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【 CASE REPORT 】

# The First Known Case of Liver Abscess Caused by *Aggregatibacter aphrophilus* in Japan

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**Abstract:**

A 48-year-old man presented with a sustained fever. Abdominal computed tomography revealed multilocular liver abscesses. He underwent percutaneous needle aspiration, yielding straw-colored pus. Gram staining revealed Gram-negative coccobacilli. The organism grew only on chocolate II agar in a 7% carbon dioxide atmosphere. Identification of *Aggregatibacter aphrophilus* was confirmed using mass spectrometry and 16S rRNA gene sequencing. He was successfully treated with antibiotics. Liver abscess caused by *A. aphrophilus* is extremely rare. We herein report the first such case in Japan. Even fastidious organisms, such as *A. aphrophilus*, should be correctly identified using mass spectrometry or 16S rRNA gene sequencing for adequate treatment.

**Key words:** *Aggregatibacter aphrophilus*, liver abscess, mass spectrometry

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## Introduction

*Aggregatibacter aphrophilus* is a species of Gram-negative coccobacilli formerly belonging to the genus *Haemophilus* (1, 2). It is a slow-growing, aerobic, non-motile bacillus (3, 4) and a commensal organism of the human oral cavity and pharynx, belonging to the HACEK group, which includes *Haemophilus* species, *Aggregatibacter* species, *Cardiobacterium hominis*, *Eikenella corrodens,* and *Kingella* species.

*A. aphrophilus* sometimes causes systemic diseases, particularly bone and joint infections, spondylodiscitis, and endocarditis (5-7), but rarely causes liver abscesses. *A. aphrophilus* is difficult to identify using culture methods; however, it is important to identify pathogenic microorganisms in order to provide adequate antimicrobial therapy for pyogenic liver abscess. If bacteriological identification by conventional culture methods fails, 16S rRNA gene sequencing or mass spectrometry may be helpful.

We encountered a rare case of liver abscess caused by *A. aphrophilus*. This is, to our knowledge, the first such case reported in Japan. We herein report the details of the case and discuss the methods for the accurate identification of *A. aphrophilus*, including mass spectrometry and 16S rRNA gene sequencing.

## Case Report

A 48-year-old man presented to our hospital with a 5-day history of a fever of 39.0℃. He had a history of gout and took 10 mg febuxostat orally regularly. He had never smoked and rarely drank alcohol. He had not traveled recently and had never had sexual contact with anyone other than his wife. He had no recent dental treatment history. A physical examination at admission revealed a temperature of 37.2℃; the other vital signs were normal. An abdominal examination was normal. He had no tenderness, and the liver was not palpable. There were no signs of endocarditis, such as heart murmur, Osler's nodes, or Janeway lesions.

Laboratory findings revealed an elevated white cell count ($10.6\times10^3$/μL) and C-reactive protein level (13.96 mg/dL). The serum aspartate aminotransferase (AST) level was elevated at 47 IU/L, alanine aminotransferase (ALT) was 64

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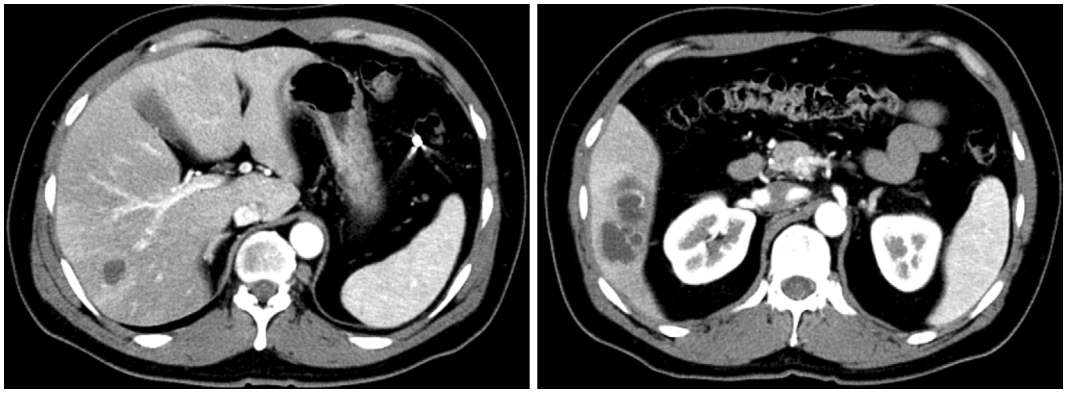


**Figure 1.** **Computed tomography (a: the cranial lesion, b: the caudal lesion). Abdominal computed tomography (CT) revealed two hypodense lesions at the right liver lobe with peripheral enhancement. The caudal lesion was larger and multilocular.**

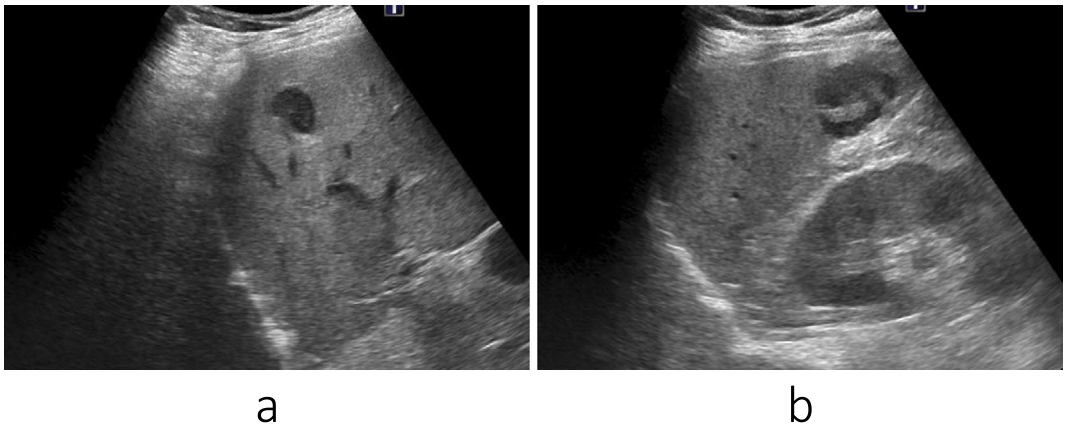


**Figure 2.** **Ultrasonography (a: the cranial lesion, b: the caudal lesion). Abdominal ultrasonography revealed hypoechoic lesions with mixed echogenicity. The caudal lesion was larger and multilocular.**

IU/L, alkaline phosphatase (ALP) was 365 IU/L, γ-glutamyltransferase (γ-GT) was 89 IU/L, and lactate dehydrogenase (LD) was 236 IU/L. The total bilirubin level was normal (0.36 mg/dL). Serum urea, creatinine, electrolytes, and coagulation were within normal ranges. He had no immunodeficiency, including human immunodeficiency virus infection.

Abdominal computed tomography (CT) revealed two multilocular hypodense lesions in the right liver lobe with peripheral enhancement (Fig. 1). Abdominal ultrasonography showed two hypoechoic lesions with mixed echogenicity (Fig. 2). These imaging tests did not show any signs of biliary tract disease, such as biliary obstruction, gallstones, and thickening of the gallbladder wall. Based on these findings, we suspected that he had developed a liver abscess, probably bacterial. He was admitted for a further work-up and therapy using intravenous antibiotics.

He was initially treated with intravenous piperacillin/tazobactam 4.5 g every 8 hours, and his temperature tended to decline. Two sets of blood cultures were taken before starting antibiotic treatment. He underwent percutaneous needle aspiration of the larger hepatic lesion for a microbiological examination in addition to treatment on hospital day 3. Straw-colored high-viscosity pus (3 mL) was obtained and sent for a microscopic examination, Gram staining, and culture. As only 3 mL of pus could be aspirated, his abscess was not drained continuously with a percutaneous catheter. Trophozoite amebae were not detected in the pus on a microscopic examination. However, Gram staining of the pus revealed Gram-negative bacilli phagocytosed by neutrophils (Fig. 3).

The following day, the organism had grown only on chocolate II agar in a 7% carbon dioxide atmosphere and had not grown on Drigalski agar modified or trypticase soy agar with 5% sheep blood. Gram staining of the colony showed Gram-negative coccobacilli (Fig. 4). Based on these findings, the cultured organism was strongly suspected to be *Haemophilus* spp. or *Aggregatibacter* spp. Mass spectrometry was performed to identify the bacteria prior to final culture results. *A. aphrophilus* was identified using matrix-

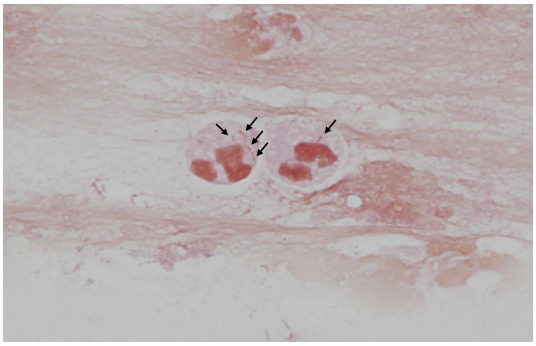

**Figure 3. Gram-staining of the pus. Gram-staining of the pus revealed probable Gram-negative bacilli phagocytosed by neutrophils (arrows indicate the bacteria).**

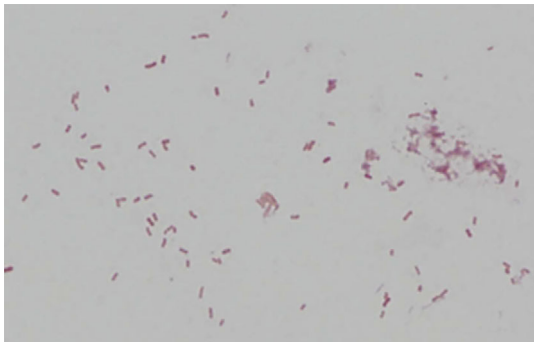

**Figure 4. Gram-staining of the grown colony. Gram-staining of the grown colony revealed Gram-negative coccobacilli.**

**Table. Antimicrobial Susceptibility of *Aggregatibacter aphrophilus*.**

| Antimicrobial agent | MIC (mg/mL) | SIR result |
|---|---|---|
| Ampicillin | 0.25 | S |
| Ampicillin-sulbactam | 0.5/0.25 | S |
| Amoxicillin-clavulanic acid | 0.25/0.12 | S |
| Penicillin | 0.25 | S |
| Ceftriaxone | <0.12 | S |
| Cefotaxime | <0.12 | S |
| Meropenem | <0.12 | S |
| Azithromycin | 4 | S |
| Clarithromycin | 8 | S |
| Ciprofloxacin | <0.03 | S |
| Levofloxacin | <0.03 | S |
| Chloramphenicol | <4 | S |
| Rifampin | <0.5 | S |
| Trimethoprim-sulfamethoxazole | <0.25/4.75 | S |

Susceptibility testing of *A. aphrophilus* was performed according to Clinical and Laboratory Standards Institute (CLSI) document M45-3rd Edition alternate method: HTM (Haemophilus Test Medium) was used instead of CAMHB-LHB.

assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). It showed a score value 2.325; the cut-off value proposed by the manufacturer for species-level identification is score value 2.0. We performed 16S rRNA gene sequencing to confirm, and *A. aphrophilus* was identified here as well.

Susceptibility testing was performed according to the Clinical and Laboratory Standards Institute (CLSI) document M45-3rd Edition alternate method, which recommends Haemophilus Test Medium (HTM) instead of standard medium (8) because of a failure to grow in Cation-Adjusted Mueller Hinton Broth-lysed horse blood (CAMHB-LHB). The identified bacterial pathogen was regarded as sensitive to various antimicrobial agents (Table).

Esophagogastroduodenoscopy and total colonoscopy did not show any sign of underlying disease that could cause liver abscess, such as enteritis. A transthoracic echocardiogram showed no vegetations. Five sets of blood cultures showed no growth despite incubation being extended for 21 days. We used a BD BACTEC 9050 (Becton Dickinson, BD Biosciences, Tokyo, Japan) as the blood culture system. *A. aphrophilus* was not isolated from pharyngeal or stool culture. An examination by a dentist, including panoramic tomography, revealed no ongoing dental infection. Ultimately, no origin of infection or underlying disease was found.

On hospital day 7, the antibiotics were changed to intravenous administration of 3 g ampicillin/sulbactam every 6 hours based on susceptibility testing (Table). On hospital day 11, his temperature rose to 40.0℃, and his examination values, including liver function tests and inflammation markers, worsened. At the same time, he complained of ankle pain. He underwent arthrocentesis by an orthopedic surgeon, and calcium pyrophosphate crystals were confirmed in the synovial fluid aspirate. Bacteria could not be confirmed by Gram staining or culture of the synovial fluid. Based on these findings, a diagnosis of acute pseudogout was made. The fever may have been caused by the pseudogout.

He was re-administered piperacillin/tazobactam considering the possibility of exacerbation of the liver abscess. Subsequently, piperacillin/tazobactam was switched to ampicillin/sulbactam again on hospital day 11 because his clinical condition promptly improved. In total, intravenous antibiotics were administered for 22 days, and then he started taking oral cefalexin 1,500 mg/day and metronidazole 1,500 mg/day on hospital day 23. He was discharged on hospital day 28, 5 days after the start of oral antibiotics. Abdominal CT six days after discharge revealed the disappearance of the abscess cavity. On the same day, he finished his course of antibiotics.

## Discussion

We reported the first case of liver abscess caused by *A. aphrophilus* in Japan. In most cases, liver abscesses are amebic or pyogenic. Pyogenic liver abscess usually occurs secondary to biliary tract disease or colonic disease, including malignancy or infectious disease. The pathogenic bacteria of pyogenic liver abscess are often caused by *Klebsiella pneumoniae* or *Escherichia coli* (2). By contrast, liver abscesses caused by *A. aphrophilus* are extremely rare. Infections by *A. aphrophilus* such as infective endocarditis can

occur following dental treatment, as it is a normal commensal of the human oral cavity and pharynx.

To our knowledge, in the literature, only three cases of liver abscess caused by *A. aphrophilus* have been reported (9-11). One of three patients had undergone a recent minor dental procedure, and the others had not. Our patient had no oral pathology, such as dental infection or a history of recent dental treatment. Thorough screening for other possible sources of infection, including biliary tract disease, colonic disease, and infective endocarditis, revealed no predisposing cause. The previous report demonstrated that 32% of patients with *A. aphrophilus* infection did not have any predisposing factors (4). Pyogenic liver abscess can be caused by *A. aphrophilus* even in patients without predisposing factors, such as a recent dental procedure or dental infection.

In most cases, it is difficult to identify *A. aphrophilus* with conventional bacterial culture tests because of its fastidious and slow-growing nature; nevertheless, clinicians should expend effort to identify the pathogen correctly in order to provide adequate antimicrobial therapy.

The presence of this organism may go undetected unless appropriate examinations are performed. On Gram staining, *Haemophilus* or *Aggregatibacter* spp. appear as Gram-negative coccobacilli, while Enterobacteriaceae, such as *Klebsiella pneumoniae* and *Escherichia coli*, appear as moderately-sized or large rods. In terms of culture tests, *Aggregatibacter* spp. grows on chocolate agar requiring 5-10% carbon dioxide for primary isolation; it does not grow on BTB or blood agars (5). Our case also showed such characteristics.

If *Aggregatibacter* spp. is suspected based on these findings and bacteriological identification by conventional culture methods fails, mass spectrometry or 16S rRNA gene sequencing may be helpful. *A. aphrophilus* may have been been overlooked in cases of liver abscess because proper examinations were not performed. While there are few reports of liver abscesses caused by this organism at present, there may be more in actuality.

There have been several studies concerning the performance of MALDI-TOF-MS for the identification of *A. aphrophilus*. Powell et al. reported the misidentification of *A. aphrophilus* as *Aggregatibacter segnis* or as *Haemophilus parainfluenzae* using a bioMérieux Vitek MALDI-TOF mass spectrometer (bioMérieux, Durham, USA) (12), while we used the MALDI Biotyper (Bruker Daltonics, Bremen, Germany) for the identification of the organism. Our case therefore cannot be simply compared to theirs.

Couturier et al. reported the misidentification of *A. aphrophilus* as *H. influenzae* with an identification score of 1.860 (<2.0) using the MALDI Biotyper software program, version 2.0 (Bruker Daltonics), for the spectra analysis (13). In contrast, no misidentifications were reported using versions 3.0 or 3.1 of this software program (13, 14).

Overall, previous studies have suggested that misidentification using MALDI-TOF-MS is uncommon if high identification scores are obtained using the latest version of the analysis software program, despite the fact that *A. aphrophilus* tends to yield a low identification score (13-15). In our case, we used the MALDI Biotyper software program, version 3.1 (Bruker Daltonics), for the identification, and an identification score of 2.325 (≥2.0) was obtained. The results of our analysis with MALDI-TOF-MS in the present case therefore seem reliable. Nevertheless, it is necessary to study more cases involving *A. aphrophilus* in order to evaluate the usefulness of MALDI-TOF-MS for the identification of this organism.

Pyogenic liver abscesses require treatment with antibiotics in addition to drainage. It is important to identify the pathogenic microorganism in order to make appropriate antibiotic choices. In patients with liver abscess, the detection rate of blood culture was 44%, whereas that of abscess culture was as high as 83% (7). Abscess culture should be performed early to direct proper treatment (2). According to previous reports, *Aggregatibacter* spp. are susceptible to cephalosporins, tetracyclines, and aminoglycosides. Resistance to ampicillin is not uncommon; however, amoxicillin combined with a β-lactamase inhibitor has been effective (3). In our patient, susceptibility testing showed *A. aphrophilus* to be sensitive to various antimicrobial agents, including ampicillin. In our case, broad-spectrum antibiotics were used because the identified organism was rare. However, it may be better to use a narrower-spectrum antibiotic. Furthermore, because our case is most likely to be a hematogenous infection considering the characteristic of identified organism, anaerobic bacteria may not need to be covered by antibiotics.

Liver abscesses caused by *A. aphrophilus* are extremely rare, and there are few clinical and bacteriological data available for evaluating the effectiveness of treatment. Consequently, there is no consensus concerning the most appropriate antibiotic choice. In previous reports, liver abscesses caused by *A. aphrophilus* have been treated with ciprofloxacin, gentamicin, and ampicillin/sulbactam (9, 10). Based on the present findings, we believe that amoxicillin or ampicillin with a β-lactamase inhibitor, such as ceftriaxone or cefotaxime, or a fluoroquinolone may be effective in patients with *A. aphrophilus* infections (5). More clinical data regarding *A. aphrophilus* infection should be gathered by identifying this organism using appropriate examinations, such as mass spectrometry or 16S rRNA gene sequencing.

## Conclusion

We encountered a case of liver abscess caused by *A. aphrophilus*, which is extremely rare and difficult to identify using common culture methods. It is important to identify even fastidious organisms in order to administer adequate antimicrobial therapy for pyogenic liver abscess. Clinicians should be aware that MALDI-TOF-MS or 16S rRNA gene sequencing can be helpful for identifying fastidious organisms, such as *A. aphrophilus*.

**The authors state that they have no Conflict of Interest (COI).**

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