

Acute Cellulitis Caused by *Neisseria skkuensis*

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A 73-year-old man visited our hospital because of pain with swelling and redness on the right foot dorsum. He was diagnosed with liver cirrhosis and nodular hepatic cellular carcinoma. Lower extremity CT scan and MRI showed abscess formation in the right foot dorsum. Gram-negative cocci were recovered from the culture of drained pus at the site and

identified as *Neisseria skkuensis* by 16S rRNA gene sequencing. Here, we report the first case of cellulitis due to *N. skkuensis* and provide a literature review. (Ann Clin Microbiol 2015;18:33-36)

Key Words: Cellulitis; *Neisseria*; RNA; Ribosomal, 16S; Sequence analysis

INTRODUCTION

The members of genus *Neisseria* are Gram negative coccal or rod-shaped bacteria, presenting as single or in pairs [1]. Most members of genus *Neisseria* have high affinity to mucosal membranes of mammals including humans, so that can cause opportunistic infections. According to "List of prokaryotic names with standing in nomenclature" (<http://www.bacterio.cict.fr/n/Neisseria.html>), the genus *Neisseria* consists of 29 species. Of these, *N. meningitidis* and *N. gonorrhoeae* are treated as the most important human pathogens.

However, several *Neisseria* species other than these two species were also reported as human pathogens [2-6]. In addition to these pathogens, *N. skkuensis* was designated as a novel species which cause foot ulcer of diabetic patient in 2010 [7]. Previous reports described *N. skkuensis* infections in diabetic foot ulcer and endocarditis with underlying disorders such as diabetes and liver cirrhosis, respectively [7-9]. We report the first case of cellulitis due to *N. skkuensis* in the patient with liver cirrhosis and hepatic cellular carcinoma who was diagnosed incidentally.

CASE REPORT

A 73-year-old man who was generally healthy visited the Emergency Department at our hospital, due to a week history of pain with swelling, redness, and heat sensation of right foot dorsum area and abnormal findings in liver function tests done at the local clinic. On the arrival, the patient's vital signs were as follows: blood pressure, 118/78 mmHg; pulse rate, 82 pulses/min; respiratory rate, 16 breaths/min; and body temperature, 36.5°C. Physical examination revealed tenderness, swelling, redness, and heat sensation on right foot dorsum. Complete blood cell count tests revealed pancytopenia with peripheral leukocyte count of 3.7×10^9 cells/L [Reference range (RR), $5.2\text{--}12.4 \times 10^9$ cells/L], hemoglobin of 8.2 g/dL (RR, 12.0-18.0 g/dL), and platelet of 65.0×10^9 cells/L (RR, $130.0\text{--}400.0 \times 10^9$ cells/L). Total bilirubin was 1.54 mg/dL (RR, <1.2 mg/dL) with direct bilirubin 0.73 mg/dL (RR, <0.4 mg/dL), total protein 6.1 g/dL (RR, 6.7-8.3 g/dL), and albumin 2.5 g/dL (RR, 3.2-4.8 g/dL). He had coagulopathy with a prolonged prothrombin time of 16.0 sec (RR, 10-14 sec), international normalized ratio of 1.57, activated partial thromboplastin time of 49.5 sec (RR, 20-38 sec), and D-dimer of 3.92 $\mu\text{g/mL}$ (RR, 0-0.5 $\mu\text{g/mL}$). C-reactive protein concentration was 4.25 mg/dL (RR, <0.5 mg/dL) with alkaline

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phosphatase of 214 U/L (RR, 104-338 U/L), aspartate transaminase of 36 U/L (RR, 8-38 U/L), and alanine transaminase of 23 U/L (RR, 4-44 U/L). Abdominal computed tomography (CT) scan showed liver cirrhosis with splenomegaly, varix, ascites, and a nodular hepatic cellular carcinoma in segments 5 and 6 of liver which were confirmed with liver magnetic resonance imaging (MRI) workup also. Lower extremity angiography CT showed subcutaneous swelling and strands in the right lower leg. MRI on right ankle showed compatible findings with cellulitis and associated abscess formation at right foot dorsum. After appropriate debridement and cleansing of the affected area, focal incision was made and pus specimen was collected from the leading edge of the lesion for subsequent culture.

The organism from the culture grew well in aerobic condition on sheep blood agar at 35°C in an atmosphere of 5% CO₂ after 24 hour incubation. Grayish circular non-hemolytic colonies grew on sheep blood agar and chocolate agar. The Gram stain revealed Gram-negative cocci. This organism was oxidase and catalase positive, consistent with most *Neisseria* species [1]. The Vitek 2 system using NH cards (bioMérieux, Marcy-l'Étoile, France), however, identified this organism as *Oligella urethralis* with 90% probability, which normally grows slowly on blood agar and appears as pinpoint colonies after 24 hours incubation mostly causing urinary tract infection or urosepsis [1]. In order to confirm the identification of this isolate, 16S rRNA gene sequencing was conducted. Two sub-regions of the 16S rRNA gene were amplified using the following primer sets: forward

27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and reverse, 800R: 5'-TACCAGGTATCTAATCC-3' and forward, 518F: 5'-CCA GCAGCCGCGGTAATACG-3' and reverse, 1492R: 5'-TACGG YTACCTTGTTACGACTT-3'. A 1348-bp sequence of the 16S rRNA gene was obtained, and compared with sequence databases in the EzTaxon database (<http://www.ezbiocloud.net/>) and the GenBank database (<http://www.ncbi.nlm.nih.gov/blast>) [10]. The 16S rRNA sequence of the isolate revealed 100% similarity (1316 of 1316 bases) to a type strain of *N. skkuensis* (GenBank accession number, FJ763637) and 97.39% similarity (1269 of 1303 bases) to a type strain of *N. animalis* (GenBank accession number, AJ239288). Then we further identified the detailed biochemical characteristics of this strain with API NE and API 50 CHB/E kits (bioMérieux, Marcy-l'Étoile, France). As a result, we could identify the isolate as *N. skkuensis*. A phylogenetic tree was constructed with the neighbor-joining method by using Molecular Evolutionary Genetics Analysis (MEGA) software version 5.05 (Fig. 1).

Antimicrobial susceptibility test was performed using disk-diffusion method. The isolate was susceptible to benzylpenicillin, ampicillin, cefotaxime, ceftriaxone, levofloxacin, tetracycline, and trimethoprim/sulfamethoxazole according to Clinical and Laboratory Standards Institute guidelines for testing *N. gonorrhoeae* and *N. meningitidis* [11].

The patient was treated with intravenous ampicillin-sulbactam 750 mg four times a day for 13 days. On the eighth day of hospital admission, the follow-up culture of pus revealed no

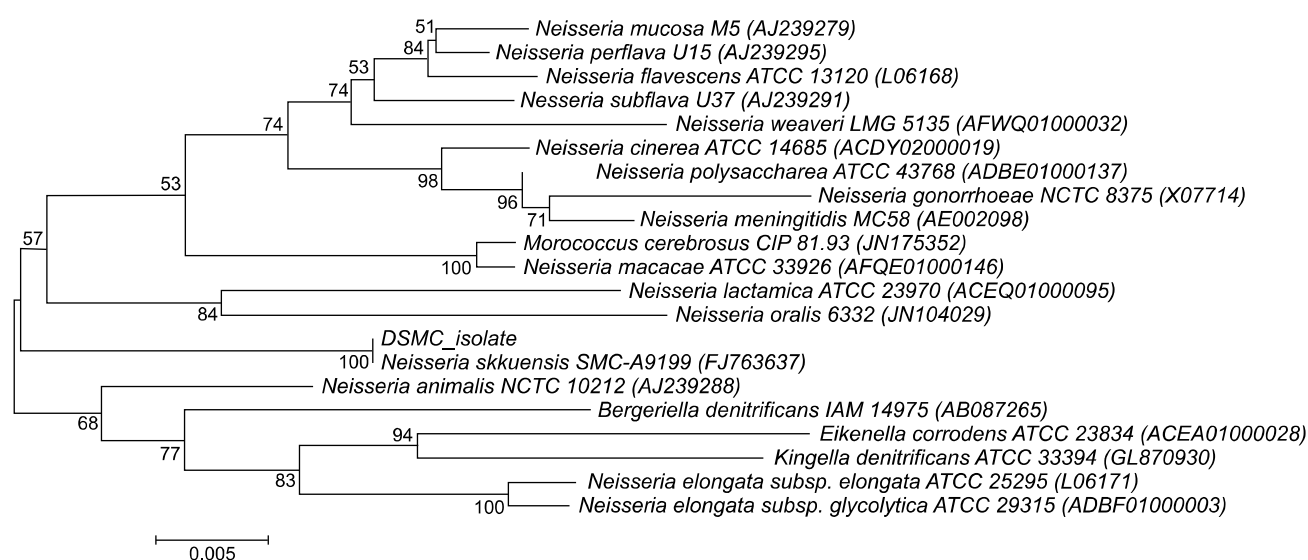


Fig. 1. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences of our *N. skkuensis* and 20 similar organisms. The bar represents 0.5% sequence divergence.

Table 1. Biochemical characteristics of the studied strain compared with reported strain and other species [1]

Species	Oxidase	Catalase	Nitrate reduction	Glucose assimilation	Acid from					
					Ribose	Glucose	Fructose	Maltose	Sucrose	Gluconate
This strain	+	+	+	—	+	+	+	+	+	+
Lee et al. [7]	+	+	—	+	+	+	+	—	+	+
<i>N. gonorrhoeae</i>	+	+	—		ND	+	—	—	—	ND
<i>N. meningitidis</i>	+	+	—	ND	ND	+	—	+	—	ND
<i>O. urethralis</i>	+	ND	—	ND	ND	—	ND	—	ND	ND

Abbreviations: +, positive; —, negative; V, variable; ND, not determined.

growth, so the incision site was closed with sutures. On the thirteenth day of hospital admission, the administration of intravenous ampicillin-sulbactam was stopped, and the treatment was started with oral amoxicillin-clavulanate 625 mg three times a day. The patient was recovered well, discharged and followed up through the out-patient clinic.

DISCUSSION

N. skkuensis, a novel species of *Neisseria* genus, was first described in 2010 [7]. In the first report, this organism was isolated from blood and pus from the foot ulcer of the diabetic patient. Then the second report, which was the first case of *N. skkuensis* infection in liver cirrhosis, *N. skkuensis* was isolated from blood of the patient with liver cirrhosis due to chronic hepatitis B infection and chronic kidney disease caused by glomerulonephritis [8]. In that case, the patient received mitral valve replacement due to infective endocarditis caused by methicillin-resistant *Staphylococcus aureus* more than 1 year ago, but the patient was diagnosed as prosthetic valve endocarditis due to *N. skkuensis* which was identified by the molecular methods. In clinical point of view, previous studies showed that *N. skkuensis* would be an important pathogen in immunocompromised patients. Our patient had liver cirrhosis and hepatic cellular carcinoma, similar with the case of Park et al. [8] which had liver cirrhosis and chronic kidney disease, other than diabetes mellitus. It is certain that this novel species is an important opportunistic pathogen in patients with underlying diseases such as diabetes mellitus and liver cirrhosis.

This species was first identified as *Oligella urethralis* by Vitek 2 NH card with phenotypic characteristics of oxidase and catalase positivity and negative for glucose assimilation. Like our case, the case of *N. skkuensis* from the foot of diabetic patient was first identified as *O. urethralis* using Vitek 2 NH card

also [9]. Since *O. urethralis* has been isolated mainly from the urinary tract, the identification was needed to confirm with molecular method. Then we further identified the detailed characteristics of *N. skkuensis* with API NE and API 50 CHB/E kit (bioMérieux). The assimilation of glucose tested in API NE was negative in our strain whereas positive in Lee et al. [7]. However, the production of acid from ribose, glucose, fructose, mannitol, sucrose and gluconate was same as in the previous report [7] (Table 1). Although some of the biochemical characteristics of our strain are contrary to the previous reports, these might be the variability of this species. Further studies are carefully needed to collect the data of *N. skkuensis* since there are only two published reports and our case.

In conclusion, we described the first case of cellulitis due to *N. skkuensis* in hepatic cellular carcinoma and liver cirrhosis, but could be the second case of infection by *N. skkuensis* in liver cirrhosis, identified by 16S rRNA sequence analysis. Furthermore, it would be important that when *Neisseria* species was cultured from pathological site such as wound or blood of immunocompromised patients, we should consider that organism as a pathogen, rather than a contaminant. Then, we should identify the organism by molecular method such as gene sequencing in accompany with biochemical analysis.

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=국문초록=

*Neisseria skkuensis*에 의한 급성 봉와직염 1예

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평소 건강하게 지내오던 73세 남자환자가 부종과 발적을 동반한 오른 발등의 통증을 주소로 내원하였다. 하지의 컴퓨터 단층 촬영과 자기공명영상을 통해 환자의 오른 발등에 농양이 발견되었고, 입원하는 기간 동안 간경화와 결절성 간세포 암종으로 진단받았다. 오른 발등 병변의 고름을 배농하여 배양한 결과, 그람 음성 알균이 분리되었고, 16S rRNA 유전자 염기서열분석을 통하여, *Neisseria skkuensis*로 동정되었다. 저자들은 *N. skkuensis*에 의한 봉와직염 1예를 문헌고찰과 함께 보고하는 바이다. [Ann Clin Microbiol 2015;18:33-36]

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