JCM Journals.ASM.org

CASE REPORT

Psychrobacter sanguinis: an Unusual Bacterium for Nosocomial Meningitis

Rémi Le Guern,[a] Frédéric Wallet,[a,c] Emmanuel Vega,[b,c] René J. Courcol,[a,c] Caroline Loïez[a,c]

Institute of Microbiology, University Hospital Center, Lille, France[a]; Department of Neurosurgical Intensive Care, University Hospital Center, Lille, France[b]; University of Lille Nord de France, Lille, France[c]

We report the first case of postneurosurgical meningitis due to *Psychrobacter sanguinis*, identified only by 16S rRNA analysis. *Psychrobacter* spp. usually live in deep sea environments and cold habitats. 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. After hospital admission to an emergency department, clinical examination revealed nuchal rigidity but no focal neurological symptoms. There was no hyperthermia or altered consciousness (Glasgow coma scale evaluated at 14). 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 put in place under local anesthesia to relieve the elevated intracranial pressure. 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 cerebrospinal 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 an increased concentration of proteins (1.64 g/liter) and decreased glucose (0.19 g/liter, with glycemia = 1.32 g/liter). Mild hypoglycorrhachia occurs frequently after subarachnoidal hemorrhage; therefore, it was not considered indicative of meningitis. No bacteria were shown on Gram stain, and the cultures remained sterile after 5 days of incubation (chocolate agar in a CO_2 atmosphere and brain heart infusion [BHI] broth under aerobic conditions at 37°C). On day 15, the patient presented hyperthermia at 39°C, with confusional syndrome and drowsiness. A new lumbar puncture was performed, and the CSF culture allowed a definitive diagnosis of postneurosurgical meningitis. CSF analysis showed hyperleukocytosis (5,900 white blood cells [WBC]/mm^3), profound hypoglycorrhachia (0.07 g glucose/liter, with glycemia = 1.78 g/liter), and elevated protein levels (1.53 g/liter). Direct examination revealed few Gram-negative coccobacilli. On the basis of the evolution of CSF parameters (Table 1), empirical antimicrobial therapy was initiated immediately using a combination of meropenem and fosfomycin at 2 g 3 times per day and at 4 g 4 times per day, respectively. A single dose of amikacin (25 mg/kg of body weight) was also administered. No bacterial growth was observed after 48 h of incubation. After 4 days of incubation, the BHI broth became turbid due to the growth of Gram-negative coccobacilli. The BHI broth was subcultured onto blood agar incubated at 37°C, chocolate agar incubated at 37°C and 5% CO_2, and blood agar incubated at 37°C in an anaerobic atmosphere. The Gram-negative coccobacilli grew on the blood and chocolate agar only under aerobic conditions after 48 h. These colonies were catalase, oxidase, and urease positive. Unfortunately, matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF) (Bruker Daltonik S. A., Wissembourg, France) using FlexControl software (version 3.0) gave no reliable identification (*Psychrobacter* sp.; score = 1.065). To identify this agent, sequencing of the 16S rRNA gene was performed and a 1,500-bp DNA fragment was amplified using universal primers 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1525R (5′-AAAGGAGGTGATCCAGCC-3′). DNA sequence analysis was performed with leBIBI and BLAST systems. There was 100% identity for 1,400 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 (1). A phylogenetic tree was created using the ARB neighbor-joining algorithm (Fig. 1). Attempts at phenotypical identification using the Vitek2 GN card (bioMérieux) and API 20NE (bioMérieux) were not successful. *In vitro* antimicrobial susceptibility was determined by Mueller-Hinton agar disc diffusion incubated under aerobic conditions at 37°C, using the critical zone diameters recommended by CA-SFM 2013 (Comité de l'Antibiogramme de la Société Française de Microbiologie; http://www.sfm-microbiologie.org/). Our isolate of *P. sanguinis* was susceptible to amoxicillin, ticarcillin, cefepime, ceftazidime, imipenem, meropenem, amikacin, ciprofloxacin, and trimethoprim-sulfamethoxazole. Therefore, meropenem and fosfomycin were switched to amoxicillin (4 g 3 times per day), as part of the antimicrobial therapy de-escalation strategy. Amoxicillin treatment was continued until the patient had received 15 days of antimicrobial therapy. The patient experienced a rapid

Received 24 April 2014 **Returned for modification** 22 May 2014
Accepted 25 June 2014
Published ahead of print 2 July 2014
Editor: E. Munson
Address correspondence to Caroline Loïez, caroline.loiez@chru-lille.fr.

doi:10.1128/JCM.01197-14

TABLE 1 Timeline of CSF parameters

Day	No. of WBC[a]/mm^3	Gram stain result	Culture result	Glycorrhachia (g glucose/liter)	Glycemia (g glucose/liter)	Protein level g/liter
8	680	Negative	Sterile	0.19	1.32	1.64
15	5.900	Gram-negative coccobacilli	*P. sanguinis*	0.07	1.78	1.53
19	12	Negative	Sterile	0.54	1.43	0.92

[a] WBC, white blood cells.

recovery under treatment. The fever subsided 4 days after the initiation of meropenem and fosfomycin treatment. A lumbar puncture performed the same day (on day 19) revealed only 12 WBC/mm^3 and a normal level of glucose (0.54 g/liter). The protein level was still slightly elevated (0.92 g/liter). No other complications were observed during the hospitalization.

We report the first case of postneurosurgical meningitis due to *P. sanguinis*. *Psychrobacter* spp. usually live in deep sea environments and cold habitats, such as Antarctic ice or Siberian permafrost. Members of the *Psychrobacter* genus are small Gram-negative coccobacilli. *Psychrobacter* spp. have been observed in marine environments such as deep sea (2) or sea ice (3) and in a wide range of food products, including seafood (4, 5) and cheese (6). *P. sanguinis* is a new species described in 2012; the type strain was isolated from the blood of an 84-year male in the United States (7). Because *P. sanguinis* is a recently described species, data are lacking concerning its pathogenicity. *P. sanguinis* was first isolated from the blood cultures of 4 patients in New York (7). However, *P. sanguinis* is probably an environmental bacteria and was recovered from seaweed samples in India (GenBank accession no. JX501674 and JX501676). Whereas *Psychrobacter* spp. usually grow on marine agar, which contains 3.5% NaCl, our *P. sanguinis* isolate failed to grow on this specific medium, as described by Wirth et al. (7). Moreover, our isolate was able to grow between the temperatures of 4°C and 37°C. Other *Psychrobacter* species have been found in various environmental sites and are considered only rare opportunistic human pathogens. *P. arenosus* is a species considered to be closely related to *P. sanguinis* on the basis of their 16S rRNA sequences and was described in 2004 (8). *P. arenosus* was isolated from costal sea ice in Japan (9) and was associated with a published case of bacteremia after blood transfusion in France (10). 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 postneurosurgical meningitis. This was a case of nosocomial meningitis because the bacteria was identified in a CSF analysis performed more than 48 h after the hospitalization, but the nature of the bacteria suggested an extrahospital contamination source. One hypothesis is that the bacteria were present in the patient environment, perhaps before the hospitalization, 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 instructions from the hospital staff. It is also possible that the mode of entry was transient bacteremia, but it is less likely. Four sets of blood cultures were drawn during the hospitalization and remained sterile.

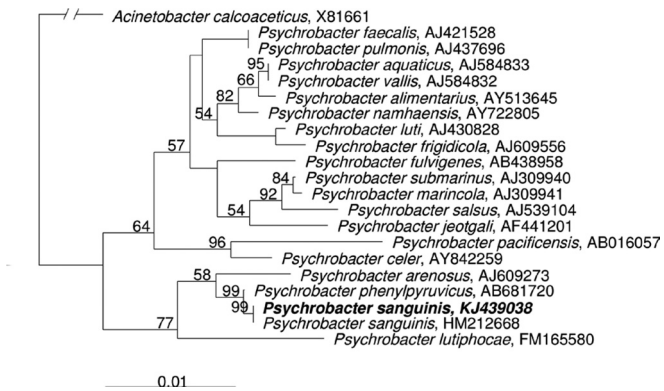

FIG 1 Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences from 19 *Psychrobacter* type strains, *Psychrobacter sanguinis* isolate KJ439038 from our study, and an *Acinetobacter calcoaceticus* type strain as a root. Bootstrap values (>50%) are indicated at branch nodes.

Nucleotide sequence accession number. The 16S rRNA gene sequence data of this *P. sanguinis* strain was deposited in GenBank under accession number KJ439038.

ACKNOWLEDGMENT

We have no conflicts of interest to disclose.

REFERENCES

1. **Ludwig W, Strunk O, Westram R, Richter L, Meier H, Yadhukumar Buchner A, Lai T, Steppi S, Jobb G, Forster W, Brettske I, Gerber S, Ginhart AW, Gross O, Grumann S, Hermann S, Jost R, Konig A, Liss T, Lussmann R, May M, Nonhoff B, Reichel B, Strehlow R, Stamatakis A, Stuckmann N, Vilbig A, Lenke M, Ludwig T, Bode A, Schleifer KH.** 2004. ARB: a software environment for sequence data. Nucleic Acids Res. **32:**1363–1371. http://dx.doi.org/10.1093/nar/gkh293.
2. **Maruyama A, Honda D, Yamamoto H, Kitamura K, Higashihara T.** 2000. Phylogenetic analysis of psychrophilic bacteria isolated from the Japan Trench, including a description of the deep-sea species Psychrobacter pacificensis sp. nov. Int. J. Syst. Evol. Microbiol. **50:**835–846. http://dx.doi.org/10.1099/00207713-50-2-835.
3. **Bowman JP, McCammon SA, Brown MV, Nichols DS, McMeekin TA.** 1997. Diversity and association of psychrophilic bacteria in Antarctic sea ice. Appl. Environ. Microbiol. **63:**3068–3078.
4. **Broekaert K, Heyndrickx M, Herman L, Devlieghere F, Vlaemynck G.** 2013. Molecular identification of the microbiota of peeled and unpeeled brown shrimp (Crangon crangon) during storage on ice and at 7.5°C. Food Microbiol. **36:**123–134. http://dx.doi.org/10.1016/j.fm.2013.04.009.
5. **Noor Uddin GM, Larsen MH, Guardabassi L, Dalsgaard L, Dalsgaard A.** 2013. Bacterial flora and antimicrobial resistance in raw frozen cultured seafood imported to Denmark. J. Food Prot. **76:**490–499. http://dx.doi.org/10.4315/0362-028X.JFP-12-402.
6. **Coton M, Delbés-Paus C, Irlinger F, Desmasures N, Le Fleche A, Stahl V, Montel MC, Coton E.** 2012. Diversity and assessment of potential risk

factors of Gram-negative isolates associated with French cheeses. Food Microbiol. **29:**88–98. http://dx.doi.org/10.1016/j.fm.2011.08.020.

7. **Wirth SE, Ayala-del-Río HL, Cole JA, Kohlerschmidt DJ, Musser KA, Sepúlveda-Torres LDC, Thompson LM, Wolfgang WJ.** 2012. *Psychrobacter sanguinis* sp. nov., recovered from four clinical specimens over a 4-year period. Int. J. Syst. Evol. Microbiol. **62:**49–54. http://dx.doi.org/10.1099/ijs.0.029058-0.
8. **Yarza P, Richter M, Peplies J, Euzeby J, Amann R, Schleifer KH, Ludwig W, Glöckner FO, Rosselló-Móra R.** 2008. The all-species living tree project: a 16S rRNA-based phylogenetic tree of all sequenced type strains. Syst. Appl. Microbiol. **31:**241–250. http://dx.doi.org/10.1016/j.syapm.2008.07.001.
9. **Romanenko LA, Lysenko AM, Rohde M, Mikhailov VV, Stackebrandt E.** 2004. *Psychrobacter maritimus* sp. nov. and *Psychrobacter arenosus* sp. nov., isolated from coastal sea ice and sediments of the Sea of Japan. Int. J. Syst. Evol. Microbiol. **54:**1741–1745. http://dx.doi.org/10.1099/ijs.0.63096-0.
10. **Caspar Y, Recule C, Pouzol P, Lafeuillade B, Mallaret MR, Maurin M, Croize J.** 2013. *Psychrobacter arenosus* bacteremia after blood transfusion, France. Emerg. Infect. Dis. **19:**1118–1120. http://dx.doi.org/10.3201/eid1907.121599.