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Postneurosurgical meningitis due to *Psychrobacter sanguinis*

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ABSTRACT

We report the first case of a post-neurosurgical meningitis due to *Psychrobacter sanguinis*. *Psychrobacter* spp. have been observed in marine environments and in a wide range of food products. Despite a strict questioning of the patient and the medical staff, we did not find the source of this bacterium.

CASE REPORT

A 64-year-old woman living near the English Channel suddenly experienced severe headaches and vomiting. Clinical examination revealed nuchal rigidity but no focal neurologic signs. There was no hyperthermia, nor altered consciousness (Glasgow Coma Scale evaluated at 14). A computed tomography angiography (CTA) showed a subarachnoid hemorrhage with enlargement of ventricles, due to a basilar artery aneurysm. The patient was immediately transferred to a neurosurgery unit. An external ventricular drain was set on to relieve the elevated intracranial pressure, under local anesthesia. She did not receive any prophylactic antibiotic therapy. Endovascular coiling was performed on day 1 after admission to seal off the basilar aneurysm.

A leak of cerebro-spinal fluid (CSF) was observed at the drain insertion site, on day 3. The patient kept touching the drain insertion site with her bare hands, despite repeated instructions. On day 8, the external ventricular drain was removed due to the persistence of the CSF leak. There was no local sign of inflammation or infection. However, the patient presented hyperthermia (38.5°C) the same day and a lumbar puncture was performed. The CSF was hemorrhagic, with increased proteins concentration (1.64 g/L) and decreased glucose (0.19 g/L). A mild hypoglycorrhachia occurs frequently after subarachnoidal hemorrhage, therefore it was not considered as indicative of meningitis. No bacteria was shown on Gram-stain, and the cultures remained sterile.

On day 15, the patient presented hyperthermia at 39°C, with altered sensorium and drowsiness. A new lumbar puncture allowed a definitive diagnosis of post-neurosurgical meningitis. CSF analysis showed a hyperleukocytosis (5900 WBC/mm³), a profound hypoglycorrhachia (0.07 g/L) and elevated protein levels. Direct examination revealed few Gram-negative coccobacilli. An empiric antimicrobial therapy was initiated immediately using a combination of meropenem and fosfomycin at 2g x 3 per day and 4g x 4 per day, respectively. A single dose of amikacine (25 mg/kg) was also administered. No bacterial growth was observed at 48h after incubation. On day 4, the brain heart infusion (BHI) broth became turbid due to the growth of Gram-negative coccobacilli. The BHI was subcultured onto blood agar incubated at 37°C, chocolate agar incubated at 37°C and 5% CO₂, and onto blood agar incubated at 37°C in an anaerobic atmosphere. The Gram-negative coccobacilli only grew on the blood and chocolate agar under aerobic conditions after 48 hours.

These colonies were catalase, oxidase and urease positive. Unfortunately, MALDI-TOF (Bruker Daltonik S. A., Wissembourg, France) using FlexControl software (version 3.0) gave no reliable identification : the Biotyper database contains the spectra of 5,627 species and is regularly updated by the Bruker Company. To identify of this agent, sequencing of the 16S rRNA gene was performed and a 1,500-bp DNA fragment was amplified using the universal primers 8F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1525R (5'-AAAGGAGGTGATCCAGCC-3'). DNA sequence analysis was performed with leBIBI and BLAST. There was a 100% identity on 1400 bp with the *Psychrobacter sanguinis* type strain (Genbank accession no. HM212668). Partial 16S sequences of 19 *Psychrobacter* species type strains and our isolate were analyzed using the ARB software environment (5). A phylogenetic tree was created using the ARB neighbor-joining algorithm (Figure 1).

In vitro antimicrobial susceptibility was determined using agar disc diffusion, using the critical zone diameters recommended by CA-SFM 2013 (Comité de l'Antibiogramme de la

Société Française de Microbiologie; <http://www.sfm.asso.fr>). Our isolate of *P. sanguinis* was susceptible to amoxicillin, ticarcillin, cefepim, ceftazidim, imipenem, meropenem, amikacin, ciprofloxacin and trimethoprim/sulfamethoxazole. Therefore, meropenem and fosfomycin were switched to amoxicillin (4g x 3 per day), as part of antimicrobial therapy de-escalation strategy. Amoxicillin was continued until the patient had received 15 days of antimicrobial therapy.

The patient experienced a rapid recovery under treatment. The fever subsided 4 days after the initiation of meropenem and fosfomycin. A lumbar puncture performed the same day revealed only 12 WBC/mm³, and a normal level of glucose (0.54 g/L). Proteins levels were still slightly elevated (0.92 g/L). No other complications were observed during the hospitalization.

We report the first case of a post-neurosurgical meningitis due to *P. sanguinis*. Members of the *Psychrobacter* genus are small Gram-negative coccobacillus. *Psychrobacter* spp. have been observed in marine environments like deep-sea (6) or sea ice (1), and in a wide range of food products including seafood (2) and cheese (4). *P. sanguinis* is a new species described in 2012: the type strain was isolated from the blood of an 84-year male in the USA (8). Because *P. sanguinis* is a recently described species, there is a lack of data available concerning its pathogenicity. *P. sanguinis* was first isolated from the blood cultures of 4 patients in New York (8). However, *P. sanguinis* is probably an environmental bacteria, and was recovered from seaweeds in India (Genbank accession no. JX501674 and JX501676). *P. sanguinis* is able to grow on marine agar, which contains 3.5% NaCl to simulate sea water, and its temperature range is 4-37°C (8). Other *Psychrobacter* species have been found in various environmental sites, and are only considered as rare opportunistic human pathogens. *P. arenosus* is a species closely related with *P. sanguinis* based on their 16S rRNA sequence,

and was described in 2004 (9). *P. arenosus* was isolated from costal sea ice in Japan (7), and associated with one published case of bacteremia after blood transfusion in France (3).

In our case report, investigations were performed to identify the source of *P. sanguinis* but it is difficult to understand how *P. sanguinis* was able to cause a post-neurosurgical meningitis. One hypothesis is that the bacteria was present in the hospital environment and entered the central nervous system by colonization of the external ventricular drain. Indeed, the patient frequently touched the drain with her bare hands despite repeated instruction from the hospital staff. It is also possible that the mode of entry is a transient bacteremia, but it is less likely. Four set of blood cultures were drawn during the hospitalization and remained sterile.

This first report of *P. sanguinis* meningitis emphasizes the need to incubate culture medium for more than 48h in post-neurosurgical patients. Neurosurgery can lead to infection with rare bacteria of low pathogenicity levels, even one week after an external ventricular drain was removed.

Nucleotide sequence accession numbers:

The 16S rDNA sequence data of this *P. sanguinis* strain was deposited to Genbank under accession number KJ439038.

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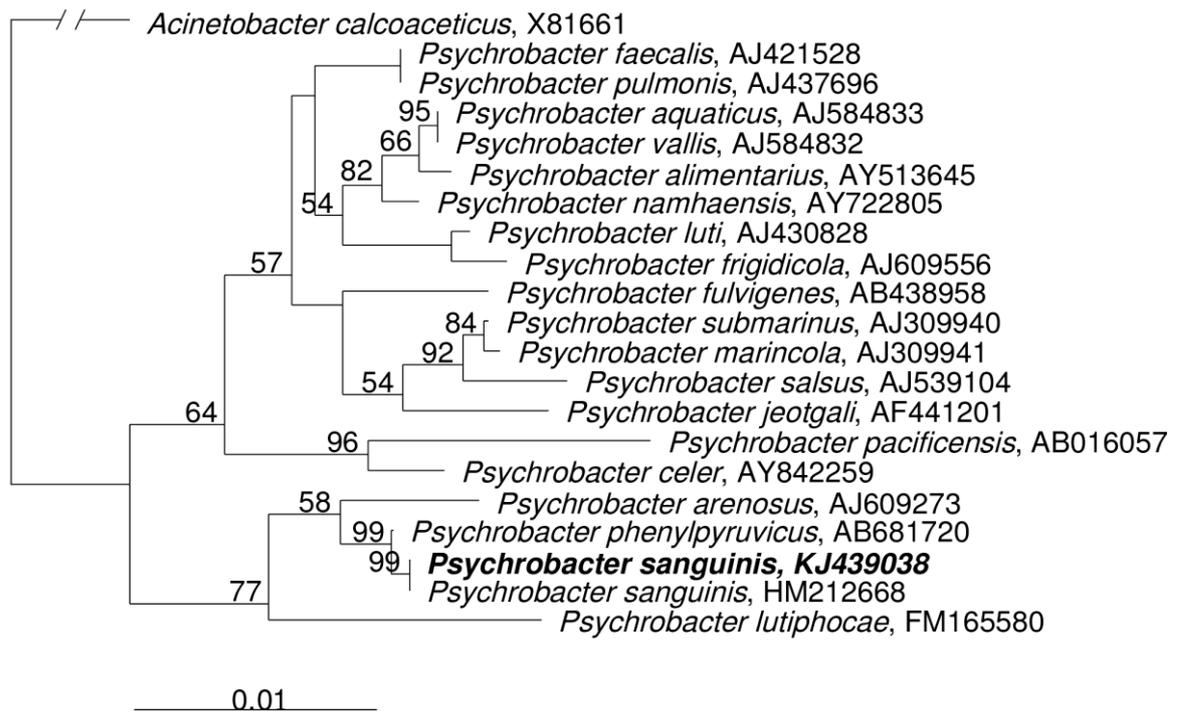


Figure 1. Neighbor-joining phylogenetic tree based on 16S rRNA sequences from 19 *Psychrobacter* type strains, the *Psychrobacter sanguinis* isolate KJ439038 from our study, and an *Acinetobacter calcoaceticus* type strain as a root. Bootstrap values (> 50%) are indicated at branch nodes.