A Case of Diabetic Mellitus Foot Infection by a Newly Reported Neisseria Skkuensis: Case Report

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Abstract. In pus and wound samples collected from the right second toe of a 61-year-old woman with diabetes mellitus (DM), gram-negative diplococci bacterium was observed. However, the bacterium could not be identified by conventional microbiological methods and mass spectrometry. In the partial 16S rRNA gene sequence analysis, the bacterium showed a 100% identity match with GenBank sequence FJ0763637.1 (Neisseria skkuensis). N. skkuensis, SMC-A9199 strain, was reported as a novel species in 2010 based on its phenotypic characteristics and the 16S rRNA gene sequence, which was isolated from the blood and wound pus of a DM patient with a foot ulcer. The second reported N. skkuensis was identified from the blood cultures of a patient with endocarditis. To the best of our knowledge, this is only the third report of N. skkuensis.

Key words: diabetes mellitus, Neisseria skkuensis, 16S rRNA, sequencing

Introduction

The genus Neisseria comprises 29 species, according to Euzéby’s “List of Prokaryotic Names with Standing in Nomenclature” (http://www.bacterio.net/Neisseria.html). Most human Neisseria species are considered normal inhabitants of the upper respiratory tract and cause disease in an opportunistic fashion [1]. However, Neisseria meningitidis and Neisseria gonorrhoea are considered clinically significant pathogens, and infect only humans [1].

Several molecular-based methodologies are being implemented to provide genus and species identification for isolates that do not fit any recognized biochemical profiles, for strains generating only a “low likelihood” or “acceptable” identification according to commercial systems, and for taxa that are rarely associated with human infectious diseases [2]. One such method is the sequencing of 16S rRNA gene [3,4]. 16S rRNA gene sequencing has been used to identify novel and emerging pathogens. As a rule of thumb, there should be a difference of 1 bp per 100 bases sequenced to warrant a new species name [5]. Given more recent evidence, values between 98.7% and 99.0% are recommended for separating species [5-7]. Neisseria skkuensis, SMC-A9199 strain, was reported as a novel species in 2010 based on its phenotypic characteristics and 16S rRNA gene sequence [8]. The species was isolated from the blood and wound pus of a diabetic patient with a foot ulcer. It is closely related to N. animalis NCTC10212 and reveals pairwise similarity <97.0% with other species [8]. It is positive for oxidase and catalase tests and does not reduce nitrate. Acid is produced from glucose, sucrose, and fructose while it is not from maltose, and lactose. Here we report another case of Neisseria skkuensis in a DM patient (KUMC-1415 strain).

Case Reports

KUMC-1415 strain was isolated from the wound pus of a diabetic patient with a foot ulcer. A 61-year-old woman with diabetes mellitus (DM) presented in the Emergency Department at Korea University Anam Hospital, Seoul, Republic of Korea, with complaints of pain and swelling in the right second toe ulcer. Eleven years ago the patient was diagnosed with DM and had DM medications prescribed at local hospital. Eight months ago, she was admitted to the Orthopedic Department at our hospital due to the pain and swelling in her right foot ulcer.

On admission, the patient had a temperature of 37.1°C, blood pressure of 120/80 mmHg, pulse of 88/min, and respiratory rate of 20 breaths/min. Laboratory investigation showed a Hb of 11.6 g/dL, leukocyte count of
12.9×10^9/L, platelet count of 447×10^9/L, C-reactive protein (CRP) level of 8.51 mg/dL, blood urea nitrogen (BUN)/creatinine of 9.9/0.79 mg/dL, and total protein/albumin level of 7.8/3.9 g/dL. In pus and wound samples collected from the right second toe, gram-negative diplococci (4+) were observed. They grew well aerobically on sheep blood agar at 35°C in an atmosphere of 0.5% CO_2 after 24-hour incubation. Large (1-2 mm in diameter) grey opaque circular convex colonies with whole margins were noted. The patient received empirical antibiotic therapy with cefazodone and netilmicin sulfate.

VITEK 2 NH card identified the isolate with 95% probability as *Oligella urethralis*, which is coccobacilli with pinpoint growth after 24 hour of incubation. The isolate seemed to be biochemically more closely related to *Oligella urethralis* than *N. cinerea, N. elongata, N. gonorrhoeae, N. lactamica, N. meningitidis*, and *N. sicca* that can be identified with the NH card. Due to a discrepant result generated by the Vitek-2 system, matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) analysis with the Bruker Biotyper software database (Bruker Daltonics, Bremen, Germany) was performed and the clinical isolate was identified as *N. flavescens*, with a score of 2.057 [9]. To confirm the identity of the isolate, 16S rRNA gene sequencing was conducted with ABI BigDye® Terminator v3.1 Cycle sequencing kit (Applied Biosystems, Foster City, CA, USA). PCR and sequencing kits were designed with primer sets 16S-27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 16S-518R (5′-ATTACCGCGGCTGCTG-3′) [10]. Sequences were analyzed using an ABI PRISM 3500 DX (Applied Biosystems, Foster City, CA, USA). The partial 16S rRNA gene sequence was compared with the GenBank database (http://www.ncbi.nlm.nih.gov/blast) using BLAST searches. In the first 479 base pairs of the 16S rRNA gene sequence, the isolate showed a 100% identity match with GenBank sequence FJ0763637.1 (*N. skkuensis*) [8]. The final diagnosis was DM foot infection due to *N. skkuensis*. The patient was subsequently treated with ampicillin for ten days. Ultimately, she was well enough to leave the hospital.

**Discussion**

Conventionally, phenotypic methods like gram stain, observation of growth and colony morphology on various media, analysis of manual biochemical reactions, and the automated commercially available biochemical panels have been used for bacterial identification. Unfortunately, commercial phenotypic databases are often outdated and lack current taxonomy [11]. The VITEK II commercial identification system identified our isolate as *Oligella urethralis*. The same database problem also occurs in bacterial identification by mass spectrometry. In our case, both phenotypic methods and MALDI-TOF showed a limitation in detecting *N. skkuensis*. Furthermore, it should be noted that the misidentification of *N. skkuensis* as another pathogen can interfere with appropriate treatment, resulting in increased morbidity.

In this respect, 16S rRNA gene sequencing can contribute to identifying poorly described, rarely isolated, or biochemically aberrant strains [5,12,13]. 16S rRNA gene sequencing analysis is less subjective and more robust, reproducible, and accurate than phenotypic testing [5]. 16S rRNA gene sequence analysis can not only lead to the discovery of novel bacteria but can also identify non-cultured bacteria, allowing independence from growth conditions [3,5,14].

*N. skkuensis* was known to be susceptible to the antimicrobial agents such as penicillin, cefotaxime, cefoxitin, ceftazidime, tetracycline, and ciprofloxacin [8]. Accordingly, this patient was treated with an additional antimicrobial agent, ampicillin. However, given that no break points associated with *N. skkuensis* were provided by the Clinical and Laboratory Standards Institute, antimicrobial susceptibility of *N. skkuensis* needs further evaluation with more cases [8,15].

The first reported case of *N. skkuensis* was isolated from the blood and wound pus of a DM patient with foot ulcer [8]. The second reported case of *N. skkuensis* was identified from blood cultures of the patient with endocarditis [15]. To the best of our knowledge, this is the third report of *N. skkuensis*. Interestingly, our case was also from a DM patient with foot ulcer, as in the first case. Those reported cases suggest the possibility that *N. skkuensis* might have some clinical relevance to DM foot ulcer and endocarditis. With 16S rRNA gene sequencing analysis, more reports on the isolation of *N. skkuensis* are necessary to elucidate its clinical significances.

In summary, this is the third reported case of *N. skkuensis*. 16S rRNA gene sequencing analysis successfully detected *N. skkuensis* from the wound pus of a DM patient with a foot ulcer.
References


