Brain Abscess Caused by *Tsukamurella tyrosinosolvens* in an Immunocompetent Patient

Wang-Huei Sheng,1, 2 Yu-Tsung Huang,3 Shan-Chwen Chang,2 and Po-Ren Hsueh2,3*

1Department of Internal Medicine, Far-East Memorial Hospital; 2Department of Internal Medicine, 3Department of Laboratory Medicine, National Taiwan University Hospital, National Taiwan University College of Medicine, Taipei, Taiwan

* Corresponding author. Mailing address: Depts. of Laboratory Medicine and Internal Medicine, National Taiwan University, No. 7, Chung-Shan S. Rd., 100 Taipei, Taiwan. Phone: 886-2-3123456 ext. 65355. Fax: 886-2-23224263. E-mail: hsporen@ntu.edu.tw.

Running Title: **TSUKAMURELLA TYROSINOSOLVENS BRAIN ABSCESS**

**Keywords:** Tsukamurella tyrosinosolvens, brain abscess
We described a previously healthy patient with chronic otitis media complicated with cerebellar abscess caused by *Tsukamurella tyrosinosolvens*. The organism was identified based on conventional biochemical identification method, PCR-RFLP analysis of hsp65 gene, and 16S rRNA gene sequencing. The patient was treated successfully with debridements and prolonged antibiotic therapy.
Tsukamurella species are obligate aerobic gram-positive, partially acid-fast, non-motile bacilli that belong to the order Actinomycetales (6). Tsukamurella share many features with other Actinomycetales, such as Nocardia, Rhodococcus, Gordonia, and rapidly growing Mycobacterium and might be misidentified as genera described above when standard biochemical tests are used (1). Infections caused by Tsukamurella species are rare (1, 2, 4-8, 11). Most reported cases of Tsukamurella infection were related to intra-vascular catheters or prosthetic devices and associated with immunocompromised condition (malignancies, post-chemotherapy, chronic renal failure and AIDS) (1, 2, 4-8, 11). Brain abscess caused by Tsukamurella has not been reported. We first report a previously healthy patient who had chronic otitis media complicated with cerebellar abscess caused by T. tyrosinosolvens.

CASE REPORT

A 36-year-old female farmer who denied any history of systemic medical illness had persistent purulent discharge from the right auditory channel for more than two months. Chronic otitis media was diagnosed and the patient received tympanoplasty and oral amoxicillin initially. However, poor wound healing and fever developed later accompanied by right facial pain, dizziness and limited range of motion of the right neck for four weeks. The patient then visited a local hospital and received intravenous
flomoxef (an oxacephalosporin) and reconstructive surgery of auditory meatus (meatoplasty) with drainage of pus. Gram stain of the abscess was no organisms seen but pus culture yielded a putative rapidly growing nontuberculous mycobacterium later. However, the patient’s symptoms persisted and the patient was referred to National Taiwan University Hospital (NTUH). On admission, blood pressure was 102/58 mm Hg, heart rate was 88 beats per minute and regular, respiratory rate was 22 breaths per minute, and temperature was 38°C. Right neck swelling and tenderness were noted. Neurological examination disclosed mild cerebellar ataxia. Leukocyte count was 8.96 x 10⁹/µl with 82.4% neutrophils. Hemoglobin was 11.0 g/dl. Anti-nuclear antibody, complements (C3 and C4), blood lymphocytes of CD4 and CD8 surface markers, and serum immunoglobulins IgA, IgG and IgM were all within normal limits. Blood anti-HIV test was negative. Magnetic resonance image (MRI) of the head and neck revealed complicated right otitis media, otitis interna, and mastoiditis with osteomyelitis of the skull base and first cervical spine with intracranial spread of leptomeningeal thickening at the right posterior fossa (Figure 1).

Vigorous surgical debridements with mastoidectomy and drainage of pus were performed three times after admission. The patient received six weeks of intravenous imipenem (500 mg every 6 hours) and amikacin (750 mg daily) followed by oral ciprofloxacin (500 mg every 12 hours), trimethoprim-sulfamethoxazole (160/800 mg
every 12 hours), and clarithromycin (500 mg every 12 hours). A followed-up brain MRI two months after the initiation of therapy revealed substantial improvement of lesions. During follow up at outpatient clinic, the patient’s condition was improved and the patient remained healthy and afebrile with a normal WBC count.

**Microbiology.** The isolate was a gram-positive and partially acid-fast bacillus. The isolate grew well at 37°C but not at 42°C on blood agar plate. The biochemical profile of the isolate produced by the API CORYNE Identification System (Biomérieux, Las Halles, France) disclosed *Rhodococcus* species. The isolate could hydrolyze tyrosine but couldn’t hydrolyze xanthine in Nocardia ID QUAD agar plate (Becton Dickinson, Sparks, MD, USA).

A previously described PCR-restriction fragment length polymorphism (RFLP) identification scheme that used an amplified 440-bp segment of the 65-kDa heat shock protein (hsp65) was performed by using two primers TB11 (5’-ACCAACGATGGTGTGTCCAT-3’) and TB12 (5’-CTTGTCGAACCGCATACCCT-3’) (9). The unique fragments digested by *Hinf*I (440 bp) and *Msp*I (313/135 bp) of our isolate were compatible of the identification of *Tsukamurella* species.

Further identification of the isolate was performed by 16S rRNA gene (1490 bp) sequencing using a pair of universal primers, 8FLP (5’-AGAGTTTGATCCTGGCTCAG-3’) and 1492RPL (5’-
GGTACCTGTTACGACTT-3') (10). The sequences obtained (1080 bp) were compared with published sequences in the GenBank database using the BLASTN algorithm (http://www.ncbi.nlm.nih.gov/blast). The closest match observed was obtained with *T. tyrosinosolvens* strain M12400 (GenBank accession no. AY254699.1) (maximal score 1818, E value 0.0, and maximal identity 99% [1078/1080]). The following nine strains also had E value of 0.0 and maximal identity of 99%: *T. inchonensis* strain ATCC70082 (GenBank accession no. AF283281.1; maximal score 1818), *T. inchonensis* strain ATCC25938 (GenBank accession no. AF283282.1; maximal score 1818), *T. inchonensis* strain IMMIB D-771T (GenBank accession no. X85955.1; maximal score 1818), *T. spumae* strain N1173 (GenBank accession no. AF238512.1; maximal score 1807), *T. paurometabola* (GenBank accession no. AF283280.1; maximal score 1796), *T. tyrosinosolvens* strain DSM44234 (GenBank accession no. AF238514.1; maximal score 1784), *T. tyrosinosolvens* strain M95570 (GenBank accession no. AF263916.1; maximal score 1784), *T. tyrosinosolvens* isolate D-1397 (GenBank accession no. Y12246.1; maximal score 1784), and *T. tyrosinosolvens* isolate D-1498 (GenBank accession no. Y12245.1; maximal score 1784). The isolate was in accordance with the identification of *T. tyrosinosolvens* not other *Tsukamurella* species due to the ability to decompose tyrosine and no growth at 42°C (1, 11). The second set of primers: PSL2 (forward) 5′- AGG
ATTAGATACCCCTGGTAGTCCA -3' and P13P (reverse.) 5' -

AGGCCCGGGAACGTATTCAC -3' were also used as described previously (8). The resulting sequence obtained (559 bp) gave a 100% identification for *Tsukamurella tyrosinosolvens* (GenBank accession no. AY254699.1).

**Discussion.** The clinical syndrome in reported cases of *Tsukamurella* infection included bacteremia (1), lung infection (2), conjunctivitis (3), infections of knee prosthesis and defibrillator (4, 5), cutaneous infection (6), and peritonitis (7). Most of the *Tsukamurella* infections reported were related to intra-vascular catheterizations, implantation of prosthetic devices or immunocompromised conditions (such as malignancies, post-chemotherapy, chronic renal failure and AIDS) (1, 2, 4-8, 11). Our patient might have acquired this pathogen which colonized in the ear from soil during work in the field, with otitis media subsequently developing after ear injury.

Accurate identification of *Tsukamurella* by phenotypic methods is difficult. Microbiological characteristics of *Tsukamurella*, including morphology on culture medium, slow growth and weakly acid-alcohol-fast, may lead to the diagnosis of *Corynebacterium, Rhodococcus, Nocardia, Gordonia*, or *Mycobacterium* species. A PCR-RFLP analysis of hsp65 has been reported to be useful for identification of all clinically significant species and taxa of aerobic actinomycetes (9). For identifying unusual pathogens, 16S rRNA sequencing is a relatively rapid and reliable new
molecular technique (8). However, 16S rRNA gene sequences have been found to be not discriminative enough for the identification of certain species, like Tsukamurella species because of small differences of 16S rRNA gene sequence on various Tsukamurella species. groEL gene has been reported to be useful for speciation of a variety of bacteria (11). However, in the GenBank database, the groEL gene sequences were available on only two Tsukamurella species: T. tyrosinosolvens (GenBank accession no. U90204) and T. paurometabola (GenBank accession no. AF352578). Further studies on groEL gene sequencing of multiple strains of each species of Tsukamurella should be performed to verify the suitability for Tsukamurella speciation. The ability to decompose tyrosine and negative growth at 42°C of our isolate suggested that the isolate was not T. paurometabola or T. inchonensis (1, 11).

Optimal management of infections caused by Tsukamurella remains to be determined. Although there is no recommended standard susceptibility method for Tsukamurella species, most case reports showed that Tsukamurella isolates were susceptible to amikacin, clarithromycin, imipenem, ciprofloxacin, trimethoprim-sulfamethoxazole, but resistant to penicillin, cefoxitin, third-generation cephalosporins (1, 6, 11). Based on the treatment principle for nocardiosis and atypical mycobacterial infections, combinations of several antimicrobial agents have been proposed for the treatment of Tsukamurella infections. The combination of a beta-
lactam (including carbapenem) and an aminoglycoside or rifamycins, along with removal of medical devices appears to be the treatment of choice (6). In our patient with intracranial invasion of cerebellum, we selected initial combination of intravenous imipenem and amikacin, followed by oral clarithromycin, trimethoprim-sulfamethoxazole, rifampin and ciprofloxacin.

The length of treatment for infections caused by *Tsukamurella* has not been determined and should be individualized according to clinical response. As in *Rhodococcus* or *Nocardia* infections, especially in immunocompromised hosts, frequent relapses can be expected and prolonged oral suppressive treatment is recommended. Since our patient had no evidence of immunosuppression, it seemed appropriate to maintain the treatment until symptoms relief and improvement on the image studies was documented.

In conclusion, we first report a patient who had chronic otitis media complicated with cerebellar abscess caused by *T. tyrosinosolvens*. Newer molecular biological techniques can provide accurate identification of *Tsukamurella* and contribute to the appropriate selection of definitive therapy of infection due to this organism.
REFERENCES


FIG. 1. MRI of the head and neck shows (A) multiple abscess formation involving the perivertebral space of the right upper neck, right skull base (mastoid bone, right occipital condyle and C1 lateral mass) (arrows) and (B) an intracranial abscess of the right cerebellum (arrow head) caused by *Tsukamurella tyrosinosolvens*. 