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CASE REPORT

Aggregatibacter aphrophilus pyogenic liver abscess in an immunocompetent young woman

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Aggregatibacter aphrophilus (formerly *Haemophilus aphrophilus/paraphrophilus*) is a small Gram-negative coccobacillus with fastidious growth requirements. It is a normal commensal of the human oropharynx and upper respiratory tract, and it can infrequently cause invasive human diseases, including bone and joint infections and subacute infective endocarditis. Cases of liver abscess caused by *Aggregatibacter aphrophilus* have been sparsely recorded in the English-language literature, but have not yet been reported in Taiwan. Here we present a case of *Aggregatibacter aphrophilus* pyogenic liver abscess in an immunocompetent young woman. She recovered uneventfully after repeated percutaneous abscess aspiration and antibiotic treatment for 5 weeks.

Introduction

Haemophilus species encompasses a group of small, non-motile Gram-negative cocobacilli with fastidious growth requirements. They are well adapted to the mucous membrane of the human oropharynx and upper respiratory tract, and are considered to be resident microbiota at these sites.

Among them, *H. influenzae* is a clinically important pathogen often associated with infections within the oro-nasopharyngeal territory, as well as at distant sites in the lower respiratory tract and meninges. Species other than *H. influenzae* are much less virulent and are infrequently associated with infective endocarditis in adults and with various pyogenic infections, typically among the pediatric population. On the other hand, hepatobiliary infections are

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rarely caused by *Haemophilus* bacteria, especially by non-*influenzae Haemophilus* species.[1]

Huang et al reviewed 28 cases of invasive *H. aphrophilus* infections, including eight cases diagnosed in a Taiwan hospital from 1990 to 2003.[2] Most of the cases were diagnosed as either endocarditis or bone and joint infections, yet none of them involved hepatobiliary tract or manifested pyogenic liver abscess (PLA). Notably, recent dental procedures or gingivitis were identified in a significant percentage (12/28) of those cases prior to the invasive *H. aphrophilus* infection.[2]

Here we report a rare case of an immunocompetent young lady who developed an *Aggregatibacter aphrophilus* (formerly *Haemophilus aphrophilus/paraphrophilus*) liver abscess after a procedure to repair dental caries.

Case report

A 30-year-old woman visited the hospital's emergency room with an acute onset of right upper quadrant pain and anorexia. She had had intermittent high fever associated with rigors and night sweats for 2 weeks. Six weeks previously, she had had a minor dental procedure to repair caries in her upper left central incisor.

Laboratory results showed neutrophilic leukocytosis (a total white cell count of 25.2×10^9/L), a raised C-reactive protein level (312 mg/L), and mildly abnormal liver function tests (aspartate aminotransferase 53 U/L, alanine aminotransferase 86 U/L, and alkaline phosphatase 330 U/L). Her renal function and plasma glucose level were within normal limits. A survey for viral hepatitis markers revealed positive hepatitis B virus surface antigen. Results were negative for human immunodeficiency virus antibody.

A non-contrast computed tomography scan of the abdomen showed hepatosplenomegaly with a solitary, 6.5 cm $\times$ 6 cm $\times$ 5.5 cm hypodense lesion with internal septa in the right lobe of the liver (S8) (Fig. 1A). On ultrasonography, the lesion was of mixed echogenicity (Fig. 1B).

Culture of blood obtained in the emergency room did not yield any pathogens. Microscopic stool examination was unremarkable, and negative seroreactivity for *Entamoeba histolytica* soluble antigens by an indirect hemagglutination assay ruled out a diagnosis of amoebic liver abscess.

The patient underwent percutaneous aspiration of the hepatic lesion on the following day, draining 30 mL of straw-colored pus. Gram-staining of the pus did not revealed any organisms, but a few poorly stained, Gram-negative coccobacilli were found in the broth medium (BACTEC blood culture system; BD, Sparks, MD, USA) within 24 hours of incubation.

Subsequent plating of the broth culture onto blood agar slowly produced alpha-hemolytic, small, mixed-sized colonies, whereas on chocolate agar much larger and more uniform colonies were generated. However, no colonies were found on eosin-methylene blue agar, suggesting that the isolate was a non-enteric Gram-negative bacillus (GNB). Further characterization with test panels designed for identifying common GNB revealed inconsistent results: *Kingella indologenes* (Phoenix; BD, Sparks, MD, USA) and *Pasteurella canis* (Vitek 2; bioMérieux, Marcy l'Etoile, France).

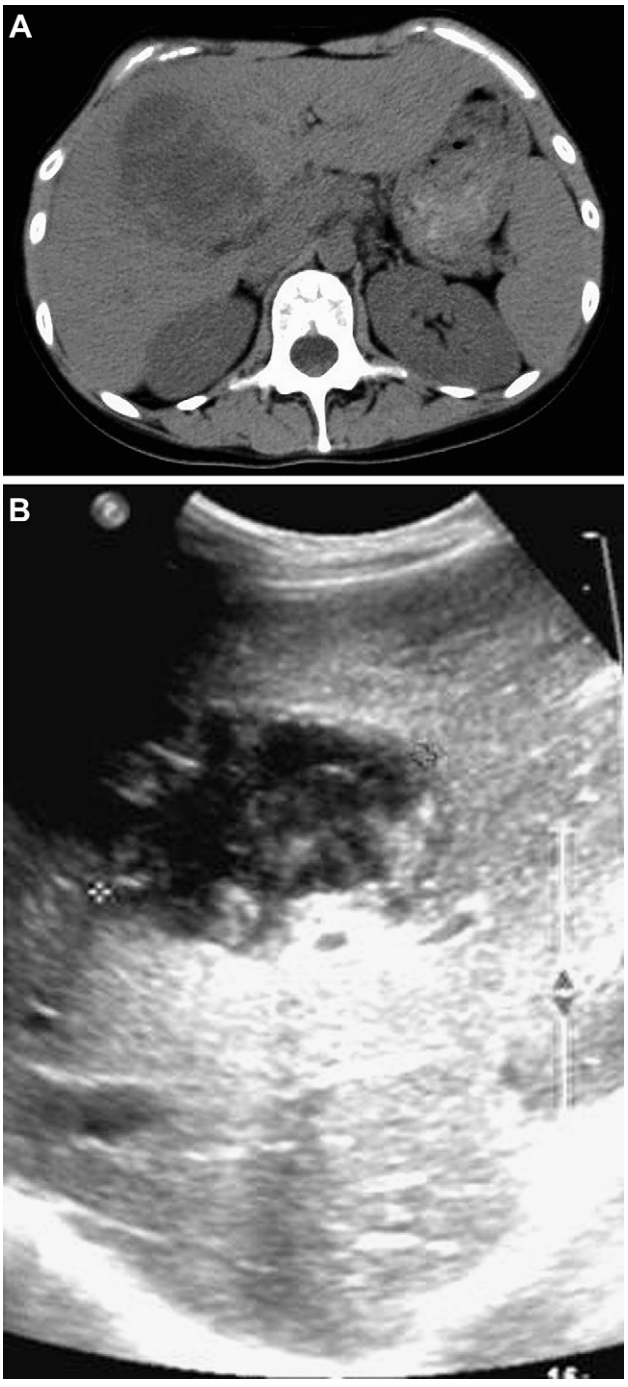

Figure 1. (A) Abdominal computed tomography without intravenous contrast performed upon first presentation revealed a large, low-attenuated lesion with internal septa in the right lobe of the liver. (B) The lesion was of mixed echogenicity.

Given the peculiar growth requirements and dubious test results, it was highly suspected that a fastidious GNB was the culprit. Therefore, three test sets designed for optimal *Neisseria/Haemophilus* identification (ViteK 2 NHI card and API NH strip, both from bioMerieux, Marcy l'Etoile, France; BBL Crystal NH ID , BD, Sparks, MD, USA) were used, all pointing to the same result of *H. aphrophilus* (now renamed *Aggregatibacter aphrophilus*), which was further confirmed by direct sequencing of a polymerase chain reaction

(PCR)-amplified 16S rRNA gene.[3] Antimicrobial susceptibility test for fastidious organisms was not available in the hospital, but a nitrocefin test for beta-lactamase was negative for the bacteria. Further survey by physical examination and a transthoracic echocardiogram did not find any evidence of infective endocarditis.

The patient was initially treated with flomoxef 1 g every 6 hours by intravenous infusion. Due to frequent hectic fever, flomoxef was replaced by ciprofloxacin 400 mg every 12 hours intravenously from the fourth day, plus gentamicin 80 mg every 12 hours intravenously from the sixth day. This led to fewer spiking febrile episodes, but the patient continued to have right upper quadrant pain and remittent fever. Therefore, needle aspiration for the liver abscess was repeated on the ninth day, which drained 75 mL of nonsterile pus.

Four days after the second drainage, the patient's fever changed from a remittent to an intermittent pattern, and her drenching night sweats and abdominal discomfort gradually resolved. Despite apparent clinical improvement, she continued to have fever occurring more frequently than before. Drug fever was highly suspected; thus, ceftriaxone 1 g every 12 hours was substituted for both antibiotics from the 19th day. Subsequently, she became afebrile 2 days later and completely recovered after a 5-weeks course of antibiotic treatment. A month later, abdominal ultrasonography showed a diminishing hepatic lesion (Fig. 2).

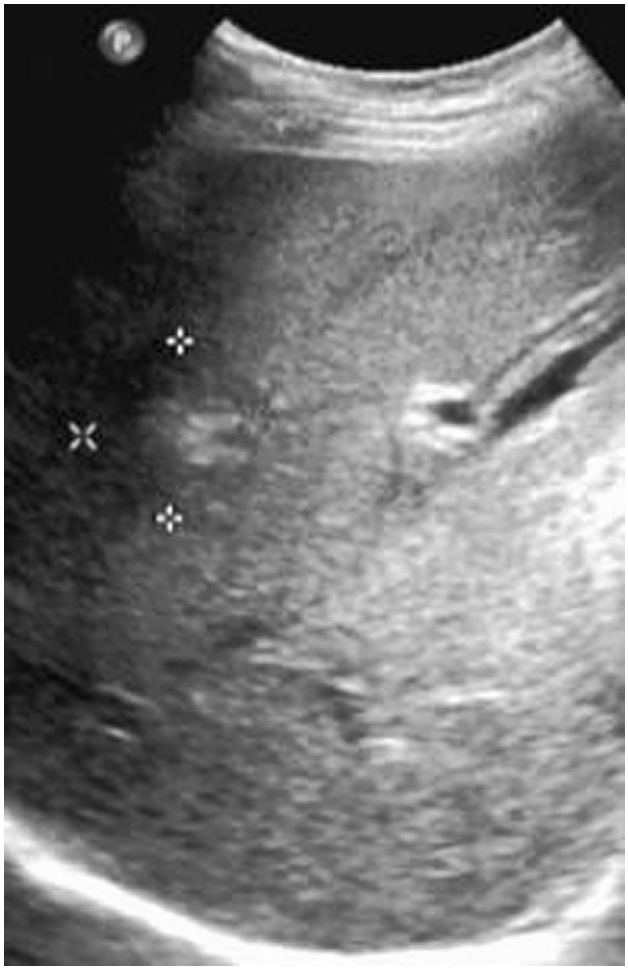

Figure 2. Sequential abdominal sonogram two and a half months after the initial imaging studies seen in Fig. 1 showed a residual mixed echoic lesion measuring 2.6 cm × 2.8 cm.

Discussion

PLA is an uncommon disease with an evolving epidemiology. Generally considered in the past to be a polymicrobial infection caused by underlying hepatobiliary or gastrointestinal abnormalities, cryptogenic PLA in patients without aforementioned risks has become predominant, especially in South-East Asia.

Most cryptogenic liver abscesses are caused by *Klebsiella pneumoniae*, and metastatic infections are not uncommon. For example, septic endogenous endophthalmitis, a potentially blinding ocular infection resulting from hematogenous spread of *K. pneumoniae*, has been seen with a frequency of 3—10% in patients with *K. pneumoniae* liver abscess.[4] In Taiwan, it is estimated by a population-based database that the annual incidence of PLA increased 50% from 11.15 per 100,000 in 1996 to 17.59 per 100,000 in 2004.[5] Analysis of the cases from a surveyed medical center, which accounted for 1.7% of the total number of cases of PLA within the study period, revealed that 80% of pathogens associated with a pyogenic infection were *K. pneumoniae*.[5]

Notwithstanding the prominent roles of enteric bacteria in PLA, a number of case reports have indicated that normal flora of low virulence within the oropharyngeal territory are as potent at causing purulent hepatic infection as they are for periodontal diseases. *Fusobacterium necrophorum*, for example, has been isolated from patients with liver abscess and periodontal infection.[6—10]

On the other hand, fastidious Gram-negative rods such as the HACEK group of bacteria (*Aggregatibacter* spp., formerly *Haemophilus aphrophilus*, *H. paraphrophilus*, and *H. segnis*, *Actinobacillus actinomycetemcomitans*, *Cardiobacterium* spp., *Eikenella corrodens*, and *Kingella* spp.) are rarely reported with PLA, despite their well-defined roles in periodontitis and in producing distant metastatic infections such as brain abscess, osteomyelitis, and infective endocarditis.

As seen in many events of the PLA, co-infection with other bacteria has frequently been observed in cases involving the HACEK bacteria.[9,11—16] For example, Sherlock et al reported a liver abscess from which *Haemophilus* (*Aggregatibacter*) *aphrophilus* was isolated concomitantly with *Mobiluncus mulieris*.[11] In our liver abscess case, however, there was no other pathogen isolated concurrently with *A. aphrophilus*.

Ariyaratnam et al also reported an unusual case of co-incident liver and brain abscesses caused solely by *Aggregatibacter paraphrophilus* in a patient with a large patent foramen ovale 2 months after dental root canal treatment.[17] Thus, polymicrobial infection is not always required for the *Aggregatibacter* bacteria to generate a liver abscess.

Aggregatibacter aphrophilus bundles together two previously separate species, *H. aphrophilus* and *H. paraphrophilus*.[18] The species are genetically related and share high degrees of similarity among their biochemical characteristics and growth requirements, with a notable

difference in their growth dependence on factor V, or nicotinamide adenine dinucleotide, an essential nutrient for most *Haemophilus* spp. except *H. aphrophilus*. Commercially prepared sheep blood agar does not include factor V; thus, most *Haemophilus* bacteria are not expected to grow on this medium, except for *H. aphrophilus*. Accordingly, *H. aphrophilus* is frequently overlooked as a potential pathogen if the bacterial isolates are recovered on the blood agar and presumptively excluded as *Haemophilus* spp.

In our case, it was initially confusing to find that the Gram-negative isolate was able to grow on the sheep blood agar but not on the eosin-methylene blue agar, as both media support for the growth of most, if not all, Gram-negative bacteria. The identification was further complicated by inconsistent results from two test panels for identifying common GNBs (Phoenix and Vitek 2).

Our case illustrated the complexity associated with the isolation and identification of the fastidious *Haemophilus* bacteria, as the slow growth rate and unique metabolic requirements rendered the protocol for identifying frequently encountered GNB of no use. As shown in our case and others,[19] neglecting these peculiar features leads to an improper use of identification test panels, which in turn gives rise to erroneous results. In this regard, direct sequencing of PCR-amplified bacterial 16S rDNA may assist the identification of slow-growing bacteria, especially for laboratories with little experience or in cases when the results are not compatible with clinical syndromes.[3]

Antibiotic choices for *A. aphrophilus* include third-generation cephalosporins, the quinolones, chloramphenicol, tetracyclines, and the aminoglycosides. Although most strains are susceptible to penicillin and ampicillin, resistant strains have been reported, including beta-lactamase-producing strains.[20] Criteria of antimicrobial susceptibility breakpoints for fastidious Gram-negative bacteria, unlike other commonly isolated bacteria, have been difficult to develop, owing to a low frequency of infection and thus few clinical and bacteriological response data available for evaluation. The Clinical Laboratory Standards Institute has published consensus guidelines for antimicrobial susceptibility testing for infrequently isolated or fastidious bacteria.[21] However, unless persistent infection, clinical treatment failure, or drug allergy or intolerance is present, susceptibility testing is not necessary, and management may follow the recommendations given in the medical literature.[22]

In conclusion, we reported a rare case of *A. aphrophilus* liver abscess in an immunocompetent young woman presumably caused by a recent dental manipulation. Interestingly, an additional case was identified 4 months later in a healthy 35-year-old man with a liver abscess, in which vigilant laboratory staff quickly turned to the right testing modules knowing no bacteria had been detected by Gram-staining of broth cultures tagged as having positive bacterial growth by the automatic culture system.

Thus, physicians caring for patients with liver abscesses, particularly young adults without diabetes mellitus or hepatobiliary comorbidity, should keep slow-growing, fastidious GNBs on high index of suspicion. As these pathogens tend to evade commonly used laboratory diagnostics, communication with the microbiology laboratory staff at the earliest time is key to correctly identifying the organism and subsequently providing adequate antimicrobial therapy.

Acknowledgments

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