

Massilia varians Isolated from a Clinical Specimen

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We report a case of *Massilia varians* isolated from a deep finger wound following orthopedic surgery on an immunocompetent patient. The bacterium was identified by 16S rDNA sequence analysis. This is the first case of *M. varians* isolated from a clinical specimen since the first report in 2008.

Key Words: *Massilia varians*; 16S rDNA sequencing; Clinical specimen

Introduction

The genus *Massilia* is a fastidious, aerobic, non-spore forming, and motile Gram-negative rod, belonging to the family *Oxalobacteraceae* [1]. Members of the genus have been mainly isolated from environmental sources [2]; however, some species are known to cause infections in immunocompromised patients [3]. Due to insufficient identification methods, infections due to *Massilia* are still challenging to clinical microbiologists and clinicians. Here, we report the first case of *Massilia varians* isolated from a deep finger wound that was successfully identified using 16S rDNA sequencing.

Case Report

A 40-year-old woman was admitted to the orthopedic sur-

gery department of a tertiary-care hospital in Seoul, Korea. This patient had injured her left thumb with a kitchen knife right before visiting the hospital. She had a history of hypertension, but did not have any other diseases. There was no redness, swelling, pus or serous discharge from her wound upon physical examination. Radiological evaluation showed no evidence of left thumb fracture. The size of the wound was 1 cm in width and 1 cm in depth. Her vital signs were as follows: body temperature, 36.5 °C; blood pressure, 120/82 mmHg; pulse, 96/min; and respiratory rate, 20/min. The laboratory results were as follows: white blood cell count, $9.69 \times 10^3/\text{mm}^3$ (reference value, 4.0×10^3 to $10.8 \times 10^3/\text{mm}^3$) with 74.4% neutrophils (reference value, 40 to 73% of total leukocytes); and erythrocyte sedimentation rate, 16 mm/h (reference value, 0 to 20 mm/h). Routine blood chemistry and electrolytes showed values within reference ranges. She was diagnosed with a rupture of the extensor pollicis longus ten-

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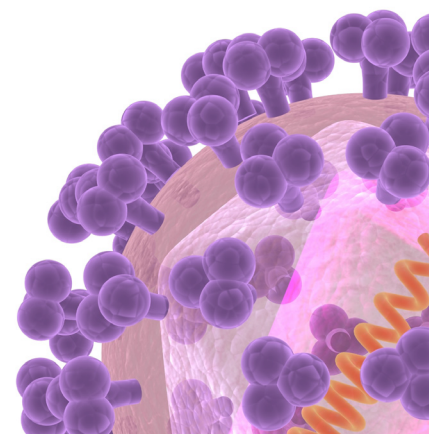
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don of the left thumb, and she underwent tenorrhaphy on the second hospital day. A deep wound culture was taken before the operation. She was discharged home the day after surgery without any complications.

The deep wound sample was inoculated onto 5% sheep blood agar (Asan Pharmaceutical, Seoul, Korea) and MacConkey agar (Asan Pharmaceutical) plates. The cultures were incubated in 6% CO₂ at 35 °C for 2 days. Grayish mucoid colonies were grown on the 5% sheep blood agar, and colorless colonies were grown on the MacConkey agar after 48 h incubation. Both cultures were positive for oxidase and catalase reactions. Gram-negative short rods were seen in stained preparations, which were confirmed using Scanning Electron Microscopy (SEM) (FE SEM S-800, Hitachi, Tokyo, Japan) at the acceleration voltage of 20 Kv. The organism was 1.5-2.0 µm in length and 0.5-1.0 µm in width with a single flagella (Fig. 1). Motility was confirmed using motility-indole-ornithine (MIO) semi-solid agar (Asan Pharmaceutical).

Biochemical features were investigated using VITEK® 2 Gram-negative (GN) Identification cards with the Vitek 2 system (bioMérieux, Marcy l'Etoile, France) and API 20NE cards (bioMérieux). The GN card identified the isolate as *Sphingomonas paucimobilis*, but with low probability (91%). The API 20NE card identified the isolate as within the *Shewanella putrefaciens* group, with low probability (90%). Data on the assimilation of carbon-sources, hydrolysis of chromogenic sub-

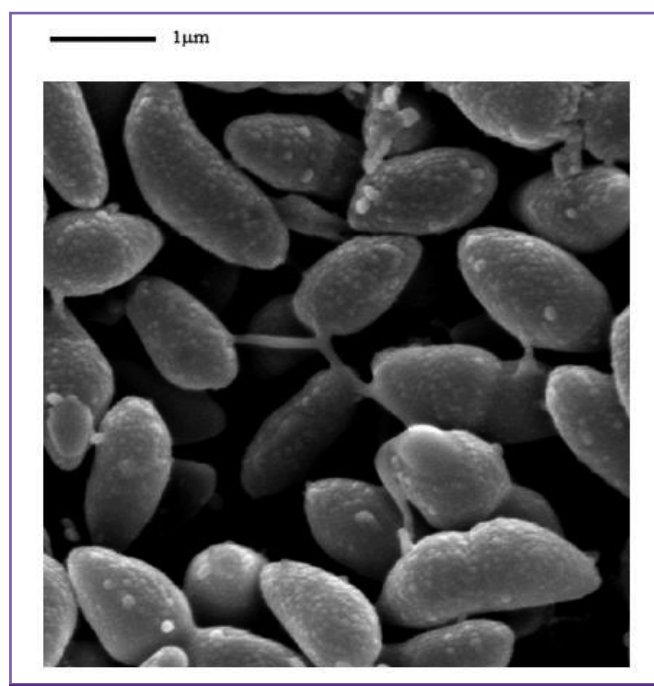


Figure 1. Electron micrograph of the *Massilia varians* isolate from the deep finger wound showing a single flagellum. Magnification, × 20,000.

strates, and enzyme activities of the isolate are described in Table 1. Matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) (Microflex™ MALDI Biotyper, Version 3.1, Bruker Daltonics, Bremen, Germany) identified the organism as *Massilia* sp. with a score of 1.621. The analysis of 16S rDNA sequence was also performed with the universal bacterial primers 27F (5'-AGA GTT TGA TCC TGG CTC AG -3') and 1541R (5'-AAG GAG GTG ATC CAG CCG CA-3'), amplifying a 1,362-bp segment. Sequences of the partial 16S rDNA fragment were 100% identical to those of strain CCUG 35299^T (GenBank accession number NR_042652.1),

Table 1. Biochemical features of the type strain CCUG 35299^T and the *Massilia varians* deep finger wound isolate.

	CCUG 35299 ^T	Isolate
Oxidase	+	+
Nitrate reduction	-	-
Urease	-	-
Indole production	-	-
Glucose fermentation	-	-
Assimilation of:		
Arabinose	+	+
Mannose	-	-
Maltose	+	+
Mannitol	-	-
Proline	+	+
Lipase	-	-
Sorbitol	-	-
Sucrose	-	-
Trehalose	-	-
Citrate	-	-
Malate	±	-
Lactate	-	-
Succinate	+	+
Histidine	-	-
Enzyme activity		
Pyrase	+	+
β-galactosidase	-	-
β-glucosidase	-	-
α-galactosidase	-	-
α-glucosidase	-	-
Glutamyl arylamidase	+	+
Alanine arylamidase	-	-
Alkaline phosphatase	-	+

+, positive; ±, weakly positive; -, negative.

the type strain of *M. varians*, when compared using BLAST (<http://blast.ncbi.nlm.nih.gov/>).

Antimicrobial susceptibility tests were performed by disk diffusion method, according to Clinical and Laboratory Standards Institute (CLSI) guideline [4]. And minimal inhibitory concentrations (MICs) were determined by Etest[®] (bioMérieux) (Table 2). The antimicrobial susceptibility patterns of the isolate were similar with those of the CCUG 35299^T type strain.

Discussion

The genus *Massilia* was first proposed by La Scola et al. in 1998 [5]. Phylogenetically, it is placed in the vicinity of *Naxibacter*, *Telluria*, *Duganella*, and *Janthinobacterium* based on

Table 2. Antimicrobial susceptibilities and E test MICs of the type strain CCUG 35299^T and *Massilia varians* isolated from deep wound

Antibiotic	Result			
	CCUG 35299 ^T		Isolate	
	Diameter (mm)	MIC (µg/mL)	Diameter (mm)	MIC (µg/mL)
Piperacillin	42	ND	42	ND
Ticarcillin	35	ND	34	ND
Ampicillin/Sulbactam	36	ND	36	ND
Cefoperazone/Sulbactam	34	ND	36	ND
Piperacillin/Tazobactam	38	ND	39	ND
Amoxicillin/Clavulanic acid	40	ND	42	ND
Cefotaxime	34	0.25	37	0.25
Ceftazidime	30	3	29	3
Ceftriaxone	41	ND	44	ND
Cefepime	35	0.5	38	0.5
Aztreonam	12	48	13	96
Imipenem	36	0.25	37	0.38
Meropenem	34	0.38	33	0.75
Amikacin	27	3	28	2
Gentamicin	26	ND	24	ND
Tobramycin	24	ND	13	ND
Ciprofloxacin	28	ND	29	ND
Levofloxacin	32	ND	35	ND
Tetracycline	35	0.25	40	0.125
Cotrimoxazole	33	0.064	35	0.064
Colistin	21	0.047	22	0.032
Tigecycline	32	0.047	38	0.047

MICs, minimal inhibitory concentrations; ND, no data available.

16S rDNA gene sequences. However, *Massilia* and *Naxibacter* show different phenotypic characteristics from *Telluria*, *Duganella*, and *Janthinobacterium* [1, 5, 6]. The genus *Naxibacter* was first described in 2005 [6], and at the time was comprised of four species; *Naxibacter alkalitolerans* [6], *Naxibacter haematophilus* [7], *Naxibacter suwonensis* [8], and *Naxibacter varians* [7]. However, the *Massilia* and *Naxibacter* genera are now grouped together based on their phenotypic and biochemical features as well as 16S rDNA gene sequence comparisons. Therefore, a proposal was made to transfer all species of the genus *Naxibacter* to the genus *Massilia* by Kämpfer et al. in 2011 [1]. The genus *Massilia* has mainly been isolated from soil, air, or water, and is comprised by at least 23 species [1, 2, 5-19] (Suppl Table 1). However, several species belonging to the genus *Massilia* have been recovered from clinical specimens [1, 3, 5, 7, 14, 20] (Suppl Table 2). *N. varians* was first identified from the eye of 90-year-old man in Tromsø, Norway in 2008 [7]. After this date, *N. varians* was referred to as *M. varians* [1]; however, there have been no further reports of *M. varians* isolations since this first report.

The deep wound isolate was misidentified as *S. paucimobilis* (91%) and *S. putrefaciens* group (90%) by the GN and API 20NE cards respectively, most likely due to lack of database information for the genus *Massilia*. MALDI-TOF MS identified the isolates at the genus level, but with a low score. Therefore, when a certain unknown bacterial isolate is identified as the genus *Massilia*, 16S rDNA sequencing is strongly recommended.

To the best of our knowledge, this is the first case of *M. varians* isolated from clinical specimen since the first report in 2008.

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Conflicts of interest

No conflicts of interest.

Supplementary material

Supplementary data including two tables can be found with this article online <http://www.icjournal.org/src/sm/ic-49-219-s001.pdf>.

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