylogenetic analysis results, the isolate in the present study (SNUAh-LA1) was most closely related to *A. hydrophila* AH10 (China) and *A. hydrophila* AKR1 (Korea). It is most likely that the pathogen infection resulted from *Potamotrygon motoro* cohabiting with ricefish (*Oryzias latipes*).

Keywords: *Aeromonas hydrophila*, *Potamotrygon motoro*, aquarium, freshwater stingray

Freshwater stingray (*Potamotrygon motoro*) belongs to the *Potamotrygonidae* family, inhabits in temperate and tropical sea throughout the world, including Atlantic rivers of South America, Equatorial Africa, Indo-China river system and the Mekong river of Laos [4]. Freshwater stingrays are frequently collected in the aquarium trade while more than 60,000 specimens are sold worldwide. In addition, more specimens have been added to the captive breeding market of Asia due to the diminished shipping costs and rise in commodity value in accordance with the growing market scale [8].

Aeromonas (A.) hydrophila can cause diseases in a numerous fish species, including grass carp (*Ctenopharyngodon idella*) [10], channel catfish (*Ictalurus punctatus*), tilapia (*Sarotherodon niloticus*) [2], and higher vertebrates [7]. Motile *Aeromonas* septicemia (MAS), which is a representative disease caused by *A. hydrophila* infection, causes symptoms such as hemorrhagic septicemia, infectious abnormal dropsy, exophthalmia, and fin and tail rot [3].

Herein, we report the isolation of *A. hydrophila* from the internal organs of captive-bred adult freshwater stingray housed at a commercial aquarium in Seoul, Korea. The freshwater stingray imported from Peru, was exhibited in a 6,000 L glass aquarium along with ricefish (*Oryzias latipes*) cap-

tured from Korea. The fish were fed properly and water temperature was maintained at $25 \pm 2^\circ\text{C}$. The water quality parameters such as dissolved oxygen, pH, and ammonia were also maintained properly. However, one of the stingrays (body-length 33 cm, body-weight 1.6 kg, total 16 of stingrays were reared in the tank) was found dead in the month of December, 2015. Dead fish showed no specific symptoms, and the other stingrays have showed no mortality so far. It was submitted to the College of Veterinary Medicine, Seoul National University for post-mortem analysis.

Fish was dissected and sterile swabs from kidney, liver, and spleen were streaked onto tryptic soy agar (TSA) (Difco, USA) plates, and incubated at 25°C for 24 h. Microbial growth was observed in plates streaked with kidney swab. It seems that single strain of bacterial colony was observed in the plate with kidney swab. The colony was re-streaked onto fresh TSA plates to obtain pure isolates. Gram-staining, oxidase test, and motility tests were performed for preliminary identification.

Vitek System2 (bioMérieux, France) was used for the identification and further characterization of the most prominent isolate SNUAh-LA1 called in this study. For 16S rRNA gene sequence, bacterial culture was sent to the genomic division

*Corresponding authors

Tel: +82-2-880-1282, Fax: +82-2-880-1213

E-mails: parksec@snu.ac.kr (SC Park), advancewoo@snu.ac.kr (JW Jun)

†The first two authors contributed equally to this work.

of Macrogen (Korea) where nucleotide sequencing reaction was performed by using ABI PRISM 3730XL Analyzer with BigDye (R) Terminator v3.1 Cycle Sequencing Kits (Applied Biosystems, USA). For accurate identification of the pathogen, species-specific multiplex PCR assay was performed [9]. After the performing the Vitek System2 analysis, the result indicated that the isolated bacteria as *A. hydrophila* or *Aeromonas caviae*. However, the multiplex PCR analysis revealed that the bacterial isolate on TSA was *A. hydrophila*. The antimicrobial sensitivity of the isolate was performed against 13 antimicrobial agents by disk diffusion method as recommended by the Clinical and Laboratory Standard Institute (USA) [5]. The 16S rRNA sequence of the bacterial strain acquired in this study was aligned with bacterial nucleotide sequence data deposited at GenBank database (the National Center for Biotechnology Information, USA). The obtained sequence highly resembled with nucleotide of the same bacterial species (KC904095, AF099021, GU204971, AM184262, GQ184148, KF358435, NR074841, CP011100, FJ462702), was aligned further using Clustal W (the Con-

way Institute UCD Dublin, Ireland) and analyzed with MEGA6 [11]. Phylogenetic analysis was performed using minimum-evolution (ME) method, and bootstrap values were calculated for ME method with 1,000 replicates. The 16S rRNA sequence of *Aeromonas salmonicida* ATCC 33658 was used as outgroup.

In the postmortem examination, bites on the fins, hemorrhages on the ventral part and congested internal organs were observed (Fig. 1). During the mating season, males often follow females with their snout close to the female vent and subsequently bite the female body and fins [11]. The resultant mating wounds were easily spread over the body surface and fins, followed by the aggressive behavior of the species. Therefore, the consequence may create a possibility of infection by etiological pathogens.

Through phylogenetic analysis, the isolated SNUAh-LA1 was the most closely related to *A. hydrophila* AH10 and *A. hydrophila* AKR1, which were reported earlier from China and Korea, respectively (Fig. 2). The present study indicates, the isolate SNUAh-LA1 is geographically more related to China or Korea while other strains in phylogenetic tree are originated from Iran, Brazil and Malaysia. As the stingrays and ricefish were reared in the same tank, it is most likely get infected by pathogen which came with the ricefish and water. The pathogen was not susceptible to the ricefish. These bacteria do not always cause disease as *A. hydrophila* is an opportunistic pathogen and could survive in the various environments [6]. As assumed, the stingray was infected with the opportunistic pathogen *A. hydrophila* SNUAh-LA1 through the wounds that developed by its reproduction behavior. Since variety of bacterial strains from various regions could be gathered in the commercial aquarium, this circumstance could increase the risk of infection when fish is reared with other species. Any bacterial strain which is harmless in a specific region may act as a major etiological factor at other region. Thus, a commercial aquarium requires close supervision and firm control at all times.

In fact, virulence of the bacterial strain cannot be characterized without performing pathogenicity test. However, the assumption indicates that *A. hydrophila* SNUAh-LA1 probably was responsible for the mortality of the fish in the present study because this was only bacterial strain isolated from the

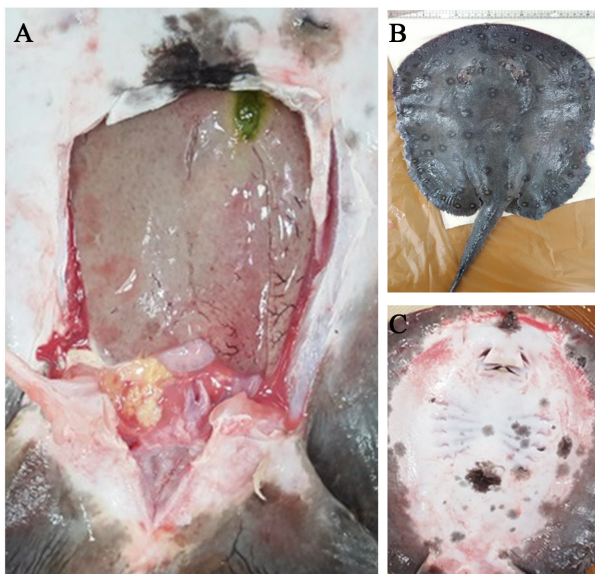


Fig. 1. Pictures of the postmortem examination. Congested internal organs (A), bites on the fins (B), and hemorrhages on the ventral part (C) were observed.

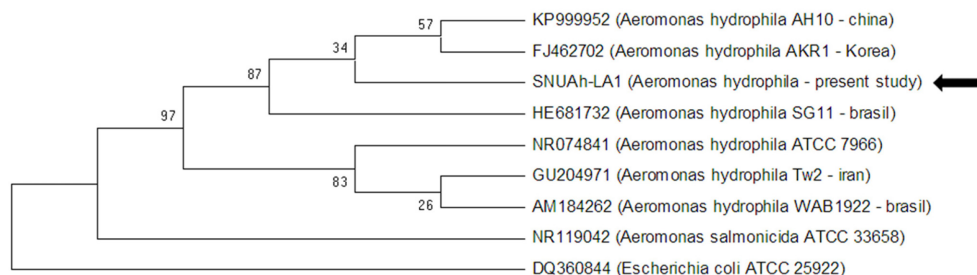


Fig. 2. Phylogenetic tree derived from minimum evolution analysis of the 16S rRNA sequences from *Aeromonas (A.) hydrophila*. Numbers at nodes indicate bootstrap confidence values (1,000 replicates). The 16S rRNA sequence of *Escherichia coli* ATCC 25922 and *A. salmonicida* ATCC 33658 were used as outgroup. The arrow represents *A. hydrophila* isolated in the present study.

Table 1. Antimicrobial susceptibility test of *A. hydrophila* isolated from the freshwater stingray

Antibiotic drugs (μg)	Results of susceptibility
Amikacin (30)	I
Amoxicillin/Clavulanic acid (30)	I
Ampicillin (10)	R
Cefotaxime (30)	S
Ceftazidime (30)	S
Ceftriaxon (30)	S
Ciprofloxacin (5)	S
Chloramphenicol (30)	S
Gentamicin (10)	S
Imipenem (10)	R
Meropenem (10)	R
Tetracycline (30)	I
Trimethoprim/sulfamethoxazole (25)	S

Each category for antibiotics (Oxoid, UK) was determined by zone diameter interpretive standards [5]. S, sensitive for antibiotic; R, resistant; I, intermediate.

organs of the dead fish. It is well studied that, *A. hydrophila* has pathogenicity to variety fishes as reported by several researchers [1, 2, 6].

In order to prevent mortality in such cases, staffs of a commercial aquarium should understand the patterns of fish behavior to avoid getting wounded. Disinfection and sterilization through chemicals or antibiotics are suggested in the case of consideration of rearing several fishes in a same tank. Performing the detection of the microorganism in aquatic environment for disinfection and sterilization is recommended, utilizing proper chemicals and antibiotics. The isolate was sensitive to the antibiotics cepheems, phenicols, tetracyclines, fluoroquinolones, folate pathway inhibitors, and Aminoglycosides, and showed resistant to penicillins and carbapenems (Table 1).

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