Short Communication

Severe Sepsis due to *Aeromonas aquariorum* in a Patient with Liver Cirrhosis

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SUMMARY: Thus far, *Aeromonas aquariorum* infection has been unrecorded in Korea. Herein, we report a fatal case of *A. aquariorum* infection in a 77-year-old male patient with liver cirrhosis. The bacterium isolated from a blood culture was initially mistaken as *Aeromonas hydrophila* using the Vitek2 identification system. In spite of intravenous ceftriaxone therapy, the patient was exacerbated by multiple organ dysfunction. By 4 days after admission, there was no hope for treatment or remission of symptoms and the patient was discharged. In the detailed microbiological investigations, the bacterium was identified as *A. aquariorum* harboring the *act* and *alt* genes, which encode cytotoxic and cytotoxic enterotoxins.

Aeromonas spp. are native to aquatic environments and can cause infections in a broad host range. *Aeromonas* infection has been implicated in a variety of clinical presentations in humans, ranging from mild conditions, including gastroenteritis and wound infections, to life threatening conditions, including septicemia and necrotizing fasciitis. In addition, the bacteria have been recognized as opportunistic pathogens leading to serious problems in patients with underlying diseases, such as cancer and liver cirrhosis (1).

The genus *Aeromonas* is a taxonomically complex group because of its phenotypic and genotypic diversity (1). Sixteen *Aeromonas* spp. were previously listed in Bergey's Manual of Systematic Bacteriology (2), and many new species have been continually described by genetic identification using housekeeping genes (*gyrB* and *rpoD*) from environmental and clinical isolates (1,3). Of these new species, *Aeromonas aquariorum* was originally isolated from ornamental fish in 2008 (3). More importantly, recent retrospective studies have reported a high prevalence of *A. aquariorum* among clinical human *Aeromonas* isolates (4–6), suggesting that it could be a human pathogen as important as *Aeromonas hydrophila*, *A. veronii*, and *A. caviae*. In Korea, *A. aquariorum* has been an unrecognized species in human and animal infection. However, *A. hydrophila*, *A. veronii*, *A. sobria*, and *A. caviae* are recognized as etiological agents of *Aeromonas* infections (7) because of inaccuracies of the present commercial systems generally used in microbiology laboratories for iden-

tification of *Aeromonas* spp. (1,7,8).

On August 8, 2010, a 77-year-old male presented to the emergency room of Gyeongsang National University Hospital. His clinical history revealed that he had periodically received care for liver cirrhosis due to alcoholism and hepatitis B virus infection since 2009. On admission, he presented with mental disorientation, general weakness, and dyspnea. His vital signs were as follows: blood pressure, 120/78 mm Hg; pulse rate, 102 beats/min; respiratory rate, 20 breaths/min; and body temperature, 36.4°C. In addition, a laboratory study revealed a leukocyte count of 15,760 cells/mL, hemoglobin level of 7.4 g/dL, platelet count of 105,000 cells/mL, serum BUN/creatinine levels of 26.1/2.01 mg/dL, aspartate aminotransferase of 150 IU/L, alanine aminotransferase of 9 IU/L, total bilirubin of 2.42 mg/dL, international normalized ratio of prothrombin time of 3.24, and an activated prothrombin time of 62.2 s. Plain abdominal erect images and chest computed tomography images were unremarkable. Brain computerized tomography revealed a small collection of subdural fluid. Two sets of blood samples collected from the patient on admission were shown to contain Gram-negative bacteria following microscopic examination using Gram staining. These bacteria were identified as either *A. hydrophila* or *A. caviae* using the Vitek2 automatic identification system with a GN card (BioMerieux, Marcy l'Etoile, France). Following this observation, we initiated intravenous (i.v.) antibiotic therapy with ceftriaxone (2 g/day); however, his symptoms progressed to multiple organ dysfunction syndrome. Dysuria was not improved by furosemide administration and supportive therapy, and mental disorientation advanced. Finally, the patient was discharged 4 days post-admission.

An initial culture from the venous blood sample identified the presence of Gram-negative bacilli. The Voges-Proskauer test was positive. These phenotypic tests,

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Fig. 1. Phylogenetic tree constructed by aligning 16S rRNA sequences (1,309 nt) derived from currently known *Aeromonas* spp. and the KBN1201762 strain isolated from the patient. Numbers at nodes indicate bootstrap values (percentages of 1,000 replicates). Bar, 0.002 substitutions per nucleotide position.

Fig. 2. Multilocus phylogenetic analysis (MLPA) tree constructed from alignments of three different genes (16S rRNA [1,309 nt], *gyrB* [877 nt], and *rpoD* [652 nt]) derived from the present and known *Aeromonas* spp. Numbers at nodes indicate bootstrap values (percentages of 1,000 replicates). Bar, 0.01 substitutions per nucleotide position.

including the Vitek2 system, lead to presumptive identification of the strain as *A. hydrophila*, which was maintained as KBN1201762 in the Culture Collection for Pathogens at Gyeongsang National University Hospital. The partial 16S rRNA gene sequences (1,309 nt) of the strain matched those of *A. aquariorum* (EU085557), *A. caviae* (HQ407268), and *A. hydrophila* (AB368776) as listed in the NCBI database. Phylogenetic trees generated using partial 16S rRNA sequences (Fig. 1) showed that the isolated strain clustered in an independent branch with *A. caviae*, *A. trota*, *A. aquariorum*, and *A. taiwanensis*, with a robust bootstrap value of 100%, when phylogeny was inferred using the neighbor-joining algorithm. In addition, phylogenetic analysis showed that the isolated strain sequence had a similarity of <99.8% to *A. caviae*, *A. trota*, *A. taiwanensis*, and *A. hydrophila*. However, the well-known *A. aquariorum* strain had a sequence similarity of 100% with the isolated strain. Detailed discrimination was performed by multilocus phylogenetic analysis (MLPA) using partial 16S rRNA, *gyrB*, and *rpoD* gene sequences (Fig. 2). In MLPA, the strain closest to the isolated bacterium was *A. aquariorum* (sequence divergence of 0.5%), followed by *A. hydrophila* (sequence divergence of 2.9%). The MLPA tree showed that the strain was distinctly separate from the independent lines of *A. trota*, *A. taiwanensis*, *A. sanarellii*, and *A. caviae* due to high levels of sequence divergence. On the other hand, the strain formed an independent phylogenetic line with *A. aquariorum* with robust bootstrap values of 100%. Based on these phylogenetic analyses, the isolated strain was identified as *A. aquariorum*. An antibiotic susceptibility test (AST) of the *A. aquariorum* strain was performed using the broth microdilution method (Sensititre panels; Trek Diagnostic Systems, Independence, Ohio, USA) for Gram-negative bacteria based on CLSI recommendations. The strain was susceptible to ciprofloxacin ($\leq 0.12 \mu\text{g/mL}$), imipenem ($\leq 2 \mu\text{g/mL}$), tetracycline ($\leq 2 \mu\text{g/mL}$), chloramphenicol ($\leq 2 \mu\text{g/mL}$), ceftriaxone ($\leq 1 \mu\text{g/mL}$), gentamicin ($\leq 1 \mu\text{g/mL}$), amikacin ($\leq 4 \mu\text{g/mL}$), nalidixic acid ($\leq 2 \mu\text{g/mL}$), and trimethoprim/sulfamethoxazole ($\leq 1/19 \mu\text{g/mL}$) but resistant to cephalothin ($\geq 128 \mu\text{g/mL}$), ampicillin ($\geq 128 \mu\text{g/mL}$), ampicillin/sulbactam ($16/8 \mu\text{g/mL}$), and ceftioxin ($\geq 128 \mu\text{g/mL}$). Enterotoxin genes (*act*, *alt*, and *ast*) were detected by PCR according to the method described by Nawaz et al. (9). The results showed that the isolated strain contained the *act* and *alt* genes, which encode cytotoxic and cytotoxic enterotoxins, respectively.

Partial sequences of the *A. aquariorum* KBN1201762 strain have been submitted to GenBank under following accession numbers; JX308270 for 16S rRNA, JX308268 for *gyrB* and JX308269 for *rpoD*.

Until 2007, clinicians regarded *A. aquariorum* as an unremarkable member of the *Aeromonas* genus (3). However, according to previous retrospective studies that aimed to isolate *Aeromonas* spp. using the house-keeping genes (*gyrB* or *rpoD* genes) from human clinical samples, the species may be a common pathogen that can be mistaken for *A. hydrophila*. The prevalence rates of *A. aquariorum* were 15.9%, 30.7%, and 50% among clinical *Aeromonas* strains phenotypically identified in

Taiwan (6), Australia (5), and Malaysia (4), respectively. However, this species cannot be identified with the current database in use with commercial phenotypic identification systems, such as the Vitek2 system (10). The present KBN1201762 strain was also misidentified as *A. hydrophila* by the Vitek2 system using the GN card. This misidentification can be explained either by non-enrollment of *A. aquariorum* in the Vitek2 system or by the genetic similarity between *A. aquariorum* and *A. hydrophila*. The Vitek2 database included only 5 different *Aeromonas* spp.: *A. salmonicida*, *A. hydrophila*, *A. veronii*, *A. caviae*, and *A. sobria* (Vitek2 Compact ver. 5.01 database). In agreement with other MLPA studies for *Aeromonas* spp. (3–6,11), our MLPA results showed that *A. hydrophila* is the closest related species to *A. aquariorum*. In addition, Martinez-Murcia et al. suggested that *A. hydrophila* subsp. *dhakensis* belonged to *A. aquariorum* (11). This genetic relationship may result in shared phenotypic characteristics between *A. aquariorum* and *A. hydrophila*. Thus, *A. aquariorum* strains may be misidentified as the related *A. hydrophila* spp. by the Vitek2 database.

Sliced raw fish is a popular food in Korea, and its consumption poses a risk for *Aeromonas* infection (1,12). In addition, previous retrospective studies have shown that liver disease significantly influences the prognosis of *Aeromonas* infections in humans (7,12,13). Although the pathogenesis of *Aeromonas* infections in patients with liver cirrhosis remains unclear, there are some reports of impaired intestinal permeability due to congestion, edema, and local hypoxia in liver cirrhosis patients (14,15). On the other hand, enterotoxins (*alt* and *ast*) are known to be important *Aeromonas* virulence factors associated with diarrhea. In addition, the *act* enterotoxin was reported to be associated with apoptosis of human intestinal epithelial cells and murine macrophages (16,17). In the present case, the patient frequently consumed alcohol with sliced raw fish before the diagnosis of hepatic cirrhosis. The isolated *A. aquariorum* harbored the *act* and *alt* genes, which encode enterotoxins. Therefore, the present fatal case may have been due to translocation of intestinal habituated *A. aquariorum* in a complicated intestinal environment because of the pathological changes due to liver cirrhosis, dysfunction of host immune cells, and bacterial virulence factors. In addition, these adverse host environments may have contributed to the failure of i.v. ceftriaxone treatment, although the *A. aquariorum* strain was shown to be sensitive to this antibiotic in the in vitro susceptible test.

To our knowledge, the present case study is the first to report a fatality caused by *A. aquariorum* in a liver cirrhosis patient. In addition, the bacterium was previously an unrecognized *Aeromonas* sp. in clinical and environmental microbiological fields in Korea. However, it is difficult to identify *A. aquariorum* with commercial systems frequently used for species-level identification of *Aeromonas* in a number of clinical microbiology laboratories. Therefore, commercial identification systems must be improved to allow detection of this species. Furthermore, physicians must consider a diagnosis of *Aeromonas* infection when caring for immunocompromised patients, because the disease can progress very rapidly and be fatal.

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Conflict of interest None to declare.

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