

# *Tsukamurella pulmonis* Bloodstream Infection Identified by *secA1* Gene Sequencing

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**Recurrent bloodstream infections caused by a Gram-positive bacterium affected an immunocompromised child. *Tsukamurella pulmonis* was the microorganism identified by *secA1* gene sequencing. Antibiotic treatment in combination with removal of the subcutaneous port healed the patient.**

## CASE REPORT

A 3-year-old female was diagnosed with acute lymphoblastic leukemia (ALL), with intermediate risk (immunophenotype pre-B), when she was 23 months old. In order to start her chemotherapy, a central venous access device (subcutaneous port [SP]) was implanted in September 2009. The patient was treated with SHOP-05 (Spanish Pediatric Hematology Society-2005) protocols for high-risk ALL, and she was in complete remission after the induction phase and completed four consolidation and reinduction phases, as was mandatory. Near the end of the second year of maintenance, when she was receiving only oral mercaptopurine (50 mg/m<sup>2</sup>/daily) and intramuscular methotrexate (20 mg/m<sup>2</sup>/weekly), the patient had a fever peak 5 h after a blood collection from the SP. The patient was treated empirically with oral cefixime (8 mg/kg of body weight/daily) when the fever started because she had had a prior isolation documented as *Klebsiella pneumoniae* that was sensitive to cephalosporins, and since she was not neutropenic and had no other signs or symptoms of interest, outpatient management was tried first. We obtained one pediatric blood culture, which grew long, slightly curved, thin, nonbranching Gram-positive rods. Culture of blood samples after 48 h of incubation at 35°C ± 2°C in 5% CO<sub>2</sub> on chocolate and blood agar showed small, rough, whitish colonies which did not produce aerial mycelium. The biochemical profile of the isolate obtained with the API Coryne identification system (bioMérieux, Marcy l'Etoile, France) after 48 h of incubation (2150004) corresponded to *Rhodococcus* spp. at a confidence level of 82.9%. By sequencing the 16S rRNA gene with the universal primers PA (5'-AGAGTTTGATCC TGGCTCAG-3') and PLO6R (5'-GCGCTCGTTGCGGGACTTA ACC-3') and the internal primer UP1R (5'-TTACCGCGGCTGC TGGCAC-3'), we obtained a sequence of 1,025 nucleotides that revealed maximal identity with *Tsukamurella pulmonis* (GenBank accession number [AB564289](https://www.ncbi.nlm.nih.gov/nucl/AB564289)). However, the 16S rRNA gene sequences of different *Tsukamurella* spp. were shown to be quite similar (99%).

Two weeks later, due to the persistence of intermittent fever in spite of cefixime therapy and two subsequent blood culture sets again positive for the same bacterium (*T. pulmonis*), the patient was admitted to the hospital. Antibiotic susceptibility was determined after 48 to 72 h of incubation at 35°C ± 2°C in ambient air by gradient diffusion (Etest; bioMérieux, Marcy l'Etoile, France) using Mueller-Hinton agar plates. Interpretations were based on

CLSI breakpoints for mycobacteria, nocardiae, and other aerobic actinomycetes but not breakpoints for meropenem (MIC > 32 µg/ml) and vancomycin (MIC, 2 µg/ml) because they were not assessed in this document. The isolate was susceptible to amikacin (MIC, 1 µg/ml), tobramycin (MIC, 4 µg/ml), ciprofloxacin (MIC, 0.25 µg/ml), clarithromycin (MIC, 0.125 µg/ml), and linezolid (MIC, 1 µg/ml) and resistant to amoxicillin-clavulanic acid (MIC > 256 µg/ml) and ceftriaxone (MIC > 32 µg/ml).

For 3 days, she received ertapenem intravenously (15 mg/kg/12 h), but due to both the onset of a generalized nonpruriginous rash and the results of susceptibility testing, ciprofloxacin (20 mg/kg/day in 2 doses) and amikacin (20 mg/kg/day) were then prescribed; the catheter was removed on the fourth day of admission. The patient improved and remained afebrile 24 h after treatment change and device removal. The treatment was completed after 7 days of ciprofloxacin and amikacin administration, and afterwards, she was treated with oral clarithromycin (15 mg/kg/day in two doses) for 15 days in her home. The device was sent for microbiological culture, but no growth was observed on brain heart infusion medium. No clinical relapse was observed during the next year, and she remains in complete uneventful remission.

In order to confirm *T. pulmonis* identification, additional phenotypic and molecular tests were performed. The isolate did not grow at 42°C, and the following results of sugars assimilation were obtained with the API 50 CH systems (bioMérieux, Marcy l'Etoile, France). The isolate was mannitol positive, inositol negative, D-sorbitol negative, arbutin negative, inulin negative, D-melezitose negative, D-arabitol negative, and L-fucose positive. The available

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TABLE 1 Sequence similarity values for 16S rRNA sequences and *secA1* sequences of *Tsukamurella* species and our clinical isolate of *T. pulmonis*, HUMV-11090126<sup>a</sup>

<i>Tsukamurella</i> species	Sequence similarity (%) with:					HUMV-11090126
	<i>T. paurometabola</i>	<i>T. pseudospumae</i>	<i>T. tyrosinosolvens</i>	<i>T. strandjordii</i>	<i>T. pulmonis</i>	
<i>T. paurometabola</i>		91.5	90.4	ND	90	90.4
<i>T. pseudospumae</i>	99		96	ND	93.4	91.7
<i>T. tyrosinosolvens</i>	99.3	99.5		ND	92.1	91.2
<i>T. strandjordii</i>	99.5	99.3	99.7		ND	ND
<i>T. pulmonis</i>	99.5	99.6	99.7	99.5		98
HUMV-11090126 <sup>b</sup>	99.6	99.6	99.7	99.7	100	

<sup>a</sup> 16S rRNA sequences are in the lower left, and *secA1* sequences are in the upper right. ND, no data. 16S rRNA GenBank accession numbers were as follows: for *T. paurometabola*, NR074458; for *T. pseudospumae*, NR042800; for *T. tyrosinosolvens*, AY238514; for *T. strandjordii*, AF283283; and for *T. pulmonis*, AY741505 (the accession number for the *secA1* gene sequences are indicated in the text).

<sup>b</sup> The 16S rRNA is 1,012 bp; the *secA1* gene is 230 bp.

partial *secA1* gene sequences of three *Tsukamurella* species (deposited by researchers at Westmead Hospital in Sydney, Australia), namely, *Tsukamurella tyrosinosolvens* (GenBank accession number GU179146), *Tsukamurella pseudospumae* (GU179143), and *Tsukamurella pulmonis* (GU179144), and the complete *secA1* sequence of the *Tsukamurella paurometabola* type strain, ATCC 8368 (CP001966), were aligned by using the Clustal W program (v. 1.81). Two candidate primers, Tsuka1 (5'-CTACCTGCGCGA CAACATG-3') and Tsuka2 (5'-CGATCGTCTTCTTGCGGATG-3'), which correspond to *T. paurometabola secA1* gene positions 546 to 564 and 756 to 775, respectively (GenBank accession no. CP001966, nucleotides 1106448 to 1109201), were designed by using primer 3.

The PCR amplification of the *secA1* gene of our isolate (HUMV-11090126) (annealing temperature of 60°C) showed the predicted amplicon of 230 bp. Subsequent sequencing with the same primers and comparison of the obtained sequence with GenBank entries, by using the BLASTn algorithm, gave 98% homology with *T. pulmonis* (accession no. GU179144), followed by *T. pseudospumae* (GU179143) (91.7%), *T. tyrosinosolvens* (GU179145, GU179146, GU179147, GU179148, GU179149) (91.2%), and *T. paurometabola* (CP001966) (90.4%) (Table 1).

The genus *Tsukamurella* includes aerobic Gram-positive and modified acid alcohol-fast-positive rods belonging to the aerobic actinomycetes, usually found in soil, sludge, and arthropods (1).

Yassin et al. isolated and described the species of interest, *T. pulmonis*, in 1996 from the sputum of a 92-year-old woman with a history of lung tuberculosis (2).

*T. pulmonis* is a rare human pathogen associated with oncologic and immunosuppressed patients (3, 4), and a variety of infections have been associated with this bacterium: pneumonia (5), conjunctivitis (6), keratitis (7), and catheter-related bacteremia (3, 8). In our case, *T. pulmonis* was isolated from an immunosuppressed child in three different blood culture sets, but the primary source of the infection could not be identified. Even though the long-term central venous catheter culture was negative, the first fever peak was registered after blood was collected for a hemato-

logical analysis, and the patient improved as the catheter was removed. In consequence, it seems reasonable to consider this device associated with the beginning of episodes. Bouza et al. (9) performed a review of catheter-related bloodstream infections, and most of them required the withdrawal of the infected line, along with antibiotic treatment, to resolve the episodes.

Our isolate was susceptible to aminoglycosides, macrolides, and quinolones but resistant to amoxicillin-clavulanic acid, ceftriaxone, and presumably carbapenems. Other authors have shown a similar susceptibility pattern (3, 4, 10).

*T. pulmonis* and related species may well be underdiagnosed or misdiagnosed as a cause of significant infections owing to the difficulty of identifying the organism by phenotypic procedures (11). Two reports (4, 9) have described the same API Coryne code which we found for our isolate.

Aimed at identifying uncommon pathogens, most of all in immunocompromised hosts, 16S rRNA sequencing is a reasonably rapid and consistent molecular technique. However, in this case, the gene was not shown to be discriminative enough for the identification of *Tsukamurella* spp., since the differences in the genes are minimal among the different species of the genus (Table 1) (9, 10). In contrast, an analysis of a region of the *secA1* gene was shown to be suitable for discrimination among four important clinical species of *Tsukamurella*, as it has been demonstrated for other clinically significant species of aerobic actinomycetes (12, 13).

We could not perform a comparison with *Tsukamurella strandjordii* and *Tsukamurella inchonensis*, the other species recovered from clinical specimens, because their sequences have not been deposited in GenBank.

Nevertheless, in our case, the isolate could be differentiated from *T. inchonensis* because our species does not grow at 45°C and from *T. strandjordii* on the basis of its sugar utilization profile, since *T. strandjordii* uses inulin, arbutin, and D-melezitose as carbon sources, whereas our isolate does not (14, 15).

In conclusion, we present a case of bacteremia caused by *T. pulmonis* that was essentially identified by molecular methods, in particular, *secA1* gene sequencing, and this gene was demon-

strated to be a good target for differentiation among the most relevant pathogenic species of this genus.

**Nucleotide sequence accession numbers.** The 16S rRNA sequence and the *secA1* sequence of the *T. pulmonis* isolate were deposited in GenBank under accession numbers [KP056257](#) and [KP013182](#), respectively.

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