CASE REPORT

A 41-year-old man was admitted to our hospital with a 1-week history of febrile sense, chills, sweating, aggravation of dyspnea, and hypotension during hemodialysis. He had a complicated 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 more than 1 year ago. He denied having had dental treatment or drug abuse since mitral valve replacement.

On arrival at the emergency department, the patient’s vital signs were as follows: blood pressure, 86/52 mm Hg; 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], <0.3 mg/dl), an erythrocyte sedimentation rate of 37 mm/h (N, <22 mm/h), and a procalcitonin concentration of 152.2 ng/ml. The white blood cell (WBC) count was 9,850/mm³ with dominant segmented neutrophils (85%), hemoglobin (Hb) at 7.5 g/dl, platelet count of 57,000/mm³, blood urea nitrogen at 49.5 mg/dl, creatinine at 6.55 mg/dl (N, <1.3 mg/dl), and total bilirubin at 1.2 mg/dl (N, <1.5 mg/dl). He was coagulopathic with a prothrombin time of 23.1 s (N, 12.6 to 14.9), international normalized ratio (INR) of 2.0, activated partial thromboplastin time (APTT) of 72.7 s (N, 29.1 to 41.9), and D-dimer of 0.06 mg/liter. It was susceptible to ceftriaxone, piperacillin/tazobactam, 0.25 mg/liter; rifampin, 1 mg/liter; and ciprofloxacin, <0.06 mg/liter. It was susceptible to ceftriaxone, piperacillin/tazobactam, rifampin, and ciprofloxacin but showed intermediate resistance against penicillin. Interpretive criteria for susceptibility were those for Neisseria gonorrhoeae, because no breakpoints were provided by CLSI (2).

Antibiotic susceptibility tests were performed, and the MICs were 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; rifampin, 1 mg/liter; and ciprofloxacin, <0.06 mg/liter. It was susceptible to ceftriaxone, piperacillin/tazobactam, rifampin, and ciprofloxacin but showed intermediate resistance against penicillin. Interpretive criteria for susceptibility were those for Neisseria gonorrhoeae, because no breakpoints were provided by CLSI (2).

The patient was treated for prosthetic valve endocarditis with intravenous vancomycin at 1g every 3 days, piperacillin-tazobactam at 2.25 g four times daily, gentamicin at 100 mg daily, and oral rifampin at 900 mg daily. On hospital day four, the patient re-

We describe the first reported 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 Neisseria skkuensis by 16 rRNA gene sequence analysis.

Neisseria skkuensis, a Novel Neisseria Species

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3D system (bioMérieux, Durham, NC., USA) and grew Gram-negative cocci in all three bottles. The Vitek 2 GNI card system (bioMérieux, Durham, NC) did not identify them. For molecular identification, we performed 16S rRNA gene sequence analysis, and the organism was formally identified at the Asian-Pacific Foundation for Infectious Diseases (APFID) as Neisseria skkuensis based on the sequence of the 16S rRNA gene. The 16S rRNA gene was amplified using primer sets 16S-F2 (5’-AGAGTTGTATCMTGGCTCAG-3’) and 16S-R2 (5’-GGTTACCTTGTTACGACTT-3’). A 1,406-bp sequence of the 16S rRNA gene was obtained from our strain. The 16S rRNA gene sequence was compared with those in the EzTaxon 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 Neisseria skkuensis (accession no. FJ763637; GenBank accession no. FJ763637.1), it was 100% identical to SMC-A9199T.

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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 group, 15 species are of human origin and only Neisseria meningitidis 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. skkuensis was isolated from blood and wound pus of a diabetic patient with a foot ulcer. The bacterium was identified as a Neisseria species 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 was shown to be oxidase and catalase positive, consistent with findings for 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 to Neisseria animalis, as with our case.

We examined the genetic relationship of the two N. skkuensis isolates (one described 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 program (Applied Maths, Belgium). Isolates that produced patterns that were <85% similar were considered different. The PFGE patterns showed the two isolates were different strains from each other (Fig. 2).

To our knowledge, there have been no prior case reports of endocarditis due to N. skkuensis. In the present case, the patient had a prosthetic mitral valve, a predisposing factor for infective endocarditis, and had received hemodialysis since 2000. The previously described patient was admitted to our hospital in 2009, and N. skkuensis was identified at the Asian-Pacific Foundation for Infectious Diseases (APFID) (7). In our case, N. skkuensis was identified as a Neisseria species 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 Neisseria skkuensis based on the sequence of the 16S rRNA gene.

In conclusion, we have described the first reported case of endocarditis due to N. skkuensis. In the present case, the patient had a prosthetic mitral valve, a predisposing factor for infective endocarditis, and had received hemodialysis since 2000. The previously described patient was admitted to our hospital in 2009, and N. skkuensis was identified at the Asian-Pacific Foundation for Infectious Diseases (APFID) (7). In our case, N. skkuensis was identified as a Neisseria species 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 Neisseria skkuensis based on the sequence of the 16S rRNA gene.

In conclusion, we have described the first reported case of endocarditis due to N. skkuensis, identified by 16S rRNA sequence analysis. Although most Neisseria spp. are opportunistic pathogens, physicians should be aware of the possibility of endocarditis due to Neisseria species. More Neisseria species may cause human disease.

### TABLE 1 Results of 16S rRNA gene sequence (1,406 bp) alignment (EzTaxon server)

<table>
<thead>
<tr>
<th>Rank</th>
<th>Name</th>
<th>Authors</th>
<th>Strain</th>
<th>Accession no.</th>
<th>Pairwise similarity (%)</th>
<th>No. of different nucleotides/total nucleotides</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Neisseria skkuensis</td>
<td>Lee et al. 2010</td>
<td>SMC-A9199&lt;sup&gt;1&lt;/sup&gt;</td>
<td>FJ763637</td>
<td>100</td>
<td>0/1,406</td>
</tr>
<tr>
<td>2</td>
<td>Neisseria animalis</td>
<td>Berger 1960</td>
<td>NCTC 10212&lt;sup&gt;2&lt;/sup&gt;</td>
<td>AJ239388</td>
<td>97.491</td>
<td>34/1,355</td>
</tr>
<tr>
<td>3</td>
<td>Neisseria cinerea</td>
<td>Von Lingelsheim 1906, Murray 1939</td>
<td>ATCC 14685&lt;sup&gt;3&lt;/sup&gt;</td>
<td>ACDY02000019</td>
<td>97.021</td>
<td>42/1,410</td>
</tr>
<tr>
<td>4</td>
<td>Neisseria subflava</td>
<td>Fluge 1886, Trevisan 1889</td>
<td>U37&lt;sup&gt;4&lt;/sup&gt;</td>
<td>AJ239291</td>
<td>96.753</td>
<td>44/1,355</td>
</tr>
<tr>
<td>5</td>
<td>Neisseria meningitidis</td>
<td>Albrecht and Ghon 1901, Murray 1929</td>
<td>MC58</td>
<td>AE002098</td>
<td>96.738</td>
<td>46/1,410</td>
</tr>
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</table>
ACKNOWLEDGMENT
We have no conflict of interest to disclose.

REFERENCES

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