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A Case Report of *Tsukamurella pulmonis* Infection Misidentified as Atypical Mycobacteria

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We report a case of catheter-related bacteremia due to *Tsukamurella pulmonis*. *T. pulmonis* is a rare cause of opportunistic infection in immunosuppressed patients and in cases of indwelling foreign materials. This infection was nearly impossible to identify using conventional phenotyping methods because of its similarities to the related genera *Nocardia*, *Rhodococcus*, *Gordonia*, *Streptomyces*, *Corynebacterium*, and *Mycobacterium*. This organism was initially misidentified as *Mycobacterium aubagnense* through PCR-RFLP analysis. We correctly identified this organism using 16S rRNA sequencing combined with phenotyping tests. **(Korean J Clin Microbiol 2010;13: 93-97)**

Key Words: *Tsukamurella pulmonis*, Catheter, Bacteremia, Korea, Misidentification, Mycobacteria

INTRODUCTION

Tsukamurella species are aerobic gram-positive organisms from the order Actinomycetes and environmental organisms found in soil, water and sludge. *Tsukamurella* is clinically considered to be a rare opportunistic pathogen because most of the reported cases were related to intravascular prosthetic devices and immunosupression[1]. In the Korea, there have been only two reported cases of *Tsukamurella* infection[2,3]. These cases have included *Tsukamurella inchonensis* bacteremia in a patient who ingested hydrochloric acid and catheter-related bacteremia of *Tsukamurella pulmonis*.

Tsukamurella species share many features with *Nocardia*, *Rhodococcus*, *Gordonia*, *Streptomyces*, *Corynebacterium* and rapidly growing mycobacteria. Due to their similar phenotypic properties, differentiation and speciation within these genera are difficult using standard phenotyping tests.

T. pulmonis was first isolated from the sputum sample of a 92-year-old woman with pulmonary tuberculosis[4]. We now present the case of catheter-related bacteremia by *T. pulmonis* that was identified via 16S rRNA sequencing and phenotyping tests.

CASE REPORT

A 48-year-old man was presented with fever for the past several days. He had undergone craniotomy due to intracerebral hemorrhage 8 months earlier and was hospitalized for conservative therapy. Methicillin resistant *Staphylococcus aureus* was identified at the suction tip culture and *Acinetobacter baumannii* was identified at urine culture. The blood culture grew gram-positive bacilli resembling a diphtheroid. The site of central catheter was noted to have minimal erythema and the catheter was changed. The findings of the remainder of his physical examination were normal. Semiquantitative culture of the catheter tip showed 15~50 colonies and the isolates from his blood and catheter tip were thought as the same gram positive bacilli. Ceftazidime and vanco-

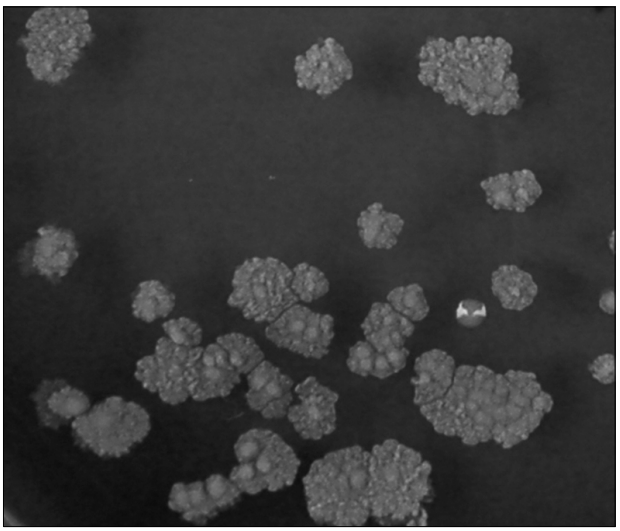

Fig. 1. *Tsukamurella pulmonis* colonies on blood agar isolated from the blood culture The colony was yellow, dry, irregular and rough.

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mycin were administered as empirical therapy, but intermittent low grade pyrexia was noted in the patient and subsequent five follow up blood cultures also showed the same organisms. It was decided to remove the catheter because it was believed to be the focus of the infection. After removing the catheter, the fever disappeared and the blood culture no longer showed this organism.

Blood cultures became positive after 54~67 hours incubation and the isolate aerobically grew on blood agar at 36°C. This organism initially appeared flat and spreading after about 48 hours and grew to irregular, rough, and dry yellowish colonies when incubation was extended (Fig. 1). The specimen was negative for acid-fast stain, but showed positivity to a modified acid-fast stain. It was catalase-positive and alkaline-slant/alkaline-deep on triple sugar iron agar.

Phenotypic identification of these gram positive organisms was performed using VITEK 2 identification card (bioMérieux, Durham, NC, USA) and API Corynebacterium system (bioMérieux, Marcy l'Etoile, France). The isolates could not be identified with VITEK 2 identification test and gave the profile 4550004 in the API Corynebacterium system, which gave an identification of *Brevibacterium* sp. (58.4%), followed by *Arthrobacter* sp. (17.6%), *Rhodococcus* sp. (12.2%). DNA was extracted and PCR-restriction fragment length polymorphism (RFLP) analysis was performed using Myco-ID (Molecules & Diagnostics, Wonju, Korea) to rule out *Nocardia* because of the colonies' morphological similarities and positivity of modified acid fast stain. Digestion of the *rpoB* amplicon by *MspI* (Boehringer Mannheim Biochemicals, Manheim, Germany) revealed restriction fragments compatible with *Mycobacterium aubagnense* sp. nov pattern which was proposed for a novel, rapidly-growing *Mycobacterium* in 2006. It was unreasonable to be assured of *Mycobacterium* when considering the patient's clinical manifestation and the organism's characteristics.

In order to make exact identification, the isolate was subsequently forwarded for molecular identification through PCR amplification with the primers 16SF (5'-GCR KTC YTA ATA CAT GCA AGT CGA-3'), 16SR (5'-TTT CAC GAA CAA CGC GAC AA-3')[5] and direct sequencing of the partial region of the 16S rRNA gene on the ABI Prism 3100 DNA sequencer (Applied Biosystems, Foster City, CA, USA). On BLAST analysis, the sequence analysis allowed reliable identification of *T. pulmonis* (AY741505, AY714240 and AY254698, 99.8% identity, 483/484 bases) followed by *T. tyroinsolvens* (AY238514, 99.2% identity, 480/484 bases), *T. inchonensis* (AF28318, 99.2% identity, 480/484 bases), *T. strandjordi* (AF283282, 99.2% identity, 480/484 bases) and *T. paurometabola* (AF283280, 99% identity, 479/484 bases) (Fig. 2). As seen above, the differences among the 16S rRNA gene sequences of various *Tsukamurella* species are small and therefore specific phenotypic tests were used to confirm *T. pulmonis*. Sugar assimilation results were obtained using API 50 CH systems (bioMérieux, Marcy-l'Etoile, France) and the tolerance at various temperatures were tested. The organism grew at 28°C and 35°C but not at 45°C. Testing for sugar utilization with the API 50 CH system showed excellent correlation with the published data of tests using conventional biochemical kits[6-8]. This test was performed twice and evaluated by two observers. Test was generally reproducible on repeated testing and inconsistent results were considered to be negative. Results of sugar assimilation and temperature tolerance test are as seen in Table 1. It can be distinguished from *T. tyroinsolvens*, *T. paurometabola* and *T. strandjordie* by assimilation results of D-mannose, inositol, D-mannitol, D-sorbitol, arbutine, inulin, D-melezibose and D-arabitol, and also can be distinguished from *T. inchonensis* through the absence of growth at 45°C and lack of assimilation of inositol

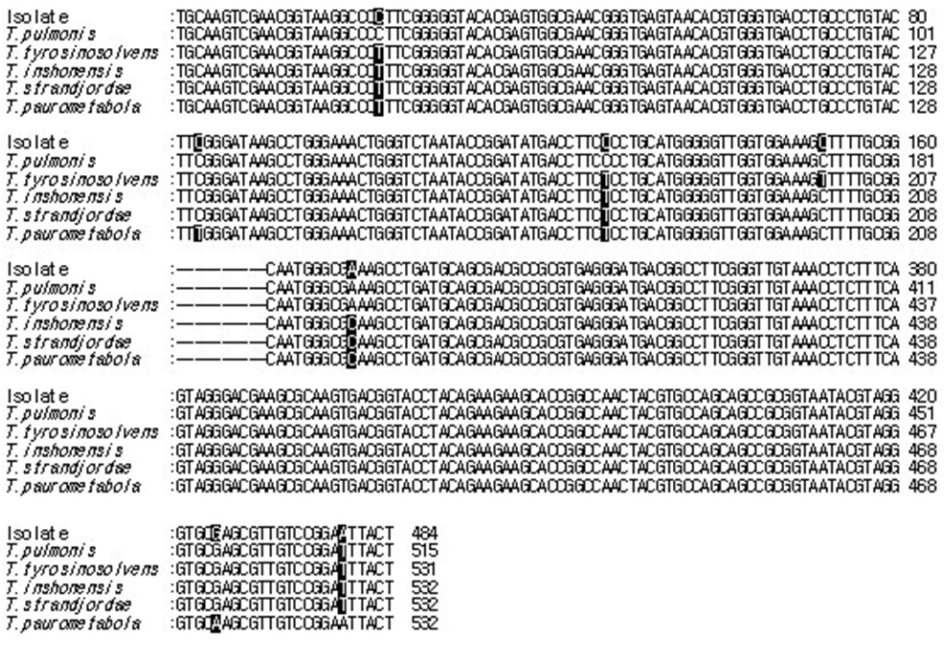

Fig. 2. Comparison of the DNA sequences of the 16S rRNA genes from the isolate and *Tsukamurella* species. The accession numbers of the sequences are as follows: *T. pulmonis*, AY741505; *T. tyrosinosolvens*, AY238514; *T. inshonensis*, AF283181; *T. strandjordae*, AF283283; and *T. paurometabola*, AF283280. The nucleotide sequences were deposited in GenBank

Table 1. Phenotype characteristics of the isolate compared to those of other *Tsukamurella* species*

		Tsukamurella species†				
	Isolate	*T. pulmonis*	*T. tyrosinosolvens*	*T. inchonensis*	*T. starndjordie*	*T. paurometabola*
Growth at 45°C	−	−	−	+	−	−
Utilization as sole carbon source						
D-mannose	+	V	+	+	−	−
Inositol	−	−	−	+	+	−
D-mannitol	+	+	+	+	+	−
D-sorbitol	+	+	+	+	+	−
Arbutin	−	−			+	−
Inulin	−	−	+	+	+	−
D-melezibose	−	−	+	+	−	−
D-arabitol	+	+	+	+	V	−

*+, presents; −, absent; V, variable; †For details, see references 6∼8.

and D-melezibose[6-8].

DISCUSSION

Tsukamurella species have emerged over the last decade as rare but significant causes of serious infections in immunocompromised individuals. The most common *Tsukamurella* infections in humans are indwelling device-related infections especially catheter-related bacteremia[1,7,9]. Others are cutaneous infection, meningitis, lung infection, peritonitis, knee prosthesis infection and conjunctivitis[7-10]. Optimal management is uncertain due to the paucity of cases, but a combination of beta-lactam and aminoglycoside, along with the removal of medical devices appears to be the preferential treatment[1,9].

Their species are suspected initially with isolation of a gram-positive bacillus that has microbiological characteristics similar to *Tsukamurella* species, but differentiation and speciation are difficult in most clinical microbiology laboratories. The genus is phylogenetically related to genera *Nocardia*, *Gordonia*, *Streptomyces*, *Rhodococcus*, *Corynebacterium* and *Mycobacterium*. At the genus level, the differentiation of *Tsukamurella* from similar species relies on gas-liquid chromatography or HPLC and differences in mycolic acid sizes and menaquinone compositions are invaluable[6,8,11]. In addition to this methodology, molecular approaches are being developed. These include molecular analysis of the 16S rRNA, 16S-23S intergenic spacer region, *groEL* and heat shock protein gene[12,13]. But 16S rRNA gene sequencing is not discriminative enough for speciation within this genus because the species share high 16S rRNA gene sequence similarities[7,13]. *T. pulmonis* could be distinguished on the basis of its sugar utilization profile and temperature tolerance in our case.

Tsukamurella are gram-positive, aerobic and partially acid-fast as a result of the presence of mycolic acid in the cell envelope. However, it may rarely exhibit substantial acid-fast staining similar to that of the *Mycobacterium* species. The misidentification of *Tsukamurella* as *Mycobacterium* has been previously reported in several studies[11,13,14]. At the initial evaluation we performed RFLP analysis of *rpoB* gene to exclude *Norcardia*. Phylogenetic trees were inferred with the PHYLIP 3.5 software package and made identification of *M. aubagnense* (98% similarity) possible. The sequences of *M. aubagnense* were not registered on the BLAST database making comparative analyses impossible and the *rpoB* sequence results showed only 83% similarity between *M. gondii* on the NCBI databases and our isolate. When considering positivity to modified acid-fast stain, colonial morphology and patient's symptoms, we could not absolutely define it as mycobacteria. Finally, by additionally executing 16s rRNA sequencing and phenotyping testing, we identified it as *T. pulmonis*.

If this organism is isolated in pulmonary infections, there is high possibility of misidentification. *T. pulmonis* is rarely misidentified as mycobacteria in pulmonary infection cases[4,10,13,14]. The presentation of pulmonary infections with *Tsukamurella* bears a striking similarity to the clinical syndrome seen with mycobacterial infections and also the intensity of acid-fast staining is variable, often confused with mycobacteria. We can usually suspect misidentification of species due to ineffective therapy with antimycobacterial drugs and failure to identify this organism on mycobacterial molecular assays[4,10,14]. But there have been no cases of *Tsukamurella* being misidentified as mycobacteria in commercial mycobacterial molecular identification assays and to our knowledge, this is the first case.

The molecular analysis of the 16S rRNA gene was used as the first-line method for the identification of unusual mycobacterial isolates[15], but only the 16S rRNA gene assay was shown to be insufficient for identifying mycobacteria and so the *rpoB* gene assay was developed as a suitable tool for accurate identification of nontuberculous mycobacteria (NTM)[16-18]. However, as evident in our case, the misidentification of genetically closely related organism such as *Tsukamurella* was found to be possible even in the *rpoB* gene assay. Care must be taken to reliably identify all acid-fast bacilli from specimens which are being investigated for mycobacteria to exclude other acid-fast organisms from phylogenetically related genera such as *Tsukamurella*. The importance of making an accurate microbiological diagnosis is underscored by

the fact that many of the antibiotics used to treat tuberculosis or nontuberculous mycobacteria are ineffective for other species.

In our case, it was initially considered as a contaminant or a saprophytic carriage rather than true pathogen and the organism was not identified as *Tsukamurella* until the patient's condition improved. Due to the persistence of fever and subsequent blood culture results, this organism was considered to be a real pathogen. It is therefore important that *Tsukamurella* species are suspected to be true infections in the immunosuppressed patient especially with an indwelling catheter and gram-positive bacilli in blood. Good clinical outcomes are achievable by combining appropriate antibiotics with removal of a catheter as the case with our patient.

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=국문초록=

비정형성 마이코박테리아로 잘못 동정된 *Tsukamurella pulmonis* 균혈증 1예

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본 저자들은 카테터와 연관된 *Tsukamurella pulmonis* 균혈증을 경험하여 보고하고자 한다. *Tsukamurella*는 드물게 면역억제자들에게 기회감염균으로 작용하며 특히 카테터 등 생체내 인공장치는 독립적인 위험인자로 작용한다. *Tsukamurella*는 계통발생학적으로 *Nocardia*, *Rhodococcus*, *Gordonia*, *Streptomyces*, *Corenybacterium*, *Mycobacterium* 등과 유사하여 일반적인 표현형 검사로는 동정이 어렵다. 본원의 48세 남자환자는 처음 *rpoB* 유전자를 이용한 PCR-RFLP에서는 *Mycobacterium aubagnense*로 잘못 동정되었다. 그러나 16S rRNA 염기서열 분석과 다른 *Tsukamurella* 종과 구별하기 위해 시행한 생화학적 검사로 *T. pulmonis*를 동정할 수 있었다. **[대한임상미생물학회지 2010;13:93-97]**

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