Prosthetic valve Endocarditis due to *Neisseria skkuensis*, novel *Neisseria* species

So Yeon Park, Seung Ji Kang, Eun-Jeong Joo, Young Eun Ha, Jin Yong Baek, Yu Mi Wi, Cheol-In Kang, Doo Ryeon Chung, Kyong Ran Peck, Nam Young Lee, and Jae-Hoon Song

Division of Infectious Diseases, Samsung Medical Center, Skungyunkwan University School of Medicine, Seoul, Republic of Korea

Division of Infectious Diseases, Chonnam National University Hospital, Chonnam National University School of Medicine, Gwang-ju, Republic of Korea

Asian-Pacific Foundation for Infectious Diseases (APFID), Seoul, Republic of Korea

Division of Infectious Diseases, Samsung Changwon Hospital, Sungkyunkwan University School of Medicine, Changwon, Republic of Korea

Department of Laboratory Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea

Running head: Endocarditis caused by *Neisseria skkuensis*

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* Corresponding author: Kyong Ran Peck, MD

Professor of Medicine

Division of Infectious Diseases, Department of Internal Medicine

Samsung Medical Center, Sungkyunkwan University School of Medicine,

50 Irwon-ro 81, Gangnam-gu, Seoul 135-710, Republic of Korea

Phone: +82-2-3410-0322

Fax: +82-2-3410-0064

Email address: krpeck@skku.edu
Abstract

We reported the first case of endocarditis due to *Neisseria skkuensis*. The organism from the blood cultures taken on admission day was identified initially as unidentified gram negative cocci by VITEK2. Finally, it was identified as a *Neisseria skkuensis* by 16S rRNA gene sequence analysis.
A 41-year old man was admitted to our hospital with a 1-week history of febrile sense, chills, sweating, aggravation on dyspnea, and hypotension during hemodialysis. He had a complicated past history, including liver cirrhosis caused by chronic hepatitis B infection and chronic kidney disease due to glomerulonephritis. He had received entecavir since 2009 and hemodialysis since 2000. In addition to these, he had undergone a mechanical mitral valve replacement due to infective endocarditis caused by methicillin resistant Staphylococcus aureus before one year ago. He denied dental treatment and drug abuse since mitral valve replacement.

On arrival at the emergency department, the patient’s vital sign were blood pressure, 86/52 mmHg; respiratory rate, 22 breaths per min; and temperature, 37 °C. Physical examination revealed metallic heart sounds without murmur and no abdominal tenderness with positive shifting dullness. A chest radiography showed cardiomegaly and pulmonary edema. Laboratory investigations revealed a C-reactive protein concentration of 10.0 mg/dl (normal [N], <0.3mg/dl), an erythrocyte sedimentation rate of 37mm/h (N, <22mm/h), a procalcitonin concentration of 152.2 ng/ml. The white blood cell (WBC) count was 9850/mm³ with dominant segmented neutrophils (85%), hemoglobin (Hb) 7.5 g/dl, platelet count 57000/mm³, blood urea nitrogen 49.5mg/dl, creatinine 6.55mg/dl (N, < 1.3mg/dl), and total bilirubin 1.2 mg/dl (N, < 1.5 mg/dl). He was coagulopathic with a prothrombin time of 23.1 seconds (N, 12.6 to 14.9), international normalized ratio (INR) of 2.0, activated partial thromboplastin time (APTT) of 72.7 seconds (N, 29.1 to 41.9), and D-dimer of 3.42 ug/ml (N, 0 to 0.5). Ascites analysis showed WBC count of 310/mm³, with 39% neutrophils, an albumin level of 1.3 g/dl, and a negative gram stain. Fluid was cultured and none grew any organism.

The transthoracic echocardiography showed well functioning prosthetic mitral valve and
moderate tricuspid valve regurgitation. There was no dilatation of the left ventricle and no vegetation was visualized. The transesophageal echocardiography showed oscillating mass lesions on prosthetic mitral valve (Fig. 1).

Blood cultures were conducted on admission day prior to antibiotic treatment. The aerobic bottles from each of three separately taken sets of blood cultures were incubated in a BacT/ALERT 3D (bioMérieux, Durham, NC., USA) and grew gram negative cocci in all three bottles. The Vitrek 2 GNI card system (bioMérieux, Durham, NC., USA) didn’t identify them. For molecular identification, we performed 16S rRNA gene sequence analysis and it was formally identified at the Asian-Pacific Foundation for Infectious Diseases (APFID) as 

*Nesseria skkuensis* based on the sequence of the 16S rRNA gene. The 16S rRNA gene was amplified using primer sets 16S-F2 (5′-AGAGTTTGATCMTGGCTCAG-3′) and 16S-R2 (5′-GGTTACCTTGTTCACGGACGT-3′). A 1406 bp sequence of the 16S rRNA gene was obtained from our strain. The 16S rRNA gene sequence was compared with those in the EzTaxone public database (http://www.eztaxon.org) (Table 1) and the GenBank database (http://www.ncbi.nlm.nih.gov/blast) using BLAST searches (1). When the sequence was compared to the 16S rRNA sequence of strain SMC-A9199T of *Nesseria skkuensis* (NC FJ763637, GenBank AC N FJ763637.1), it was 100% identical to SMC-A9199T.

Antibiotic susceptibility tests were performed and the MICs determined by the broth microdilution method in accordance with the guidelines established by the Clinical and Laboratory Standards Institute (CLSI) (2). The MICs were as follows: penicillin, 1 mg/liter; ceftriaxone, 0.12 mg/liter; *piperacillin/tazobactam* 0.25 mg/liter; *rifampicin* 1 mg/liter; and ciprofloxacin, <0.06 mg/liter. It was susceptible to ceftriaxone, *piperacillin/tazobactam*, *rifampicin*, and ciprofloxacin, but was showed intermediate resistance against penicillin. Interpretive criteria for susceptibility were those for *Nesseria gonorrhoeae* because no breakpoints were provided by CLSI (2).
The patient was treated for prosthetic valve endocarditis with intravenous vancomycin 1g every three days, piperacillin/tazobactam 2.25g four times daily, gentamicin 100mg daily, and oral rifampicin 900mg daily. On hospital day 4, the patient was received mitral valve replacement and tricuspid anuuloplasty. Gram staining and culture of mitral valve specimen removed at operation were performed. No microorganism were recovered from culture of the replaced mitral valve after surgery. We applied 16S rRNA gene sequencing to identify bacterial species in mitral valve tissue, too. However, the result was negative. We performed acid-fast stain and mycobacterial culture on specimen of mitral valve tissue taken during the operation. The results of acid-fast stain and mycobacterial culture were negative. The next day after surgery, rifampicin was stopped. On hospital day 28, a result of 16S rRNA gene sequence was identified as *Nesseria skkuensis* and we changed the antibiotics regimen. We stopped intravenous administration of vancomycin and piperacillin/tazobactam. Therapy was continued with 2g intravenous ceftriaxone daily for 6 weeks. All blood cultures performed after the beginning of antibiotics remained negative. The patient recovered fully.

The genus *Neisseria* includes a group of closely related gram negative bacteria that are primarily commensal inhabitants of the mucus membrane of mammals. Within the 15 species are of human origin, only *Neisseria gonorrhoeae* and *Neisseria meningitides* are considered important pathogens, whereas the others are opportunistic pathogen sporadically involved in infections (4). However, several *Neisseria* species other than *N. gonorrhoeae* and *N. meningitides* cause human infections. *Neisseria elongata* and *Neisseria sicca* have been isolated from endocarditis (3, 5). *Neisseria bacilliformis*, also causes endocarditis and various human infections (4, 8, 9).

A novel *Neisseria* species, ‘*Neisseria skkuensis*’ was first described in 2010 (7). *N.
The bacteria was identified as *Neisseria* spp by conventional methods, but comparative 16S rRNA gene sequence analysis along with phenotypic analysis showed that the isolate is a novel species of *Neisseria* (7). Lee et al performed phenotypic analysis and *N. skkuensis* showed oxidase and catalase positive, consistent with most *Neisseria* species (6, 7). In addition, *N. skkuensis* could produce acid from ribose, glucose, fructose, mannitol, sucrose and gluconate, but not the remaining carbohydrates (7). Based on the 16S rRNA gene sequence, that isolate was most closely related with *Nesseria animalis* same as our case.

We examined the genetic relationship of the two *N. skkuensis* isolates (one reported in reference 7 and one in this study) by pulsed-field gel electrophoresis (PFGE). PFGE DNA preparation followed standard procedure and PFGE analyses were performed following the interpretative procedures described previously (10-12). The PFGE patterns were analyzed using GelCompar II software (Applied Maths, Belgium). Isolates that produced patterns that were < 85% similar were considered different. The PFGE patterns showed the two isolates were different strain from each other (Fig2).

To our knowledge, there have been no prior case reports of endocarditis due to *N. skkuensis*. In present case, the patient had prosthetic mitral valve, predisposing factor for infective endocarditis, and had received hemodialysis since 2000. The previous reported patient was admitted to our hospital in 2009 and the *N. skkuensis* was identified at the Asian-Pacific Foundation for Infectious Diseases (APFID) (7). In our case, *N. skkuensis* was identified as *Neisseria* spp. by 16S rRNA sequence analysis in a clinical microbiology lab, but correct identification at the species level could not be obtained. It was identified at APFID as *Nesseria skkuensis* based on the sequence of the 16S rRNA gene.

In conclusion, we describe the first reported case of endocarditis due to *N. skkuensis*, identified by 16S rRNA sequence analysis. Although most *Nesseria* spp. are
opportunistic pathogens, physicians should be aware of the possibility of endocarditis due to

Neiserria species. More Neisseria species may cause human disease.
References


9. Michaux-Charachon, S., J. P. Lavigne, A. Le Fleche, N. Bouziges, A. Sotto, and P.


Financial: Nothing to disclose

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<td>MC58</td>
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Figure legends.

FIG 1 Transesophageal echocardiogram showing (0.84 cm x 0.61 cm and 0.42 x 0.3 cm) mobile vegetations (arrow) on the prosthetic mitral valve strut.

FIG 2 PFGE analysis of genomic DNA from N. skkuensis strains digested with NheI and SpeI. A and B show N. skkuensis strains digested with NheI and SpeI respectively. Two isolates showed <85% similarity. The isolates were considered unrelated to each other. N. skkuensis 1 is SMC-A9199 (reference 7). N. skkuensis 2 is the isolate from this case patient.