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A new causative bacteria of infective endocarditis, *Bergeyella cardium* sp. nov.

Kyung Mok Sohn [a,1], Kyungmin Huh [b,1], Jin-Yang Baek [d], Yeon-Sook Kim [a], Cheol-In Kang [b], Kyong Ran Peck [b], Nam Yong Lee [c], Jae-Hoon Song [b], Kwan Soo Ko [d,e,*], Doo Ryeon Chung [b,**]

[a] Division of Infectious Diseases, Chungnam National University Hospital, Daejeon, Republic of Korea
[b] Division of Infectious Diseases, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea
[c] Department of Laboratory Medicine and Genetics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea
[d] Asia Pacific Foundation for Infectious Diseases (APFID), Seoul, Republic of Korea
[e] Department of Molecular Cell Biology, Samsung Biomedical Research Institute, Sungkyunkwan University School of Medicine, Suwon, Republic of Korea

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ABSTRACT

The first cases of infective endocarditis due to a new species of *Bergeyella*, *Bergeyella cardium* sp. nov., are reported. Two strains, 13-07^T (= JCM 30115^T = NCCP 15908^T) and 13-16, were independently isolated from 2 patients in different hospitals in Korea. Initially, the isolates were identified as *Brevundimonas* spp.; however, their 16S rRNA gene sequences shared a similarity of 94.9% with *Bergeyella zoohelcum*, implying that they are a new species belonging to of the genus *Bergeyella*. The organisms might be susceptible to many commonly used antibiotics, including penicillin. The first case was successfully treated with ceftriaxone, and the second, with piperacillin/tazobactam plus amikacin.

1. Introduction

The genus *Bergeyella*, a nonfermentative gram-negative bacilli within the family Flavobacteriaceae, was first described in 1994 (Vandamme et al., 1994). Presently, only 1 species, *Bergeyella zoohelcum*, has been identified in this genus. *B. zoohelcum* is known to be part of the normal oral flora of cats, dogs, and other animals (Hugo et al., 2006). Most reported cases of human infection by these bacteria are attributed to animal bites or are associated with prolonged exposure to pets (Isotalo et al., 2000; Montejo et al., 2001; Reina and Borrell, 1992; Shukla et al., 2004). Additionally, *B. zoohelcum* bacteremia has also been reported in a patient who consumed food prepared with coagulated goat blood, and a case of cellulitis attributed to *B. zoohelcum* has been reported in a tsunami victim (Beltran et al., 2006; Kallman et al., 2006).

In this paper, we report 2 cases of infective endocarditis caused by a new species of the genus *Bergeyella*, *Bergeyella cardium* sp. nov. The bacteria were independently isolated from 2 different hospitals in Korea.

2. Cases

2.1. Case 1

A 26-year-old male was admitted to hospital (Chungnam National University Hospital, Daejeon, Korea) because of a fever and chills. The patient had been in his usual state of health, without any history of medical problems, until approximately 2 weeks before admission, when the fever and chills developed. The patient self-administered over-the-counter cold medication, but the symptoms persisted. One day prior to admission to the hospital, his primary care provider prescribed a 5-day course of amoxicillin; however, he did not comply. The patient was an office worker and did not keep any pets. He did not smoke, drink alcohol, or use illicit drugs. On examination, the patient was found to be thin, alert, and oriented to person and time. The patient's temperature was 38.6 °C, he had a blood pressure of 100/60 mm Hg, his pulse was 92 beats per minute and regular, his respiratory rate was 18 breaths per minute, and his oxygen saturation was 98% while breathing ambient air. A systolic murmur was audible at the left sternal border. There were no peripheral stigmata of infective endocarditis, and the remainder of the physical examination was normal. Laboratory evaluation revealed leukocytosis with a left shift, elevated C-reactive protein levels, and 1+ proteinuria indicated by a 1+ dipstick test result. All other laboratory results were within normal limits. Transthoracic echocardiography revealed normal left ventricular function with severe eccentric mitral regurgitation due to a prolapse of the anterior mitral valve leaflet (AMVL); a small echogenic mobile mass was found on the tip of the AMVL. The electrocardiogram and chest radiograph were normal. Blood cultures were taken from the patient on 3 consecutive days following admission. During the next 3 days, the patient was noted to be persistently febrile, but the blood cultures displayed no bacterial growth. On the fourth day after admission, ampicillin/sulbactam was administered intravenously to treat possible infective endocarditis. The following day, bacterial growth was observed on the blood cultures; the patient became afebrile but reported dyspnea. A chest radiograph

* Correspondence to: Tel.: +82-31-299-6223; fax: +82-31-299-6229.
** Correspondence to: Tel.: +82-2-3410-0323; fax: +82-2-3410-0064.
E-mail addresses: ksko@skku.edu (K.S. Ko), iddrchung@gmail.com (D.R. Chung).
[1] K. M. Sohn and K. Huh contributed equally to this work.

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showed bilateral pleural effusion and pulmonary edema. The patient underwent urgent mitral valve replacement 6 days after hospital admission. Pathological examination of a biopsy specimen of the mitral valve vegetation showed infiltration of inflammatory cells; no microorganisms were revealed by Gram staining. A culture grown from the biopsy specimen was positive for gram-negative rod-shaped bacteria. It was supposed that the causative pathogen was a member of the HACEK group of microorganisms. Treatment with ceftriaxone commenced, and treatments with other antibiotics were halted. On the 7th day following admission, the microbiology laboratory reported that the microorganism from the blood culture was a *Brevundimonas* spp.; this was identified using the Vitek2 system (bioMérieux, Durham, NC, USA). The patient's postoperative recovery was uneventful. The patient completed a 6-week course of treatment with ceftriaxone. He was discharged from the hospital in a stable condition and resumed his normal daily activities.

2.2. Case 2

A 47-year-old male was admitted to the cardiothoracic surgery department of a hospital (Samsung Medical Center, Seoul, Korea) with orthopnea. He recalled intermittent fever with chills and a productive cough that had been occurring for 5 months prior to visiting the hospital; his symptoms slightly improved after symptomatic treatment but they recurred 2–3 weeks later. The patient developed orthopnea 3 weeks before visiting the hospital. The patient's past medical history was unremarkable with the exception of an episode of paroxysmal supraventricular tachycardia approximately a year ago. He denied using any medication or illicit drugs. He had not experienced any trauma or undergone any invasive medical procedures. No remarkable travel history was reported. He did not keep pets or domestic animals at home, but he did report the presence of stray cats in a warehouse at his place of work. He stated that he had not had any direct contact with the stray cats. Echocardiography revealed oscillating vegetation attached to the aortic valve. A blood culture was started and empirical antibiotic treatment with ceftriaxone, ampicillin, and gentamicin commenced. Mitral and aortic valve replacement with mechanical valves and aortic annular reconstruction surgery were performed the next day. Intraoperatively, a 10 × 5 mm perforation with adjacent necrotic debris on the noncoronary cusp of the aortic valve was noted. Vegetation approximately 3 mm in size was found on the ventricular side of the valve. There was no sign of infection on the mitral valve. No postoperative complications occurred. The results of a histology analysis of the resected aortic valve were compatible with a diagnosis of fibromyxoid valvulopathy. While tissue cultures taken from the surgical specimens did not produce notable bacterial growth, an automated blood culture system reported the growth of microorganisms 4 days after the initial culture. Gram stain revealed the presence of gram-negative bacilli. A Vitek 2 system (bioMérieux) was used to identify the bacteria as a *Brevundimonas* spp. Based on the result, the antibiotic regimen was changed to piperacillin/tazobactam and amikacin. The result of molecular identification was reported on postoperative day (POD) 29, and the antibiotic regimen was changed to ampicillin/sulbactam on POD 33. The patient was discharged without further antibiotic treatment 4 days later. Since discharge, there has been no significant clinical event during follow-up.

3. Microbiological analysis

The strains investigated in this study, 13-07^T (= JCM 30115^T = NCCP 15908^T) and 13-16, were identified as *Brevundimonas* spp. using an automated identification system, the Vitek2 system, in the clinical microbiology laboratories of the hospitals. For a more precise identification, we independently performed a molecular identification analysis for the samples subjected to Vitek2 system based on the 16S rRNA gene sequences. The 16S rRNA gene sequences were obtained from the 2 strains, 13-07^T and 13-16 (1407 bp and 1306 bp, respectively), using primer sets 16S-F3 (5′-CAG GCC TAA CAC ATG CAA GT-3′) and 16S-R3 (5′-GGG CGG WGT GTA CAA GGC-3′) (Shin et al., 2008). The 2 strains showed the same 16S rRNA gene sequence. These 16S rRNA gene sequences were compared with the records of the EzTaxon public database (http://www.ezbiocloud.net) (Kim et al., 2012) and with the GenBank database (http://www.ncbi.nlm.nih.gov/blast) using BLAST searches. The type strain with the greatest pairwise similarity to strains 13-07^T and 13-16 was *B. zoohelcum* ATCC 43767^T, which demonstrated a sequence similarity of 94.9%. This sequence similarity strongly suggested that the 2 strains isolated from the hospital cases belonged to a novel bacterial species of the genus *Bergeyella* (Stackbrandt and Goebel, 1994), although there may be underreporting of rare organisms due to incomplete database. After retrieving the 16S rRNA gene sequences of species showing relatively high similarities to the novel species, a phylogenetic tree was constructed (Fig. 1). In Fig. 1, the 2 strains, 13-07^T and 13-16, were clustered with *B. zoohelcum*, but their grouping was not supported by bootstrap analysis. They showed very low similarities (<75%) with *Brevundimonas diminuta* ATCC11568^T, the type species of the genus *Brevundimonas*. Consequently, the phylogenetic tree supported that the 2 strains belonged to a novel species of *Bergeyella*.

Both strains grew slowly on blood and chocolate agars at 37 °C and did not grow at 24 °C and 30 °C. The strains grew at 41 °C on both blood and chocolate agars, but their growth rates were very low. The novel strains did not grow on Luria–Bertani and Mueller–Hinton agars. Thus, owing to the slow bacterial growth, antimicrobial resistance was evaluated in blood agar after 5 days of growth. The strains showed similar MIC susceptibilities in response to antimicrobial agents, with the exception of fluoroquinolones (Table 1). Both strains might be susceptible to ampicillin/sulbactam, cefepime, ceftriaxone, penicillin, and piperacillin/tazobactam, although we cannot definitely interpret the MICs due to lack of clinical experience in the treatment of the novel species. While the MICs of ciprofloxacin and levofloxacin against strain 13-7^T were 0.5 mg/L and 1 mg/L, respectively, the MICs against strain 13-16 were 2 mg/L and 4 mg/L, respectively. The cellular fatty acid (CFA) composition of the strains, which were assessed using a Hewlett Packard 6890A gas chromatograph and the MIDI aerobe method (Chem Station ver. 4.02) at MicroID (Seoul, Korea), are shown in Table 1. The most predominant CFA component of both strains was C15:0 iso (59.9% and

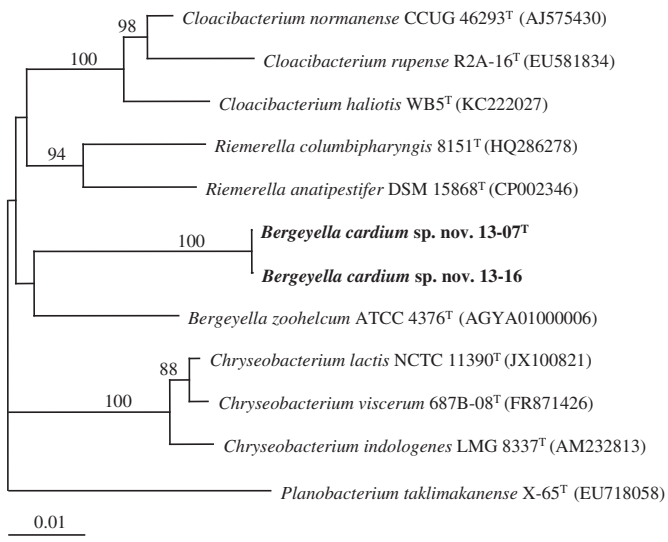

Fig. 1. Phylogenetic tree of *Bergeyella cardium* sp. nov. strains 13-7^T and 13-16, including type strains of other closely-related species based on 16S rRNA gene sequences. This tree was constructed using the neighbor-joining method, and *Planobacterium taklimakanense* X-65^T was used as an outgroup. The numbers at the branching nodes are percentages of 1000 bootstrap replications. Only values greater than 50% are shown. The scale bar represents one substitution per 100 nucleotides.