First Report of Human Acute Acalculous Cholecystitis Caused by Fish Pathogen, Lactococcus garvieae

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ABSTRACT

We report herein the first case of acute acalculous cholecystitis caused by *Lactococcus garvieae*, which is known as a fish pathogen. A 69-year-old fisherman underwent laparoscopic cholecystectomy due to severe inflammation in the gallbladder. The isolate obtained from the gallbladder was identified as *L. garvieae* by the 16S rRNA and manganese-dependent superoxide dismutase (*sodA*) gene sequence analysis.

Key words: acute acalculous cholecystitis, *Lactococcus garvieae*, 16S rRNA, manganese-dependent superoxide dismutase
A 69-year-old man presented at Inje University Ilsan Paik Hospital (Goyang, Republic of Korea) with severe upper abdominal and postprandial pains for 2 days. His medical history included gastric ulcer perforation, fatty liver and chronic alcoholism with tobaccoism. The gastric ulcer perforation had been treated with exploratory laparotomy nineteen years ago; however, the patient took no prescribed medications for fatty liver and alcoholism during that time. The patient was a fisher by occupation, and, during the middle of his life, worked at a fish culture farm located at the Han-tan and Nam-han rivers in Republic of Korea. He occasionally ingested raw rainbow trout (Oncorhynchus mykiss) at that time, and continued eating raw freshwater fish or shellfish frequently before this presentation. He had never travelled overseas.

Physical examination on admission showed crouching with a blood pressure of 130/80 mm Hg, a heart rate of 73 beats/min, a respiratory rate of 20/min, and a body temperature of 36.3°C. The patient exhibited no evidence of murmur, jugular vein engorgement or liver cirrhosis. His abdomen was mildly distended and soft, with normoactive bowel sounds, tenderness in the right upper quadrant, positive Murphy’s sign and no rebound tenderness. Results of routine tests were as follows: white cell count, 17,000 cells/µl; polymorphonuclear count, 14,340 cells/µl; hemoglobin, 15.1 g/dl; ESR (erythrocyte sedimentation rate), 15 mm/h; electrolyte (Na / K / Cl), 138 / 4.8 / 104 mEq; BUN (Blood urea nitrogen) / creatinine, 15 mg/dl / 1.1 mg/dl; AST (Aspartate transaminase) / ALT (Alanine transaminase), 94 IU/l / 73 IU/l; total bilirubin, 2.6 mg/dl; urine analysis, clear.

Abdominopelvic computed tomography (CT) findings were as follows: distension of the gallbladder, edematous wall thickening and mild hyperemic change at the gallbladder bed. In addition, attenuation of parenchyma was lower in the liver than in the spleen. There were no remarkable findings in the pancreas, kidneys, spleen or urinary bladder. No evidence of
enlarged lymph nodes or ascites was found on CT scans. Therefore, the patient underwent laparoscopic cholecystectomy with closed drainage (Baro-vac®), which showed a thickened, distended, hyperemic and edematous gallbladder. Obstructive stones were not observed in the gall bladder. There were no specific abnormalities in other organs except that the liver angle was blunt. After operation, the patient was treated with cefminox sodium (Meicelin®, 2 g bid) for 3 days and thereafter cefaclor monohydrate (Cefaclor®, 1T tid) for 5 days. The patient recovered and was discharged from the hospital on the fourth postoperative day.

Three specimens (specimen each from the patient’s bile juice, gallbladder lumen tissue and gallbladder mucosa) were obtained from the gallbladder. All cultures from the specimens yielded oxidase-negative, catalase-negative, α-hemolytic colonies of Gram-positive cocci in short chains on 5% sheep blood agar after 24 hours of incubation at 37°C. The GP card of Vitek2 System® (bioMérieux, Marcy l’Etoile, France) was used for preliminary identification of bacterial isolates, and the results indicated that those isolates were *L. garvieae* with 99% probabilities. Based on these results, one of the bacterial isolates (*L. garvieae* LG-ilanspaik-gs201105: isolated from bile juice) was subjected to further genetic identification based on 16S rRNA and manganese-dependent superoxide dismutase (*sodA*) gene sequence analysis. The partial 16S rRNA and *sodA* gene of *L. garvieae* LG-ilanspaik-gs201105 was sequenced at Macrogen Inc. (Republic of Korea) using universal primers (27F/1492R) and d1/d2 primers as previously described (17), respectively. The obtained 1343-bp 16S rRNA and 495-bp *sodA* sequences were compared with the NCBI GenBank database using the BLAST search (http://www.ncbi.nlm.nih.gov/BLAST). The 16S rRNA sequence of *L. garvieae* LG-ilanspaik-gs201105 was a 99.4% match with the 16S rRNA gene of the *L. garvieae* strain NRIC 0612 (GenBank accession No. AB362690). In addition, the *sodA* sequence of the isolate was a 99.5% match with the *sodA* sequence of *L. garvieae* strain JIP 31-90 (GenBank accession No. AM490328). The sequences of 16S rRNA and the *sodA* genes were further
compared with those of closely related Gram-positive species in the GenBank by multiple sequence alignments using CLUSTAL X (version 1.83) (22). The phylogenetic relationships were determined by the neighbor-joining method using Molecular Evolutionary Genetics Analysis (MEGA) (version 5.0) software (20), which revealed that the patient’s isolate was *L. garvieae* (FIG. 1).

The antimicrobial susceptibility of the *L. garvieae* LG-ilsanpaik-gs201105 was determined using the Etest® (bioMérieux, Marcy l’Etoile, France) on Mueller-Hinton medium with 5% sheep blood. The resistance breakpoints for *L. garvieae* were followed by the Clinical and Laboratory Standards Institute’s (CLSI) guidelines (5). The *L. garvieae* isolate were susceptible to ampicillin (MIC, 0.25 μg/ml), ceftriaxone (MIC, 1 μg/ml), chloroamphenicol (MIC, 2 μg/ml), linezolid (MIC, 2 μg/ml), ofloxacin (MIC, 2 μg/ml), tetracycline (MIC, 1 μg/ml), vancocmycin (MIC, 1 μg/ml), and intermediate to erythromycin (MIC, 0.5 μg/ml) but was resistant to clindamycin (MIC, 8 μg/ml). Therefore, to detect the potential erythromycin resistance methylase (*erm*) or macrolide efflux (*mef*) genes in *L. garvieae* LG-ilsanpaik-gs201105, PCR primers were used for the detection of *erm(A)*20, *erm(B)*17, *erm(C)*20 and *mef*16 (13). However, the *erm* or *mef* genes were not detected from the isolate.

We described the identification by 16S rRNA and sodA gene sequencing of a strain of *L. garvieae* isolated from the gallbladder of a patient with acute acalculous cholecystitis. To the best of our knowledge, this is the first case of acute acalculous cholecystitis caused by the *Lactococcus* species. *L. garvieae* (formerly named *Streptococcus garvieae* or *Enterococcus seriolicida*) is a Gram-positive, nonmotile and nonspore-forming coccus (6, 10) and is responsible for bacteremia in various fish species (24) and for mastitis in ruminants (21, 23). Since the number of cases of fatal human infections caused by *L. garvieae* has recently increased, this bacterium has received much attention as an emerging zoonotic pathogen, thus,
the genome of several *L. garvieae* clinical isolates from fish and human have been sequenced (1, 2, 16, 18, 19). Although several potential virulence factors such as capsule, NADH oxidase and superoxide dismutase have been reported on *L. garvieae* to explain these different clinical symptoms (14, 16), the bacterium is suspected to be an opportunistic pathogen in elderly immune-deficient subjects and individuals with prosthetic valves (9). In general, human infections caused by *L. garvieae* are known to be associated with significant morbidity and mortality, and most of the cases (10 of the 13 cases) exhibited bacteremia. Among the 13 *L. garvieae* infections reported in humans, the most common cases are infective endocarditis (*n* = 6; native valve [*n* = 5] and prosthetic valve [*n* = 1]) (8, 9, 12, 25-27), followed by spondylodiscitis (*n* = 2) (4, 11), liver abscess (*n* = 1) (15), secondary peritonitis (*n* = 1) (26), diverticulitis (*n* = 1) (26), septicemia with multiorgan failure (26), and prosthetic joint infection (*n* = 1) (3). However, this bacterium had never been reported as a causative agent of acute acalculous cholecystitis.

Although the clinical symptoms of the previously reported *L. garvieae* infections varied, manipulation and consumption of raw fish have been suspected as the most probable source of the infections in humans (26). In addition, skin wounds are suspected to be the portal-entry for asymptomatic bacteremia caused by *L. garvieae* as in the case which occurred in an immune-suppressed woman fishmonger who lived near the sea (3). Likewise, our patient was a fisherman and frequently ingested raw fish or shellfish before this presentation. Based on these results, even though the source of infection is still uncertain in this case, the consumption of raw fish (especially rainbow trout) has been suspected to be the most probable cause of the infection.

*L. garvieae* LG-ilsanpaik-gs201105 which was isolated from the patient’s gallbladder was almost 100% identical to previously reported bacterial isolates based on their 16S rRNA and *sodA* gene sequences. Moreover, the resistance of the isolates in this case to clindamycin is
consistent with that of a previous study which reported intrinsic resistance of *L. garvieae* to clindamycin and proposed to use this resistance as a criterion for distinguishing between *L. garvieae* and *L. lactis* (7). Therefore, based on these results, the bacterial isolates from the patient’s gallbladder were confirmed as *L. garvieae*.

Laparoscopic cholecystectomy successfully treated our patient with acute acalculous cholecystitis caused by *L. garvieae*. Our patient also received antibiotic therapy after surgery and was discharged from the hospital on the second postoperative day. However, in most of the laparoscopic cholecystectomies, bacteriologic diagnoses are not established through cultures. Therefore, physicians and clinical microbiologist should be aware of taking meticulous medical history, and bacteriologic studies could help discovering cases of acute acalculous cholecystitis in patients at high risk of *L. garvieae* infection. Indeed, due to the similarities in the phenotypic characteristics of *L. garvieae* and its clinical symptoms of acute acalculous cholecystitis to those of other genera, especially *Enterococcus* spp., culture-based molecular analysis using 16S rRNA or *sodA* genes will help to make accurate identification of *L. garvieae* infection. Further studies are currently in preparation to investigate the genome sequence of *L. garvieae* isolate LG-ilsanpaik-gs201105.

**Nucleotide sequence accession numbers.** The nucleotide sequence for 16S rRNA and the *sodA* genes of *L. garvieae* LG-ilsanpaik-gs201105 were deposited in the GenBank under accession numbers JN162117 and JN162118, respectively.

**REFERENCES**


**Figure legend**

**FIG 1** Phylogenetic analysis of the *L. garvieae* Lg-ilsanpaik-gs201105 isolated from the patient based on the partial nucleotide sequences of 16S rRNA gene (A) including the 16S rRNA sequence of *Enterococcus faecium* (AF039901) as an out-group, and *sodA* gene (B). One thousand bootstrap replicates were subjected to nucleotide sequence distance by neighbor-joining methods. All bootstrap values are displayed above the tree branches, and only bootstrap values >70% are shown.