

World Journal of *Clinical Cases*

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Obligate aerobic, gram-positive, weak acid-fast, nonmotile bacilli, *Tsukamurella tyrosinosolvens*: Minireview of a rare opportunistic pathogen

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Abstract

Tsukamurella species are obligate aerobic, gram-positive, weak acid-fast, nonmotile bacilli. They are found in various environments, such as soil, water, sludge, and petroleum reservoir wastewater, and belong to the order *Actinomycetales*. In 2016, there was a reclassification of species within the genus *Tsukamurella*, merging the species *Tsukamurella tyrosinosolvens* (*T. tyrosinosolvens*) and *Tsukamurella carboxydivorans*. *Tsukamurella* species are clinically considered to be a rare opportunistic pathogen, because most reported cases have been related to bacteremia and intravascular prosthetic devices and immunosuppression. To date, it has been isolated only from human specimens, and has always been associated with clinical disease; human infections are very rare. Reported infections have included pneumonia, brain abscesses, catheter-related bloodstream infections, ocular infections, bacteremia, and sepsis presenting with septic pulmonary emboli in patients who are immunocompromised. To date, there is no commercially available test for identification. On the other hand, sequence-based identification, including matrix-assisted laser desorption ionization time-of-flight mass spectrometry, is an alternative method for identifying clinical isolates that are either slow growers or difficult to identify through biochemical profiling. The golden standards for diagnosis and optimal management still remain to be determined. However, newer molecular biological techniques can provide

accurate identification, and contribute to the appropriate selection of definitive therapy for infections caused by this organism. Combinations of several antimicrobial agents have been proposed for treatment, though the length of treatment for infections has yet to be determined, and should be individualized according to clinical response. Immunocompromised patients often experience severe cases due to infection, and life-threatening *T. tyrosinosolvens* events associated with dissemination and/or failure of source control have occurred. Favorable prognoses can be achieved through earlier identification of the cause of infection, as well as successful management, including appropriate antibiotic therapy together with source control. Further analyses of similar cases are required to establish the most adequate diagnostic methods and treatment regimens for infections.

Key Words: *Tsukamurella tyrosinosolvens*; Gram-positive bacilli; Opportunistic infection; Sequence-based identification; Matrix-assisted laser desorption ionization time-of-flight mass spectrometry; Combination antibiotic therapy

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Core Tip: *Tsukamurella* species are obligate aerobic, gram-positive, weak acid-fast, nonmotile bacilli that are found in various environments, including soil, water, and sludge. In 2016, there was a reclassification of species within the genus *Tsukamurella*, merging the species *Tsukamurella tyrosinosolvens* (*T. tyrosinosolvens*) and *Tsukamurella carboxydovorans*. To date, human infections are very rare, and reported infections include pneumonia, brain abscesses, catheter-related bloodstream infections, ocular infections, and bacteremia in patients who are immunocompromised. The golden standards for diagnosis and optimal management still remain to be determined. Immunocompromised patients often experience severe cases due to infection, and life-threatening *T. tyrosinosolvens* events associated with dissemination and/or failure of source control have occurred. Favorable prognoses can be achieved through earlier identification of the cause of infection, as well as successful management, including appropriate antibiotic therapy together with source control. Further analyses of similar cases are required to establish the most adequate diagnostic methods and treatment regimens for infections.

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INTRODUCTION

Tsukamurella species, which are members of the order *Actinomycetales*, can be found in a variety of different environments, including soil, water, sludge, and wastewater from petroleum reservoirs; these nonmotile bacilli are obligate aerobic, gram-positive, and weak acid-fast[1-4]. They were initially isolated from bedbug mycetoma and ovaries, in 1941[5]. The name *Tsukamurella* comes from Tsukamura and Mizuno[6], the microbiologists who described the first *Gordona aurantiaca* strain in 1971, which had been isolated from the sputum of a patient suffering from a chronic lung pathology[5-7]. The genus *Tsukamurella* currently contains 12 species with validly published names that include *Tsukamurella tyrosinosolvens* (*T. tyrosinosolvens*), and was first described by Collins et al[8] in 1988. Following a 2016 reclassification of *Tsukamurella* species, the species *T. tyrosinosolvens* and *Tsukamurella carboxydovorans* have been merged[4]. The genus is phylogenetically related to other mycolic acid-containing genera of the order *Actinomycetales*, including *Nocardia*, *Gordonia*, *Streptomyces*, *Rhodococcus*, *Corynebacterium*, and *Mycobacterium*, because of their similar phenotypic properties[1,7,9,10].

ROUTE OF INFECTION AND SYMPTOMS

Tsukamurella species infections are rare in humans, as the species is a type of saprophyte bacterium[5]. This genus has the potential to cause various infections in humans, and more and more infections have been reported in Asia, America, Europe, and Africa, suggesting a global emergence of diseases caused

by this group of bacteria[11]. *Tsukamurella* spp. are clinically considered a rare opportunistic pathogen, as most reported cases have been related to bacteremia and intravascular prosthetic devices, such as catheters or cardiac pacemaker implants, and immunosuppression (hematological malignancy, post chemotherapy, chronic renal failure, graft-versus-host disease after bone marrow transplant, and acquired immunodeficiency syndrome)[1,12-14]. Catheter-related infection range varies, from infections of the local insertion site to metastatic deep-seated infections[9]. In addition, a case has been reported of a septic pulmonary embolism, secondary to a central venous catheter-related bloodstream infection (CRBSI)[15]. Other examples include cutaneous infection, meningitis, brain abscess, lung infection, peritonitis, knee prosthesis infection, and ocular infection[2,11,16]. Among these, a striking similarity has been noted between the clinical features of lung disease with *Tsukamurella* and those of mycobacterial infections[16]. There is a great likelihood that *Tsukamurella* lung disease incidence is significantly underestimated in areas with high tuberculosis incidence rates; it is conceivable that *Tsukamurella* has greater community prevalence than currently recognized[16].

To date, *T. tyrosinosolvens* has been isolated only from human specimens, and has been associated with clinical disease in all cases; human infections are very uncommon[7]. The infections reported have included pneumonia, brain abscesses, CRBSI, ocular infections, bacteremia, and sepsis presenting with septic pulmonary emboli[1,7,15,17].

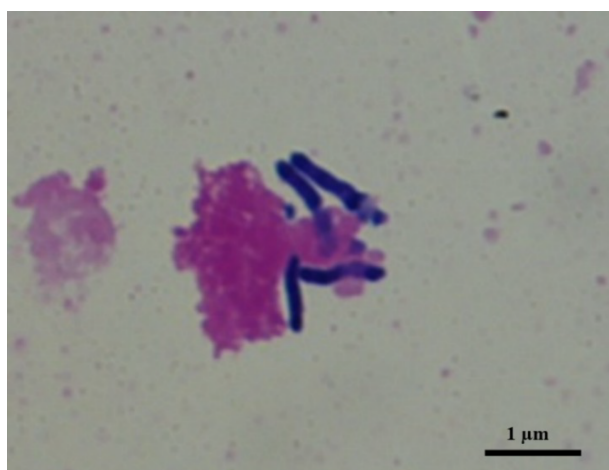
On the other hand, there has been a reported case of coinfection with *T. tyrosinosolvens* and another microorganism (e.g., *Mycobacterium bovis* pneumonia) in an immunocompromised patient[17].

EXAMINATIONS

To date, no test to identify *Tsukamurella* spp. is commercially available[7]. The genus bears a phylogenetic relation to other *Nocardia*, *Gordonia*, *Streptomyces*, *Rhodococcus*, *Corynebacterium*, *Mycobacterium*, and other mycolic acid-containing genera of the order *Actinomycetales*; because of similarities in their phenotypic properties, it remains difficult for the majority of clinical microbiology laboratories to identify individual species using ordinary biochemical tests[1,9,10,18]. As a result, identification using conventional biochemical assays has proven unsuccessful, and it is not feasible to accurately identify *Tsukamurella* through phenotypic methods[1,7,11]. Here, to help share a better understanding of this pathogen through our experience, we show gram staining of *T. tyrosinosolvens* from aerobic bottles, in blood cultures from a blood sample of a CRBSI patient at our institution ($\times 1000$ magnification). Numerous rod-shaped bacteria are confirmed (Figure 1). We also show colonies of *T. tyrosinosolvens* on a blood agar plate, in a blood sample from a CRBSI patient at our institution. This confirmed the development of flat, huge, dry, and white- to light-cream-colored colonies, in addition to crow's feet in the overlapping portions (Figure 2).

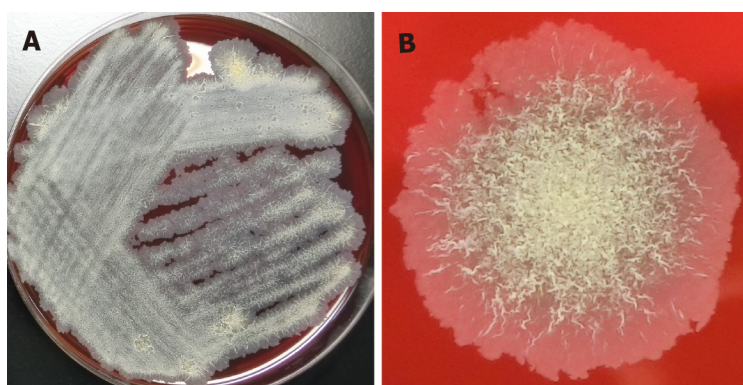
One alternative method for identifying clinical isolates that are either slow growers or difficult to identify through biochemical profiling is sequence-based identification[7]. Because clinical laboratories most frequently use the 16S rRNA gene as a target for molecular identification, and for the identification of unusual pathogens, sequencing serves as a comparatively rapid and reliable new molecular technique[1,7,13,18]. Previous studies have shown that the majority of *Tsukamurella* species have highly similar 16S rRNA gene sequences in common ($> 99\%$ nucleotide identities); as a result, these species cannot be confidently identified using this gene target[18]. Despite this, 16S rRNA gene sequences have been found to be insufficiently discriminative for the purpose of identifying certain species, including *Tsukamurella* species, due to the minor differences that exist within various *Tsukamurella* species' 16S rRNA gene sequences[1,7,9]. However, in previously reported criteria used to interpret partial 16S rRNA gene sequences, the sequence provided 100% identification for *T. tyrosinosolvens* (GenBank Accession numbers AY238514, Y12246, Y12245, and Y12247)[9].

An isolate digested by *Hinf*I (440 bp) and *Msp*I (313/135 bp) leaves unique fragments compatible with *Tsukamurella* species identification[1]. Other bacterial genera have been successfully identified through other gene targets, including *ssrA* (stable small RNA), *secA* (the secretion ATPase), *rpoB* (beta-subunit of RNA polymerase), and *groEL* (heat shock protein 60)[5,18,19]. On the other hand, the *groEL* gene is reportedly useful for speciation of *T. tyrosinosolvens*[1,7,11]. However, two gene sequences for *Tsukamurella* species were found to be available within the GenBank database: *T. tyrosinosolvens* (GenBank accession No. U90204) and *T. paurometabola* (GenBank accession No. AF352578)[1]. This indicates that further studies of *groEL* gene sequencing of multiple strains of each *Tsukamurella* species would be merited, as verification of suitability for speciation of *Tsukamurella*[1,11]. Furthermore, regarding the biodegradation of hydrocarbons, two alkane catabolic pathways have been discovered within the *T. tyrosinosolvens* PS2 genome, unlike the above-mentioned representatives of the genus *Tsukamurella*[4]. One of these pathways contains an alkane 1-monooxygenase gene (*alkB*; GenBank accession number KZL97795), two rubredoxin genes (*rubA*; GenBank accession numbers KZL97794 and KZL97793), one rubredoxin-reductase gene (*rubB*), and one regulatory protein *TetR* gene (*alkU*; GenBank accession number KZL97792)[4]. This pathway resembles that of the alkane-degrading actinomycete *T. tyrosinosolvens* strain MH1, in accordance with the complete genome[4]. In addition, a gene known to perform a key alkane degradation role was found within the *T. tyrosinosolvens* PS2 genome (GenBank



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Figure 1 Gram staining of *Tsukamurella tyrosinosolvens* from aerobic bottles, blood cultures (× 1000 magnification). Blood sample from a catheter-related blood stream infection patient, at our institution. Numerous rod-shaped bacteria are confirmed.



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Figure 2 *Tsukamurella tyrosinosolvens* on a blood agar plate. The blood sample was taken at our institution, from a patient with a catheter-related blood stream infection. A: The development of flat, huge, dry, and white- to light-cream-colored bacterial mass is confirmed; B: A close-up image. The development of flat, huge, dry, and white- to light-cream-colored colonies, in addition to crow's feet in the overlapping portion, is confirmed.

accession number KZL95198), homologous to cytochrome P450 alkane 1-monooxygenase, from the *Gordonia* spp. strain TF6 (GenBank accession number BAF95905)[4]. Consequently, these two systems may benefit this particular strain in highly polluted environments, and may additionally offer insights into the ecological role played by this bacterium[4].

PCR sequencing is not yet used in clinical laboratories as a routine method of identification, due to its expensive, time-consuming nature and its technical demands[11]. To date, none of the 60 isolates have been correctly identified at the species level using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) with the original Bruker database V.6.0.0.0[11]. By using the Bruker database, expanded with 15 type and reference strains that cover all 11 *Tsukamurella* species recognized to date, 59 of the 60 isolates were accurately identified at the species level, with scores of ≥ 2.0 [11]. Therefore, MALDI-TOF MS should prove beneficial for the routine identification of *Tsukamurella* species in clinical microbiology laboratories, once the database has been optimized[11]. However, because of its inability to correctly identify species of *Tsukamurella*, it is necessary to continually expand the MALDI-TOF MS databases to add more gram-positive rods[11]. *Tsukamurella* isolates have also been identified at the species level through PCR-restriction fragment length polymorphism analysis, as well as through 16S rRNA gene sequencing[1,7,20,21]. One identification scheme based on PCR-restriction fragment length polymorphism, using an amplified 440-bp segment of *hsp65*, has been described as being performed with the primers TB11 (5'-ACCAACGATGGTGTGTCCAT-3') and TB12 (5'-CTTGTCGAACCGCATACCT-3')[1].

Regarding other genetic analysis, it has been confirmed through DNA-DNA hybridization that these are distinct from the other known species within the genus *Tsukamurella* ($26.2\% \pm 2.4\%$ to $36.8\% \pm 1.2\%$ DNA-DNA relatedness), in line with results of in silico genome-to-genome comparison (32.2% - 40.9% Genome-to-Genome Distance Calculator and 86.3% - 88.9% average nucleotide identity values)[19].

DIAGNOSIS

For diagnoses, physicians should ask for detailed information about patients' working and living environments, as well as their histories of animal contact, and perform routine bacterial smear tests[16]. The first step towards a reliable diagnosis of *Tsukamurella* infections is a high level of suspicion, and a low threshold for microbiological sampling[16]. Ideally, molecular diagnostic assays should serve as the routine laboratory method[16]. Given the rising number of *Tsukamurella* infections, new *Tsukamurella* species implementations in matrix-assisted laser desorption ionization time-of-flight databases need to be more discriminant[22].

TREATMENT

The optimal management of *Tsukamurella* bacterial infections has yet to be determined[1]. Based on treatment principles for nocardiosis and atypical mycobacterial infections, a number of combinations of antimicrobial agents have been proposed as potential treatments for *Tsukamurella* infections[1,5]. To date, there remains no recommended standard susceptibility method for *Tsukamurella* species; however, in most case reports, a susceptibility to amikacin, clarithromycin, imipenem, ciprofloxacin, and trimethoprim-sulfamethoxazole has been noted in *Tsukamurella* isolates, as well as a resistance to penicillin, cefoxitin, and expanded-spectrum cephalosporins, determined through a standard *in vitro* antibiotic disk diffusion susceptibility assay[1,9]. Immunocompromised patients often experience severe cases due to infection, and life-threatening *T. tyrosinosolvens* events associated with dissemination and/or failure of source control have occurred[14,15]. Favorable prognoses can be achieved through earlier identification of *T. tyrosinosolvens* as the cause of infection, as well as successful management, including appropriate antibiotic therapy with source control[9,14]. In particular, good clinical outcomes are possible for CRBSI caused by *Tsukamurella* species, through a combination of appropriate antibiotics and catheter removal; this is likely to be the most effective management strategy for patients[1,2,9].

The length of treatment for *Tsukamurella* bacterial infections has yet to be determined, and should be individualized based on clinical response[1]. On the other hand, expect frequent relapses, especially in hosts who are immunocompromised; it is recommended to administer prolonged antibiotic treatment and oral suppressive treatment[1,17].

INFECTION CONTROL

Multiple lapses in infection control have been identified; the most likely cause of the outbreak was the clinic improperly preparing the saline flush syringes[23]. It has been demonstrated, through this outbreak, that oncology patient bloodstream infections can be the result of improper infection control practices; this serves to highlight the critical necessity of increased attention to, and supervision of, infection control measures in outpatient oncology settings[23].

CONCLUSION

This mini-review aims to highlight the difficulty of identifying the genus *Tsukamurella*. The golden standards for diagnosis and optimal management still remain to be determined. However, accurate identification can be achieved through newer molecular biological techniques, thus contributing to appropriate selection of definitive therapy for infections due to this organism. Further analysis of similar cases is required in order to establish the most adequate diagnostic methods and treatment regimens for *T. tyrosinosolvens* infections. In addition, future studies should aim to establish better guidelines for the effective management of *Tsukamurella* infections.

FOOTNOTES

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