Ann Clin Microbiol Vol. 20, No. 3, September, 2017
https://doi.org/10.5145/ACM.2017.20.3.74
pISSN 2288-0585 · eISSN 2288-6850

First Case of *Psychrobacter sanguinis* Bacteremia in a Korean Patient

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Psychrobacter sanguinis has been described as a Gram-negative, aerobic coccobacilli originally isolated from environments and seaweed samples. To date, 6 cases of *P. sanguinis* infection have been reported. A 53-year-old male was admitted with a generalized tonic seizure lasting for 1 minute with loss of consciousness and a mild fever of 37.8°C. A Gram stain revealed Gram-negative, small, and coccobacilli-shaped bacteria on blood culture. Automated microbiology analyzer identification using the BD BACTEC FX (BD Diagnostics, Germany) and VITEK2 (bioMérieux, France) systems indicated the presence of *Methylobacterium* spp., *Aeromonas salmonicida*, and the *Moraxella* group with low discrimination. The GenBank Basic Local Alignment Search Tool and an Ez-Taxon database search revealed that the 16S rRNA gene sequence of the isolate showed 99.30% and 99.88% homology to 859 base-pairs of the corresponding sequences of *P. sanguinis*, respectively (GenBank accession numbers JX501674.1 and HM212667.1). To the best of our knowledge, this is the first human case of *P. sanguinis* bacteremia in Korea. It is notable that we identified a case based on blood specimens that previously had been misidentified by a commercially automated identification analyzer. We utilized 16S rRNA gene sequencing as a secondary method for correctly identifying this microorganism. **(Ann Clin Microbiol 2017;20:74-79)**

Key Words: Bacteremia, *Psychrobacter sanguinis*, 16S ribosomal RNA

INTRODUCTION

Psychrobacter spp. are a Gram-negative, small, aerobic, non-motile, chemoheterotrophic, and halotolerant coccobacilli bacteria [1]. *Psychrobacter* spp. have been isolated from diverse habitats such as deep sea water, sea ice, marine sediments, and a wide range of food products including seafood and cheese [1-4]. *Psychrobacter* isolates from humans are generally not clinically relevant. On occasion they have served as opportunistic pathogens in humans and have been isolated in clinical samples from brain tissue, urine, ears, wounds, cerebrospinal fluid (CSF), and blood. Most clinical isolates belong to the species *P. immobilis* and *P. phenylpyruvicus* [5-10].

In 2012, *P. sanguinis* was first described as a novel species based on four clinical specimens, and the type strain was isolated from the blood of an 84-year-old male in New York [11]. Because *P. sanguinis* is a recently described species, data are lacking concerning its pathogenicity, and its clinical significance is not clear. Since the initial description of this microorganism, six cases of *P. sanguinis* infection have been reported in humans, specifically blood specimens and CSF [11-13].

To the best our knowledge, this is the first report of a *P. sanguinis* bacteremia case identified by 16S rRNA gene sequencing in Korea.

CASE REPORT

A 53-year-old male was admitted to the emergency department after suffering one generalized tonic seizure for 1 minute with loss of consciousness. The patient had a 20-year history of

Received 28 April, 2017, Revised 28 July, 2017, Accepted 11 August, 2017

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chronic alcoholism with fatty liver, an 8-year history of gastrotomy with peptic ulcer, and had been admitted to the intensive care unit 8 years prior due to head trauma caused by a traffic accident. He lived in an urban area of Seoul and denied having traveled or having had any animal or fish contact in the past year. His vital signs were as follows: blood pressure, 135/72 mmHg; pulse rate, 89 beats/min; respiratory rate, 20 breaths/min; and body temperature, 36.5-37.8°C (Fig. 1). Laboratory test results were as follows: Hb, 110.0 g/L; white blood cell count, 17.52×10^9/L (neutrophils, 81.6%; lymphocytes, 10.3%; monocytes, 7.3%; eosinophils, 0.3%; and basophils, 0.5%); and platelets, 365×10^9/L. Aspartate aminotransferase (AST) and alkaline phosphatase (ALP) levels were elevated to 0.97 μkat/L and 1.75 μkat/L, respectively. Serum lactate dehydrogenase (LDH), creatine kinase-MB (CK-MB), and creatine phosphokinase (CPK) levels were elevated to 4.56 μkat/L, 8.64 μg/L, and 8.27 μkat/L, respectively. The initial serum C-reactive protein (CRP) level of 30.48 nmol/L was within the reference interval, but increased to 189.53 nmol/L on follow-up 13 hours later test. At that time, his body temperature was 37.4°C. This patient showed hyponatremia (115 mmol/L), hypochloremia (73 mmol/L), and hypoosmolality (249 mmol/kg). Diffusion-weighted magnetic resonance imaging (diffusion MRI) showed an old infarction in the right posterior corona radiate, but no intracerebral hemorrhage, mass or extra-axial blood/fluid collection. CSF analysis showed the following: colorless; pH of 7.6; RBCs, 0.033×10^9/L; WBCs, 0.002×10^9/L; specific gravity, 1.006; total protein, 42.4 mg/dL; glucose, 77 mg/dL; and chloride, 115 mmol/L. No evidence of infection or malignancy, and CSF culture study didn't performed due to not ordered and lack of remained sample volume. Further clinical and neurological examinations for mild fever and mental status were difficult due to a lack of patient compliance, repeated denials and swearing.

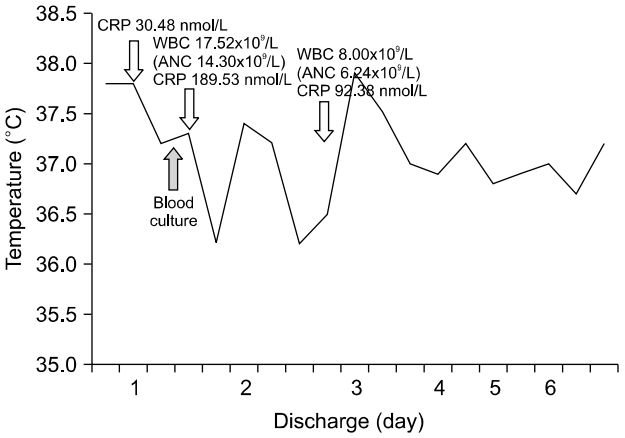

Fig. 1. Graph showing change in body temperature, white blood cell count (WBC), absolute neutrophil count (ANC) and C-reactive protein (CRP) levels during admission days.

Because of the persistent mild fever despite fever-reducing supportive care with fluid therapy and ice bag applications, we immediately performed blood and urine cultures. Two blood culture sets were taken from two different vein sites on day 1 at the emergency department. However, he did not take antibiotics except supportive care. After 1 day of incubation, one aerobic blood culture of two blood culture sets showed growth of microorganisms. Two days later, non-pigmented, circular, smooth, and non-hemolytic colonies of unequal sizes were observed only on the blood agar plate (Fig. 2A). And no growth was observed on the MacConkey agar plate. Gram staining of a purified colony revealed Gram-negative coccobacilli-shaped bacteria that were positive for oxidase (Fig. 2B). Automated microbiology analyses using the BD BACTEC FX system (BD Diagnostics, Heidelberg, Germany) and the VITEK2 system

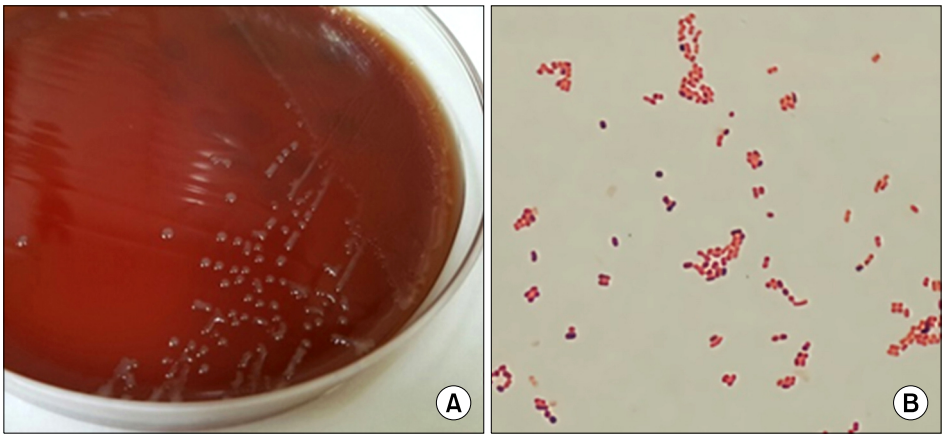

Fig. 2. Colonial and microscopic morphology of *Psychrobacter sanguinis*. (A) Non-pigmented and non-hemolytic colonies with circular and smooth edges on a blood agar plate. (B) Gram-negative coccobacilli (Gram stain, ×1,000).

(bioMérieux, Marcy l'Etoile, France) indicated the presence of *Methylobacterium* spp., *Aeromonas salmonicida*, and the *Moraxella* group, with low (<50%) discrimination and a questionable level of confidence due to low biofrequency. An antimicrobial susceptibility test of the isolate could not be performed due to a lack of established criteria for interpretation of the results. On admission day 3, the patient showed rhabdomyolysis and serum CPK was elevated to 323.88 μkat/L. Mild fever continued for three days and the maximum body temperature up to 37.8°C with serum CRP level of 92.38 nmol/L on follow-up test, but we did not start antibiotic therapy until his condition gradually improved. He recovered from rhabdomyolysis with a reduced CPK level (83.65 μkat/L) and no additional seizure episode, but his orientation was decreased in that he did not respond quickly with information about location and time, and he gave delirious, irrelevant answers. The patient's mild fever lasted 37.1-37.2°C but his and his family's compliance was poor, and he repeatedly denied follow-up procedures for blood cultures and clinical examinations. He requested discharge after 6 days of admission, after which he was lost to follow-up by the outpatient department. The etiologic diagnosis was established after discharge by means of molecular analysis.

To identify the bacteria, molecular identification was performed by DNA amplification and sequencing analysis of the 16S rRNA gene according to CLSI guidelines [14]. In the PCR reaction, the 16S rRNA gene was amplified using the following primer pair: forward, 27F: 5′-AGA GTT TGA TCM TGG CTC AG-3′ and reverse, 1492R:5′-TAC GGY TAC CTT GTT ACG ACT T-3′. The PCR reaction was performed with 20 ng of genomic DNA as the template in a 30-μL reaction mixture using EF-Taq (SolGent, Daejeon, Korea) as follows: activation of Taq polymerase at 95°C for 2 minutes; 35 cycles of 95°C, 55°C, and 72°C for 1 minute each; and finishing with 10 minutes at 72°C. The amplification products were purified with a multiscreen filter plate (Millipore Corp., Bedford, MA, USA). The sequencing reaction was performed using a PRISM BigDye Terminator v3.1 cycle sequencing kit with using the following primer pair: forward, 785F: 5′-GGATTAGATACCCTGGTA-3′ and reverse, 907R: 5′-CCGTCAATTCMTTTRAGTTT-3′. The DNA samples containing the extension products were added to Hi-Di formamide (Applied Biosystems, Foster City, CA, USA). The mixture was incubated at 95°C for 5 minutes, followed by 5 minutes on ice and analysis by the ABI Prism 3730XL DNA analyzer (Applied Biosystems).

We obtained a consensus sequence of 859 base-pairs (bp). The sequence was analyzed using the GenBank Basic Local

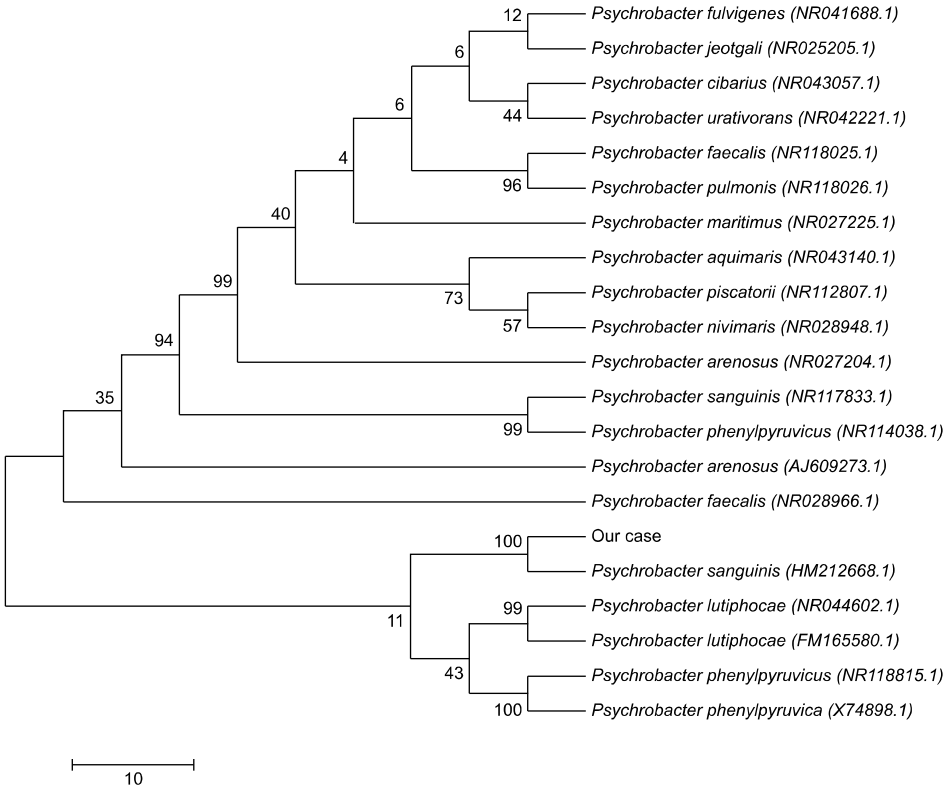

Fig. 3. Unrooted neighbor-joining phylogenetic tree based on 16S rRNA sequences of *Psychrobacter sanguinis* and 20 similar organisms.

Alignment Search Tool (BLAST) (http://www.ncbi.nlm.nih.gov/genbank) and the EzTaxon database (http://www.eztaxon.org). A GenBank BLAST search revealed that the 16S rRNA gene sequence of the isolate showed 99.30% homology of 859 bp with the corresponding sequences of *P. sanguinis* (GenBank accession numbers JX501674.1 and HM212667.1). *P. arenosus* (NR_027204.1), *P. phenylpyruvicus* (LT223678.11), and *Moraxella phenylpyruvica* (AF005192.1) were the next matches, with similarities of 97.78%, 97.67%, and 97.07%, respectively. When the sequence was submitted to the Ez-Taxon database, we found that the highest similarity was with *P. sanguinis* (HM212667.1) (99.88%). *P. arenosus* (AJ609273), *P. phenylpyruvicus* (BCUH 01000122), and *P. lutiphocae* (FM165580) were the next matches, with similarities of 98.35%, 98.13%, and 97.04%, respectively. A phylogenetic tree based on the 16S rRNA sequences of the isolate and 20 organisms with similarity to the isolate was constructed using the neighbor-joining method (Fig. 3). Phylogenetic and molecular evolutionary analyses were conducted using Molecular Evolutionary Genetics Analysis (MEGA) software version 6.0 (http://www.megasoftware.net).

DISCUSSION

Psychrobacter sanguinis is a member of the *Psychrobacter* genus, which contains small, gram-negative, non-motile, chemoheterotrophic, and halotolerant coccobacilli bacteria [1]. The genus *Psychrobacter* contains 33 species with validly published names. Species of this genus have been observed from various origins, including the deep sea, water, sea ice, pigeon feces bioaerosols, and food products including seafood, poultry, and meat [1-4]. From 2004 to 2008, human blood cultures yielded four *Psychrobacter* isolates at the Wadsworth Center in New York. As the Wadsworth Center laboratory was not able to definitively identify them and the isolates did not provide an acceptable sequence similarity match to any species in the 16S rRNA sequence database, they were assigned to a single novel species. This represented the first report of *P. sanguinis* in humans, and the type strain was isolated from the blood of an 84-year-old male in 2012 [11]. In 2014, *P. sanguinis* was isolated from the CSF of a 64-year-old female patient with post-neurosurgical meningitis, and this strain was identified only by 16S rRNA analysis [12]. In 2016, one case of meningitis associated with *Psychrobacter* was reported, and this isolate was identified by next-generation sequencing (NGS) metagenomics in the CSF of a 13-year-old male patient. The closest relative of this strain was *P. sanguinis* on the basis of a DNA fingerprinting phylogenomic study and a 16S rRNA gene phylogenetic analysis [13]. To date, a total of six cases of *P. sanguinis* human infection have been reported in the literature (Table 1).

Current phenotypic identification systems, including the VITEK2 system, are unable to identify *P. sanguinis* and may confuse it with other organisms such as in this case, including *Methylobacterium* spp., *Aeromonas salmonicida*, and the *Moraxella* group. The genus *Psychrobacter* currently belongs to the family *Moraxellaceae* [15]. In addition, *M. phenylpyruvica* has been reclassified to the genus *Psychrobacter* as *P. phenylpyruvica*, based on a sequence analysis of its 16S rRNA genes [16]. Published articles describing microorganisms misidentified by conventional methods are increasing [17]. It is evident that the number of newly-identified bacterial species is growing. Consequently, the performance of commercially available biochemical platforms that allow an exact identification is decreasing, partly due to sequence database limitations. The unambiguous identification of unusual strains in clinical micro-

Table 1. Summary of previously reported cases with *Psychrobacter sanguinis* from human specimens

Patient	Sex/Age	Diagnosis	Specimen	Conventional cultures	*P. sanguinis* identification methods	Reported year	Country	Reference
1	M/84	Unknown	Blood	Unknown	16S rRNA gene sequencing	2012	USA	[11]
2	M/50	Unknown	Blood	Unknown	16S rRNA gene sequencing	2012	USA	[11]
3	F/61	Unknown	Blood	Unknown	16S rRNA gene sequencing	2012	USA	[11]
4	M/66	Unknown	Blood	Unknown	16S rRNA gene sequencing	2012	USA	[11]
5	F/64	Meningitis	Cerebrospinal fluid	No reliable identification (*Psychrobacter* sp. score=1.065)	16S rRNA gene sequencing	2014	France	[12]
6	M/13	Meningitis	Cerebrospinal fluid	Unknown	NGS metagenomics	2016	Mexico	[13]
7	M/53	Unknown	Blood	*Methylobacterium* spp., *Aeromonas salmonicida*, *Moraxella* group	16S rRNA gene sequencing	2017	Korea	Present report

biology is increasingly needed to improve our knowledge of tissue reservoirs, routes of transmission, antibiotic susceptibility, and enhancement of treatments, and to assist in the identification of novel bacterial species. Identification by conventional methods of rare bacteria and bacteria with unusual phenotypic profiles needs to be confirmed using a reliable tool such as 16S rRNA gene sequencing [18].

In this case, 16S rRNA gene sequencing was required for accurate bacterial identification. Our study shows that sequencing of 16S rRNA and analyses using various databases are useful tools for the identification of unknown isolates. We report a first case in which *P. sanguinis* was isolated from human blood in Korea. In the six previously reported cases, we didn't detailed review for first reported case and the other three cases due to a lack of information in the literature [11] and two cases with meningitis were described as a probable infection [13] or a definite infection [12]. These cases were confirmed as a single organism with *P. sanguinis* infection by using molecular methods, and were treated with antibiotics. Only one patient [12], antibiotic susceptibility testing was performed, which was susceptible to amoxicillin, ticarcillin, cefepime, ceftazidime, imipenem, meropenem, amikacin, ciprofloxacin, and trimethoprim-sulfamethoxazole. Therefore, amoxicillin treatment with 4g three times per day was continued until the patient had received 15 days of antimicrobial therapy. The patient experienced a rapid recovery under treatment with no other complications [12].

Our study had several limitations. In our case, we attempted to identify the source of *P. sanguinis*, but it has proved difficult to ascertain how *P. sanguinis* caused bacteremia. It is likely that the bacteria were present in the patient's environment given that he had a mild fever on arrival at the emergency department, but this is less likely because blood cultures were drawn during hospitalization and sterile blood sampling. It is also possible that the patient had transient bacteremia due to his fever seemed to be improved with supportive care during admission day 2, but this is less likely because 37.8°C fever checked at admission day 3 and maintained 37.1-37.2°C before discharge. Lastly, although gram-negative coccobacilli-shaped bacteria was growth in his blood culture under infection-related conditions with elevated WBC, CRP levels and body temperature. It is seen as a weak point in confirming pathogenic microorganism because of being detected only one aerobic blood culture. However, patient and his family's compliance were poor and patient repeatedly denied follow-up procedures, we could not further work-up for blood cultures and clinical examinations.

Aerobic microbes are the most common cause of clinical infections such as community-acquired bacteremia and opportunistic infections, and aerobic bacteremia can be serious and even life-threatening in patients with weakened immune systems [19, 20]. Chronic heavy drinking increases the risk of infection by disrupting the immune system [21-23]. Although *P. sanguinis* is rarely reported as a pathogenic microorganism, it should be carefully considered in patients suffering from chronic alcoholism with fatty liver and nutritional imbalance. We isolated *P. sanguinis* from blood and successfully identified it using molecular methods in clinical laboratories.

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=국문초록=

한국인에서 발생한 *Psychrobacter sanguinis* 균혈증 1예

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*Psychrobacter sanguinis*은 그람 음성 호기성 짧은 간균으로 해조류 표본과 환경에서 분리되는 균입니다. 지금까지 *P. sanguinis* 감염은 6건이 보고 되었습니다. 53세 남자 환자가 의식 상실을 동반한 1분간 지속되는 전신 강직 발작과 37.8℃의 경미한 발열로 입원하였습니다. 혈액배양을 통한 그람 염색에서 그람 음성의 작고 짧은 간균 형태의 균이 보였습니다. BD BACTEC FX (BD Diagnostics, Germany) 및 VITEK2 (bioMérieux, France) 시스템을 이용하여 자동화 된 미생물 분석기로 낮은 식별을 보이며 *Methylobacterium* spp., *Aeromonas salmonicida*와 *Moraxella* group으로 동정되었습니다. 이에 대하여 균을 확인하기 위해 분자 식별 검사를 수행하였고, GenBank Basic Local Alignment Search Tool과 Ez-Taxon 데이터베이스 검색 결과, 분리 균주의 16S rRNA 유전자 서열은 *P. sanguinis* (GenBank accession numbers JX501674.1 and HM212667.1)의 해당 염기 서열 859 염기쌍과 99.30% 및 99.88%의 상동성을 보였습니다. 아직까지 국내에는 이 균에 의한 인체 감염이 보고된 바가 없어, 이것은 한국의 *P. sanguinis* 균혈증의 최초의 사례입니다. 이전에 상업적으로 자동화된 식별 분석기로 잘못 식별된 혈액 표본을 바탕으로 한 사례를 확인했습니다. 우리는 이 균을 정확하게 확인하기 위하여 2차적 방법으로 16S rRNA 유전자 서열을 활용했습니다. [Ann Clin Microbiol 2017:20:74-79]

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