Aggregatibacter aphrophilus misidentified as Brucella spp. in an immunocompetent patient with brain abscess

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Abstract
Aggregatibacter aphrophilus rarely causes brain abscesses. Here we report a case of the brain abscess caused by Aggregatibacter aphrophilus. Cultivated gram negative coccobacilli from cerebral abscess were initially misidentified as Brucella spp. because it gave false positive agglutination with anti-Brucella sera. Definite identification was made with MALDI-TOF assay. Right to left shunt through the pulmonary arteriovenous malformation was speculated to be the underlying cause for the brain abscess. The patient was treated successfully with ampicillin-sulbactam after failing ceptriaxone treatment.

Keywords: Aggregatibacter aphrophilus, brain abscess, brucella.

Introduction
Aggregatibacter aphrophilus (formerly Haemophilus aphrophilus) is an aerobic gram-negative coccobacillus that belongs to the HACEK (Haemophilus aphrophilus/paraphrophilus, Actinobacillus actinomycetem comitans, Cardiobacterium hominis, Eikenella corrodens and Kingella spp.) group. Being a commensal organism in the oropharyngeal flora, it rarely causes clinical infection. It was first described by Khairat et al. in 1940 as a causative pathogen in a case of fatal infective endocarditis.2 In rare cases of a clinical infection, A. aphrophilus mainly causes osteoarticular or ophthalmic infections or infective endocarditis.3 Skin and soft tissue infections, central nervous system (CNS) infections, empyema and a hepatic abscess caused by A. aphrophilus have also been reported.3

In addition, Brucella spp. are gram negative coccobacilli and cause brucellosis, an endemic zoonosis in Turkey. It may manifest itself in various ways such as a CNS infection and a brain abscess.4

Here we describe a case of a patient with a brain abscess culture of the abscess drainage material yielded a gram negative coccobacillus that was initially identified as Brucella spp. Further analysis using matrix-assisted laser desorption ionisation time of flight mass spectrometry (MALDI-TOF MS) identified the causative organism as A. aphrophilus.

Case Report
Three weeks before admission, 30 years old otherwise healthy Caucasian woman had a sudden onset seizure, which lasted for four minutes. She did not seek medical help at that time. Two days later, she developed a new epileptic attack. After she presented to the emergency room of the state hospital, she was diagnosed to have epilepsy. Contrast enhanced cerebral magnetic resonance imaging (MRI) was performed which revealed a frontal mass. She was transferred and admitted to Neurosurgery at Hacettepe University Hospital for further investigations and treatment in March, 2014. On admission she was alert and had normal vital signs. Her neurologic examination revealed left hemiparesis, decreased motor strength (4/5) at upper extremities, hyperactive deep tendon reflex responses in the left extremities, cerebral ataxia, Babinski reflex and clonus in her left foot. Four days after the first MRI, a new cerebral MRI for surgical navigation was obtained and showed a lesion at the right precentral gyrus which was enlarged when compared to former images. Spectroscopic examination was in accordance with frontal abscess. She was diagnosed with pyogenic frontal abscess and ampicillin-sulbactam 4x1.5g was started empirically.

The abscess was drained surgically. Gram stain of the pus revealed polymorphonuclear leucocytes. Cultivation of the exudates on chocolate agar plate yielded small, round and opaque colonies after incubation in 5% CO2 at 35°C for 48 hours. Gram stain of the culture was performed and small gram-negative coccobacilli were seen. Primarily X and V factors were performed onto triptic soy agar (TSA) to exclude Haemophilus spp., but it was X and V factor independent. Colony morphology was similar to Brucella spp. colonies (small, round and opaque colonies with smooth margins). Cultivated organism gave agglutination with anti-Brucella...
mellitensis antibody therefore the microorganism was initially identified as Brucella mellitensis although its catalase and urease tests were negative. However, Brucella tube agglutination tests both from serum and cerebrospinal fluid (CSF) were found to be negative. Also, routine CSF examination was unremarkable for active inflammation. Transthoracic and transesophageal echocardiogram was found to be unremarkable for infective endocarditis. Thus, we decided to further identify the organism with Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry based Vitek MS system.

Since the initial identification of the bacteria was Brucella spp. empiric antibiotic treatment was switched to doxycycline plus rifampicin plus ceftriaxone. The patient received this combination before final identification of the organism was available. Repeated MRI scans were obtained on day 15 of ceftriaxone treatment and showed partially shrunken lesion with partially regressed cerebral oedema. After definite identification of the microorganism as Aggregatibacter aphrophilus we reviewed and figured out that there were some treatment failure reports with ceftriaxone. The patient’s treatment was immediately changed to ampicillin (12g/day) plus sulbactam (2g/day). In order to determine the possible suppurative foci at other organs thoraco-abdominal computerized tomography scan was performed which revealed arteriovenous malformations between pulmonary artery and vein. Patient received ampicillin-sulbactam treatment for an additional 4 weeks. Cerebral contrast enhanced MRI was once more obtained at the end of the 4th week and revealed that the lesion had totally regressed. Antibiotic treatment was stopped. At the follow-ups no permanent sequelae was found.

Discussion
A. aphrophilus is a small, fastidious, gram-negative, capnophilic coccobacillus. It produces small, round and glistening colonies on chocolate plate. It is an oxidase and catalase negative and X and V factor independent bacterium. Being a commensal oral flora as a component of interdental material, A. aphrophilus rarely causes clinical infection.1

Brucellosis is an endemic zoonosis in Turkey, particularly among the inhabitants of southeastern Anatolia. In Turkey, particularly in patients from Southeastern Anatolia, when cell culture analysis yields oxidase-positive, gram-negative coccobacilli from CNS samples, Brucella spp. Are the prime suspects in differential diagnosis. As suggested by our equipment supplier (Remel Europe Ltd., Kent, UK), the suspect microorganism was tested with anti-B. melitensis antibodies, which formed a precipitate with the patient’s serum. Thus, our patient initially received a diagnosis of a brain abscess caused by Brucella spp. Nevertheless, negative results of catalase and urease tests and disappearance of detectable antibodies against Brucella spp. in the serum and CSF samples of the patient according to the Brucella STA kit prompted us to identify the organism using MALDI-TOF MS.

MALDI-TOF MS is a rapid and accurate tool for identification of various microorganisms and was recently shown to be effective in identification of HACEK species.5 A report from Japan also indicated that A. aphrophilus may be misidentified with phenotype-based methods and further identification procedures were warranted.6 Thus, we performed MALDI TOF MS and identified the pathogenic organism as A. aphrophilus.

Among the clinical problems caused by A. aphrophilus, osteoarticular complications3,6 and endocarditis3 are the most common. CNS infection is a rare type of an A. aphrophilus-related disease. Ahamed et al. reported the presence of a brain abscess caused by A. aphrophilus in an otherwise healthy patient, this condition was successfully treated with surgical drainage and systemic antibiotics.7 Page et al. reported nine cases of a brain abscess caused by H. aphrophilus and found that these types of abscess are common among patients with congenital heart diseases.8 In the present case, we evaluated the possible underlying cardiac disease by means of transthoracic and transesaophageal echocardiography. We could not demonstrate any underlying heart disease or microbial growth suggestive of infective endocarditis.

Because A. aphrophilus has been reported in deep-seated infections,9 for further testing we attempted contrast-enhanced CT. Although we could not demonstrate the involvement of any organ or tissue other than the brain abscess, results of the CT scan shed light on the possible pathogenesis of the brain abscess in this case. We found this report to be intriguing, a commensal microorganism led to a brain abscess without any apparent cardiac or other involvement. Our past experience with a patient who had patent foramen ovale suggested that a right to left shunt through the patent foramen ovale may be the contributing factor of the A. aphrophilus brain abscess.10 In this case, we demonstrated an arteriovenous malformation between the pulmonary artery and vein, which led us to suspect that a possible
right to left shunt through this malformation contributed to the brain abscess.

Although the isolated microorganism was found to be sensitive to ceftriaxone, the patient did not respond to ceftriaxone treatment. Therefore, we changed the treatment to ampicillin-sulbactam. The patient completely recovered without any persistent sequelae after one month of high dose ampicillin-sulbactam.

**Conclusion**

To conclude, attending physicians must bear in mind that A. aphrophilus may lead to CNS infections in people with right to left shunts. Physicians are supposed to explore the underlying structural abnormality especially in patients without infective endocarditis. Second, MALDI-TOF is a rapid and accurate tool for the identification of many microorganisms including HACEK group. Ampicillin/sulbactam is the agent-of-choice especially in the treatment of HACEK group organisms.

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**Conflict of Interest:** No.

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**References**