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Cellulitis Caused by a Novel *Cupriavidus* Species Strain J1218 Identified by Whole Genome Sequencing

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We report a case of cellulitis caused by a novel *Cupriavidus* species identified using whole-genome sequence analysis. Subcutaneous tissue biopsies from the left lower leg of a 67-year-old man who suffered from cellulitis were cultured. Round, convex, gray and non-hemolytic colonies were recovered after 72-h incubation. 16S rRNA sequence analysis showed 98.6% similarity with *Cupriavidus basilensis* DSM 11853(T) in the NCBI database and 99.9% similarity with *C. basilensis* KF708 in the EzBioCloud database. Genomic analysis using the MiSeq platform (Illumina, USA) and the TrueBac ID database (ChunLab, Korea) revealed that the average nucleotide identity (ANI) of this strain with *C. basilensis* DSM 11853(T) was 87.6%. The patient was treated with oral cefditoren pivoxil for 9 weeks. This study is the first to report cellulitis caused by *Cupriavidus* species strain J1218. **(Ann Clin Microbiol 2019;22:105-109)**

Key Words: Cellulitis, *Cupriavidus*, Whole-genome sequencing, 16S rRNA

INTRODUCTION

Cupriavidus is a genus of aerobic gram-negative glucose-non-fermenting bacillus common in the environment but rarely isolated from clinical specimens. Clinical manifestations of infection with *Cupriavidus* species are varied and include pneumonia, bacteremia, meningitis, and septicemia in children [1-3]. Muscular abscess in a renal transplant recipient and airway infection with a *Cupriavidus* species in patients with cystic fibrosis suggest that immunodeficiency may be a prior condition for infection by this genus [3-7].

We describe the first case of class II cellulitis in an immunocompetent patient caused by a novel *Cupriavidus* species identified using whole-genome sequencing.

CASE REPORT

A 67-year-old man complained of fever, redness, swelling, and pain in his left lower leg for 2 weeks; the lesion did not respond to antibiotic therapy. He had no underlying disease other than benign prostatic hyperplasia. He was often exposed to freshwater during fishing and gardening. Erythema remained after 10 days of intravenous and oral ceftriaxone treatment. Intravenous and oral levofloxacin treatment was then given for 6 weeks, but the lesion persisted. A magnetic resonance imaging (MRI) scan with enhancement of the lower leg revealed prominent swelling of the cutaneous and subcutaneous layer and non-enhancing fluid in the mid- to distal calf. Tissue was obtained from the lower leg via ultrasound-guided biopsy for bacterial and acid-fast bacterial culture. Round, convex, gray, non-hemolytic colonies were visible on BAP agar after 72 h incubation at 35°C (Fig. 1). Vitek MS IVD v3.0 (bioMérieux, Manchester, UK) failed to identify these colonies, while the Vitek 2 system (bioMérieux, Hazelwood, MO, USA) identified them as *Cupriavidus pauculus*. For 16S rRNA sequencing, genomic DNA was extracted using a Genedia Mycobacteria DNA Prep kit (Green Cross Medical Science co., Eumseong, Korea) and amplification of the 16S rRNA gene was performed using

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the following universal primer sets: 4F: 5′-TTGGAGAGTTT GATCCTGGCTC-3′, 534R: 5′-TACCGCGGCTGCTGGCAC-3′, 27F: 5′-AGAGTTTGATCMTGGCTCAG-3′ and 801R: 5′-GGC GTGGACTTCCAGGGTATCT-3′. The resulting sequences were compared with the 16S rRNA sequences (713 bp) of related taxa, which were obtained from the GenBank database and EzBioCloud database. Sequences showed 99.9% similarity with *C. basilensis* strain KF708 (GenBank accession no. AB109778) in EzBioCloud and 98.6% similarity with *C. basilensis* strain DSM 11853^{T} (GenBank accession no.: NR_025138) in the National Center for Biotechnology Information (NCBI) database. A neighbor-joining phylogenetic tree was constructed using BLAST pairwise alignments (Fig. 2).

Fig. 1. Circular, convex, gray non-hemolytic colonies of *Cupriavidus* sp. strain J1218 were grown on BAP after 72 h incubation at 35°C.

A paired-end library (insert size: 300 bp) was constructed from genomic DNA using a TruSeq DNA LT Sample Preparation kit (Illumina, San Diego, CA, USA). The nucleotide sequences of this strain were determined by synthesis on the MiSeq platform (Illumina). Gene discovery and functional annotation pipeline of the whole-genome assembly were performed using data from the EzBioCloud database. For average nucleotide identity (ANI) calculations, the query genome was cut into small fragments (1,020 bp), and the BLAST algorithm was used to select the highest-scoring pair between two genomic sequences. The size of the genome was 7,960,659 bp, with 795

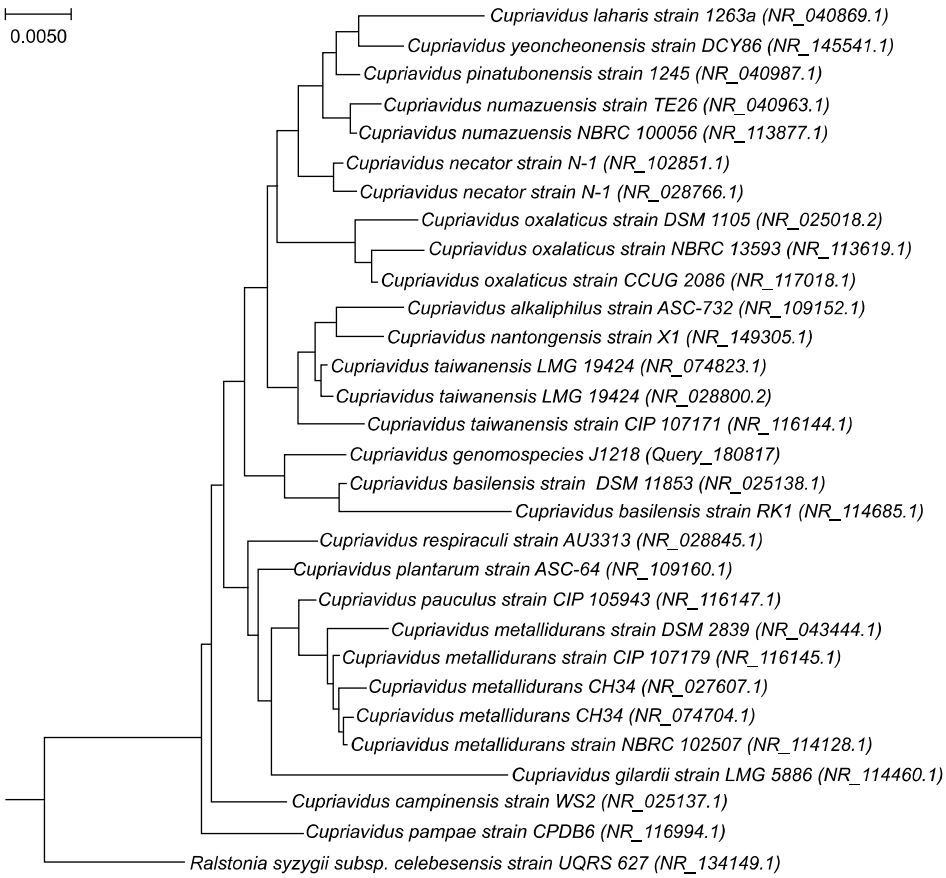

Fig. 2. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences, showing the phylogenetic relationships of *Cupriavidus* sp. strain J1218 with *Cupriavidus* and *Ralstonia* species obtained from the National Center for Biotechnology Information (NCBI) database.

Table 1. The antimicrobial susceptibility test results of *Cupriavidus* sp. strain J1218 performed by MicroScan and Sensititre panel

Antimicrobial agents	MIC (μg/mL)		Interpretation*
	MicroScan	Sensititre	
Amikacin	≤8	≤4	S
Aztreonam	16	8	S or I
Cefepime	≤1	-	S
Cefotaxime	≤1	≤0.5	S
Ceftazidime	≤1	1	S
Chloramphenicol	≤8	-	S
Ciprofloxacin	≤0.5	≤0.06	S
Gentamicin	≤2	≤0.5	S
Imipenem	≤1	≤0.5	S
Levofloxacin	≤1	-	S
Meropenem	≤1	≤0.12	S
Minocycline	≤4	-	S
Piperacillin-tazobactam	≤8	≤1/4	S
Piperacillin	≤8	-	S
Tetracycline	≤4	-	S
Tobramycin	≤2	≤2	S
Trimethoprim-sulfamethoxazole	≤2/38	≤1/19	S

*Antimicrobial susceptibility test results were interpreted according to the CLSI guideline M100.
Abbreviation: MIC, minimum inhibitory concentration.

contigs, and the GC content of the coding sequences was 68.6%.

The first taxon identified by TrueBac ID was *Cupriavidus* genomospecies BBQM_s (KF708), according to the overall genome relatedness index (OGRI). The ANI of *Cupriavidus* strain J1218 with *Cupriavidus* genomosp. BBQM was 98.6%, while it shared 87.6% ANI with *C. basilensis* DSM 11853^T, according to genome analysis using the TrueBac ID database (ChunLab, Seoul, South Korea).

The antimicrobial susceptibility test (AST) results of *Cupriavidus* strain J1218 performed using MicroScan (Beckman Coulter, Brea, CA, USA) are shown in Table 1. The strain was suspected to be resistant to ciprofloxacin because the patient's lesion persisted despite continued treatment with ceftriaxone for 10 days and levofloxacin for 6 weeks. However, an AST of *Cupriavidus* strain J1218 performed using MicroScan showed susceptibility to all antimicrobial agents, including ciprofloxacin, making the choice of treatment difficult. We performed a second AST of strain J1218 using a Sensititre DKMGN panel (Trek Diagnostic Systems; Thermo Fisher Scientific, Inc., East Grinstead, UK) to yield AST data for up to 72 h, taking into account the slow growth characteristics of this strain. However, the minimum inhibitory concentration (MIC) of ciprofloxacin was still less than 0.06 μg/mL (Table 1). Finally, the patient was successfully treated with a 9-week course of oral cefditoren pivoxil.

DISCUSSION

The criteria for prokaryotic species circumscription are traditionally defined as a DNA-DNA hybridization (DDH) value of at least 70% similarity, and a temperature within 5°C of the thermal denaturation midpoint [8]. Recently, 16S rRNA sequence similarity of less than 98.7%, and ANI values of 95-96% or a digital DDH of less than 70%, were proposed as criteria for classifying new species based on whole-genome sequencing data [9,10]. A recent study found that 12% of bacteria prospectively collected from intensive care units in a tertiary care hospital during 1 year were novel genomospecies, according to pairwise ANI analysis using BLAST [11].

In this study, we were unable identify this isolate using Vitek MS despite repeated tests. The Vitek 2 system identified this isolate as *C. pauculus*, which was included in the Vitek MS v3.0 library. We performed 16S rRNA sequencing analysis to determine the reason for the discrepancy between the Vitek 2 system and Vitek MS. *Cupriavidus* species are known to be difficult to identify by biochemical tests, making molecular assays necessary for accurate identification of this genus [4].

The 16S rRNA sequencing result was analyzed according to the Clinical and Laboratory Standards Institute (CLSI) guideline MM18-ED2 [12]. This strain was identified as *C. basilensis* strain KF708 with 99.9% similarity, and could be distinguished from *C. basilensis* strain DSM 11853 showing 98.9% similarity in the EzBioCloud. In addition, species identification based on the NCBI GenBank database limiting to sequences from type material of this strain showed a similarity of 98.6% with *C. basilensis* DSM 11853^T. The phylogenetic tree created by NCBI GenBank revealed it to be an unknown strain.

A whole genome analysis classed the sequences as *Cupriavidus* genomospecies BBQM (KF708) with 98.5% similarity. A previous reclassification study of *Cupriavidus* suggested that the standard ANI cut-off value of *Cupriavidus* spp. should be 90% [13]. In the same study, *C. basilensis* strain KF708 was reclassified as *Cupriavidus* sp. by a combination of phylogenetic analyses and whole-genome sequence analyses [13]. Genomic analysis revealed that the strain investigated in the current study was a novel *Cupriavidus* species.

Whole-genome sequencing data of this strain showed 100%

identity of *carA*, *ErmE*, Brucella suis *mprF* and OXA-63 genes. Also, it had an *adeF* gene with 75-76% similarity and the gene was associated with fluoroquinolone resistance with efflux pumps. The AST results for *Cupriavidus* strain J1218 showed that it is susceptible to all antibiotic agents according to the CLSI guideline M100 [14]. Since differences can exist between genetic and phenotypic data, further study is needed on the relationship between the AST and whole-genome sequencing data.

Patients infected with *Cupriavidus* species are usually immunocompromised [2,3,5,7]. Cases of infection with this genus have also been reported in elderly patients without obvious immunodeficiency [15,16]. The patient in the present study was immunocompetent, but had experienced frequent injuries to the lower limbs. The patient underwent sustained antimicrobial treatment and the lesion was completely healed after 6 months.

In conclusion, we identified a novel *Cupriavidus* sp. strain, J1218, using 16S rRNA-based molecular analyses and whole-genome sequencing. This is the first reported case of cellulitis caused by a novel *Cupriavidus* sp. strain J1218 requiring long-term antibiotic therapy in an immunocompetent patient.

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=국문초록=

전장유전체 염기서열분석으로 동정한 신종 *Cupriavidus* Species Strain J1218에 의한 봉와직염

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저자들은 전장유전체 염기서열분석을 이용하여 동정된 새로운 *Cupriavidus* sp.에 의한 봉와직염의 첫 사례를 보고한다. 이전에 건강하였던 67세 남자환자가 왼쪽 정강이의 봉와직염이 의심되어 피하조직을 채취하여 배양하였다. 72시간 배양한 후 원형의 볼록한, 회색 및 비용혈성 집락이 혈액한천배지에서 관찰되었다. 16S rRNA 염기서열은 NCBI 데이터베이스를 이용하여 *Cupriavidus basilensis* DSM 11853의 서열과 98.6%의 유사성을 얻었고 Ezbiocloud 데이터베이스에서는 *Cupriavidus basilensis* KF708의 서열과 99.9%의 유사성을 보였다. 유전체 염기서열분석을 MiSeq platform (Illumina, USA)을 이용해 시행하였다. TrueBac ID (ChunLab, Korea) 데이터베이스를 이용한 유전체 염기서열분석 결과 이 균종의 Average Nucleotide Identity는 *Cupriavidus basilensis* DSM 11853과 87.6%를 나타냈다. 환자는 경구 cefditoren pivoxil로 9주간 치료하여 병변이 호전되었다. 본 연구는 *Cupriavidus* species J1218 균주에 의한 봉와직염의 첫 증례보고이다. [Ann Clin Microbiol 2019;22:105-109]

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