JOURNAL OF CLINICAL MICROBIOLOGY, June 2009, p. 1953–1956 Vol. 47, No. 6
0095-1137/09/$08.00+0 doi:10.1128/JCM.00424-09

CASE REPORTS

First Report of *Tsukamurella* Keratitis: Association between *T. tyrosinosolvens* and *T. pulmonis* and Ophthalmologic Infections[▽]

Patrick C. Y. Woo,[1,2,3]* Angie H. C. Fong,[4] Antonio H. Y. Ngan,[3] Dorothy M. W. Tam,[3] Jade L. L. Teng,[3] Susanna K. P. Lau,[1,2,3] and Kwok-Yung Yuen[1,2,3]

State Key Laboratory of Emerging Infectious Diseases,[1] Research Centre of Infection and Immunology,[2] and Department of Microbiology,[3] the University of Hong Kong, Hong Kong, and Department of Ophthalmology, Queen Mary Hospital,[4] Hong Kong

Received 26 February 2009/Returned for modification 25 March 2009/Accepted 7 April 2009

We describe the first two cases of *Tsukamurella* keratitis, presented as eye pain with or without blurring of vision. One case was associated with trichiasis and the other with contact lens wear. The two isolates were identified as *T. tyrosinosolvens* and *T. pulmonis*, respectively, by phenotypic characterization and 16S rRNA sequencing.

CASE REPORTS

Case 1. An 87-year-old Chinese woman with hypertension and hyperlipidemia presented with left eye pain and blurring of vision for 1 day in August 2007. She had a history of bilateral upper lid trichiasis and had one previous episode of right eye corneal abrasion with no infection in May 2007. Examination of the left eye revealed a 1-mm by 0.5-mm epithelial defect with infiltrates in the inferotemporal quadrant of the cornea near the limbus associated with pannus. Culture of corneal scrapings recovered a gram-positive bacillus. The patient recovered after 10 days of treatment with 0.5% levofloxacin ophthalmic drops.

Case 2. A 25-year-old Chinese man with an unremarkable past medical history presented with left eye redness and pain in August 2008. He had a long history of contact lens wear with long daily duration of wear, usually more than 16 h per day. Examination of the left eye showed 360° vascular ingrowth at the corneal periphery and a 1.5-mm abscess at the inferonasal quadrant of the cornea near the limbus. There was mild anterior chamber reaction. No hypopyon was noted. Culture of corneal scrapings recovered a gram-positive bacillus and *Serratia* species. Cultures of swabs from the patient's contact lens case and of the contact lens solution recovered *Serratia* species and *Pseudomonas* species. The patient was treated with 0.5% levofloxacin ophthalmic drops and ofloxacin ointment for 3 weeks, and 0.3% gentamicin ophthalmic drops were added after 1 week in view of the contact lens case and solution culture results. The corneal abscess and anterior chamber reaction subsequently resolved, leaving a 1.5-mm corneal scar.

Microbiological data. All clinical data were collected prospectively. Clinical specimens were collected and handled according to standard protocols (12). All suspect colonies were identified by standard conventional biochemical methods (12). Biochemical reactions in the API 20C AUX and API 50 CH systems (bioMérieux, Lyon, France) were also used for identifying the two strains of gram-positive bacilli, with *Tsukamurella pulmonis* and *Tsukamurella tyrosinosolvens* used as controls, as we previously described (24). On day 2 postincubation, a gram-positive aerobic nonsporulating bacillus was recovered from the cultures of the corneal scrapings from both patients. Both isolates were acid fast according to a modified acid-fast stain. Both isolates grew on blood agar as yellow, rough, irregular, dry, but easily emulsified, 2-mm diameter colonies after 48 h of incubation at 37°C in an aerobic environment with 5% CO_2. For comparisons with those of other *Tsukamurella* species, the major phenotypic characteristics of the two isolates are summarized in Table 1. The phenotypic characteristics of the isolate recovered from patient 1 best fit the phenotypic profile of *T. tyrosinosolvens*, whereas those of the isolate recovered from patient 2 best fit that of *T. pulmonis.*

16S rRNA gene sequencing. Bacterial DNA extraction, PCR amplification, and DNA sequencing of the 16S rRNA genes of the two isolates of gram-positive bacilli were performed according to our previous publication, using LPW3606 5′-TAC TTCGGGATAAGCCTG-3′ and LPW3607 5′-ACGACTTCG TCCCAATCG-3′ (Gibco BRL, Rockville, MD) as the PCR and sequencing primers (24). The sequences of the PCR products were compared with sequences of closely related species in GenBank by multiple sequence alignment, using Clustal_X 1.83 (21). Phylogenetic relationships were determined by using the neighbor-joining method. Sequencing of the 16S rRNA genes of the two isolates showed that there was >99% similarity between the 16S rRNA gene sequences of the two isolates and those of other *Tsukamurella* species, indicating that both isolates were *Tsukamurella* species (Fig. 1). There were 0-, 4-, 4-, 6-, and 7-base

* Corresponding author. Mailing address: Department of Microbiology, the University of Hong Kong, University Pathology Building, Queen Mary Hospital, Hong Kong. Phone: (852) 28554892. Fax: (852) 28551241. E-mail: pcywoo@hkucc.hku.hk.

▽ Published ahead of print on 15 April 2009.

TABLE 1. Phenotypic characteristics of the two clinical isolates compared to those of other clinically important *Tsukamurella* species

Phenotypic characteristics	Presence or absence in clinical isolate from case:		Presence or absence of characteristic in indicated *Tsukamurella* species[a]:				
	1	2	*T. inchonensis*	*T. paurometabola*	*T. pulmonis*	*T. strandjordii*	*T. tyrosinosolvens*
Growth at 42°C	−	−	+	−	−	−	−
Hydrolysis of							
Tyrosine	+	−	−	−	−	−	+
Xanthine	−	−	−	−	−	−	V
Assimilation of							
Maltose	+	−	+	−	−	V	+
Cellobiose	−	−	+	−	−	NA	−
D-Melezitose	+	−	+	−	−	V	+
D-Sorbitol	+	+	+	−	+	NA	+
Glycerol	−	−	V	+	V	V	V
2-Ketogluconate	+	−	V	−	−	V	+
Xylitol	−	−	V	−	V	+	V
Inositol	+	−	+	−	−	+	+
α-Methyl-D-glucoside	−	−	+	−	−	V	V
D-Arabinose	+	−	V	−	V	−	V
Ribose	−	−	V	−	−	−	V
D-Mannose	−	−	+	−	+	+	V
Mannitol	+	+	+	−	+	+	+
Arbutine	−	−	V	−	−	+	V
Salicin	−	−	V	−	−	+	V
Inulin	−	−	+	−	−	+	+
L-Fucose	+	−	V	−	+	+	+
D-Arabitol	+	+	+	−	+	+	+

[a] See references 7 and 25 to 27. +, characteristic is present; −, characteristic is absent; V, variable; NA, not available.

differences between the 16S rRNA gene sequence of isolate 1 and those of *T. tyrosinosolvens* (GenBank accession no. FJ643549), *T. pulmonis* (GenBank accession no. AY741505), *T. strandjordae* (GenBank accession no. AF283283), *T. paurometabola* (GenBank accession no. Z37151), and *T. inchonensis* (GenBank accession no. AF283281), respectively. There were 3-, 3-, 5-, 5-, and 6-base differences between the 16S rRNA gene sequence of isolate 2 and those of *T. pseudospumae* (GenBank accession no. AY333425), *T. spumae* (GenBank accession no. AY238513), *T. columbiensis* (GenBank accession no. AF272835), *T. pulmonis* (GenBank accession no. AY741505), and *T. tyrosinosolvens* (GenBank accession no. FJ643549), respectively.

Keratitis, or inflammation of the cornea, is caused by both infectious and noninfectious agents. Infective keratitis, an ophthalmologic emergency that requires prompt diagnosis and expedient treatment so as to prevent visual loss, can be caused by bacteria, viruses, fungi, or parasites. Among infectious agents, bacterial pathogens are responsible for the majority of microbial keratitis (1). Most bacterial keratitis is caused by *Staphylococcus aureus*, *Streptococcus pneumoniae*, beta-hemolytic streptococci, other gram-positive bacteria such as *Bacillus* and *Propionibacterium* species, and other gram-negative bacteria such as *P. aeruginosa*. In this study, with the help of both phenotypic tests and 16S rRNA gene sequencing, we defined the first two cases of acute keratitis associated with *Tsukamurella* species.

The symptoms of *Tsukamurella* keratitis are indistinguishable from those of keratitis caused by other bacteria. Both patients presented with severe eye pain, typical of bacterial keratitis. This is in contrast to three patients from a previous report with *Tsukamurella* conjunctivitis, which did not cause eye pain (24). Notably, in contrast to keratitis caused by other, more virulent bacteria such as *S. aureus* and *P. aeruginosa*, in which there is dense suppurative stromal inflammation and necrosis at the site of bacterial invasion, keratitis in both of our patients was presented as whitish infiltrates in the peripheral parts of the cornea near the limbus, resembling that of marginal keratitis. We speculate that this may be a result of the relatively less virulent nature of the invading *Tsukamurella* species, so that there was minimal necrosis at the site of initial bacterial invasion. Instead, the inflammatory response began near the limbal vessels from which neutrophils migrate into the cornea, leading to a change in transparency. To ascertain the importance of *Tsukamurella* species as a cause of keratitis as well as whether the inflammation of most cases of *Tsukamurella* keratitis appear near the limbus, diphtheroidal gram-positive rods recovered from corneal scrapings should be subjected to detailed phenotypic characterization and 16S rRNA gene sequencing. In fact, cases of *Tsukamurella* keratitis and conjunctivitis have probably been overlooked in the past because the diphtheroidal gram-positive rods recovered from corneal scrapings and eye swabs were either regarded as contaminants or misidentified as other bacteria, such as atypical mycobacteria (20).

As in most other cases of bacterial keratitis, microtrauma to the epithelial surface of the cornea in both patients in this study predisposed them to *Tsukamurella* keratitis. In normal

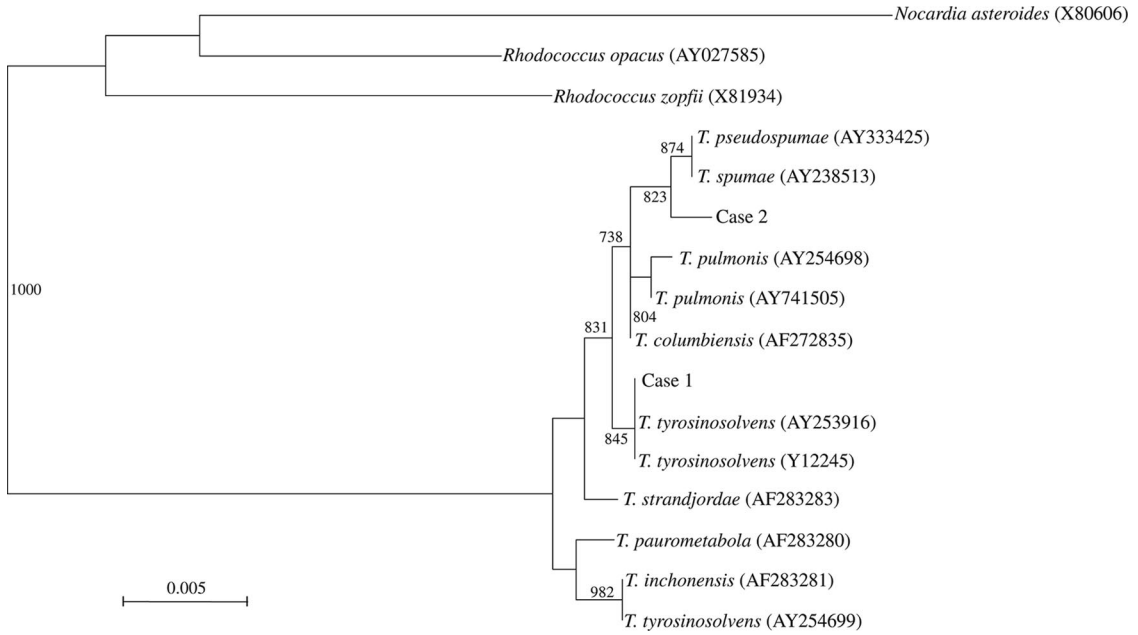

FIG. 1. Phylogenetic tree showing the relationships of the corneal-scraping isolates from our patients to related species. The tree was inferred from 16S rRNA sequence data by the neighbor-joining method, and bootstrap values were calculated from 1,000 trees. The scale bar indicates the estimated number of substitutions per 200 bases. Names and accession numbers are given as cited in the GenBank database.

individuals, the corneal epithelium and Bowman's membrane underneath prevent penetration of most infectious agents into the corneal stroma unless this natural barrier is breached by trauma. In the first patient of the present study, trichiasis caused constant rubbing of the corneal surface, resulting in an epithelial defect. In fact, she had suffered from corneal abrasion with no infection in the other eye 3 months prior to the present episode of keratitis. For the second patient, contact lens wear had led to microtrauma and an epithelial defect of the corneal surface, which was further aggravated by long hours of use, during which the contact lens can dry out and rub on the cornea (2). We speculate that the *Tsukamurella* species in the two patients first colonized their corneas. Microtrauma due to trichiasis and contact lens wear, respectively, resulted in epithelial defects which facilitated invasion by the colonized environmental *Tsukamurella* species, giving rise to keratitis.

Ophthalmologic infections due to *Tsukamurella* species are associated with *T. tyrosinosolvens* and *T. pulmonis.* Among the 10 known species of *Tsukamurella*, *T. inchonensis*, *T. paurometabola*, *T. pulmonis*, *T. strandjordii*, and *T. tyrosinosolvens* have been reported to cause infections in humans (3–11, 13–20, 22–27). Including the present two cases, five ophthalmologic infections due to *Tsukamurella* species have been described (24). All of these five cases were associated with *T. tyrosinosolvens* or *T. pulmonis* (Table 2) (24). On the other hand, of those isolated from sputum, blood, or other clinical specimens, only 16 (53%) of 30 cases with *Tsukamurella* identified to the species level were due to *T. pulmonis* or *T. tyrosinosolvens* ($P < 0.05$ by Fisher's exact test) (Table 2). We speculate that the genomes of *T. tyrosinosolvens* and *T. pulmonis* or "ophthalmologic strains" of *T. tyrosinosolvens* and *T. pulmonis* may encode adhesins for binding to unique receptors

TABLE 2. *Tsukamurella* species isolated from different clinical specimens and reported in the literature

Sources of specimens	Source or reference(s)	*Tsukamurella* species (no. of isolates)
Ophthalmologic specimens		
Eye swab	24	*T. tyrosinosolvens* (2); *T. pulmonis* (1)
Corneal scraping	Present study	*T. tyrosinosolvens* (1); *T. pulmonis* (1)
Respiratory specimens		
Sputum	9, 11, 13, 14, 16, 20, 22, 25, 26, 27	*T. paurometabola* (3); *T. pulmonis* (4); *T. tyrosinosolvens* (3)
Necrotic lung tumor	25	*T. inchonensis* (1)
Blood specimens		
Blood and catheter tip	8, 10, 17, 18	*T. paurometabola* (3); *T. pulmonis* (4); *T. strandjordii* (1); *T. tyrosinosolvens* (1)
Blood	3, 4, 6, 7, 19, 27	*T. paurometabola* (1); *T. tyrosinosolvens* (4); *T. inchonensis* (1); *T. strandjordii* (1)
Other specimens		
Cerebrospinal fluid	15	*T. paurometabola* (1)
Pus from subcutaneous abscess	23	*T. paurometabola* (1)
Skin biopsy	5	*T. paurometabola* (1)

on the conjunctival and corneal cells or, alternatively, that they may be particularly resistant to antibacterial substances in tears, such as lysozymes, lipocalin, and lactoferrin, leading to the unique susceptibility of the eye to these two species or to "ophthalmologic strains" of these two species.

Nucleotide sequence accession numbers. The 16S rRNA gene sequences of the two isolates of gram-positive bacilli recovered from the three patients have been lodged within the GenBank sequence database under accession numbers FJ755301 and FJ755300.

This work was partly supported by the University Development Fund and the Committee for Research and Conference grant, the University of Hong Kong.

REFERENCES

1. **Asbell, P., and S. Stenson.** 1982. Ulcerative keratitis. Survey of 30 years' laboratory experience. Arch. Ophthalmol. **100:**77–80.
2. **Bourcier, T., F. Thomas, V. Borderie, C. Chaumeil, and L. Laroche.** 2003. Bacterial keratitis: predisposing factors, clinical and microbiological review of 300 cases. Br. J. Ophthalmol. **87:**834–838.
3. **Chong, Y., K. Lee, C. Y. Chon, M. J. Kim, O. H. Kwon, and H. J. Lee.** 1997. *Tsukamurella inchonensis* bacteremia in a patient who ingested hydrochloric acid. Clin. Infect. Dis. **24:**1267–1268.
4. **Elshibly, S., J. Doherty, J. Xu, R. B. McClurg, P. J. Rooney, B. C. Millar, H. Shah, T. C. Morris, H. D. Alexander, and J. E. Moore.** 2005. Central line-related bacteraemia due to *Tsukamurella tyrosinosolvens* in a haematology patient. Ulster Med. J. **74:**43–46.
5. **Granel, F., A. Lozniewski, A. Barbaud, C. Lion, M. Dailloux, M. Weber, and J. L. Schmutz.** 1996. Cutaneous infection caused by *Tsukamurella paurometabolum*. Clin. Infect. Dis. **23:**839–840.
6. **Jones, R. S., T. Fekete, A. L. Truant, and V. Satishchandran.** 1994. Persistent bacteremia due to *Tsukamurella paurometabolum* in a patient undergoing hemodialysis: case report and review. Clin. Infect. Dis. **18:**830–832.
7. **Kattar, M. M., B. T. Cookson, L. D. Carlson, S. K. Stiglich, M. A. Schwartz, T. T. Nguyen, R. Daza, C. K. Wallis, S. L. Yarfitz, and M. B. Coyle.** 2001. *Tsukamurella strandjordae* sp. nov., a proposed new species causing sepsis. J. Clin. Microbiol. **39:**1467–1476.
8. **Lai, K. K.** 1993. A cancer patient with central venous catheter-related sepsis caused by *Tsukamurella paurometabolum* (*Gordona aurantiaca*). Clin. Infect. Dis. **17:**285–287.
9. **Maalouf, R., S. B. Mierau, T. A. Moore, and A. Kaul.** 2009. First case report of community-acquired pneumonia due to *Tsukamurella pulmonis*. Ann. Intern. Med. **150:**147–148.
10. **Maertens, J., P. Wattiau, J. Verhaegen, M. Boogaerts, L. Verbist, and G. Wauters.** 1998. Catheter-related bacteremia due to *Tsukamurella pulmonis*. Clin. Microbiol. Infect. **4:**51–53.
11. **Matsumoto, T., M. Shiraishi, H. Yoshimura, K. Sogen, T. Harada, C. Yoshimura, R. Aramaki, F. Yamamoto, T. Kuraki, and K. Watanabe.** 2006. *Tsukamurella tyrosinosolvens* cultured from sputum of a patient who received total gastrectomy for gastric cancer. Kekkaku **81:**487–490. (In Japanese.)
12. **Murray, P. R., E. J. Baron, J. H. Jorgensen, M. R. Louise, and M. A. Pfaller (ed.).** 2007. *Nocardia*, *Rhodococcus*, *Gordonia*, *Actinomadura*, *Streptomyces*, and other aerobic actinomycetes, p. 515–542. *In* Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, DC.
13. **Osoagbaka, O. U.** 1989. Evidence for the pathogenic role of *Rhodococcus* species in pulmonary diseases. J. Appl. Bacteriol. **66:**497–506.
14. **Perez, V. A., J. Swigris, and S. J. Ruoss.** 2008. Coexistence of primary adenocarcinoma of the lung and *Tsukamurella* infection: a case report and review of the literature. J. Med. Case Rep. **2:**207.
15. **Prinz, G., E. Bán, S. Fekete, and Z. Szabó.** 1985. Meningitis caused by *Gordona aurantiaca* (*Rhodococcus aurantiacus*). J. Clin. Microbiol. **22:**472–474.
16. **Rey, D., D. De Briel, R. Heller, P. Fraisse, M. Partisani, M. Leiva-Mena, and J. M. Lang.** 1995. *Tsukamurella* and HIV infection. AIDS **9:**1379.
17. **Schwartz, M. A., S. R. Tabet, A. C. Collier, C. K. Wallis, L. C. Carlson, T. T. Nguyen, M. M. Kattar, and M. B. Coyle.** 2002. Central venous catheter-related bacteremia due to *Tsukamurella* species in the immunocompromised host: a case series and review of the literature. Clin. Infect. Dis. **35:**e72–e77.
18. **Shapiro, C. L., R. F. Haft, N. M. Gantz, G. V. Doern, J. C. Christenson, R. O'Brien, J. C. Overall, B. A. Brown, and R. J. Wallace, Jr.** 1992. *Tsukamurella paurometabolum*: a novel pathogen causing catheter-related bacteremia in patients with cancer. Clin. Infect. Dis. **14:**200–203.
19. **Sheridan, E. A., S. Warwick, A. Chan, M. Dall'Antonia, M. Koliou, and A. Sefton.** 2003. *Tsukamurella tyrosinosolvens* intravascular catheter infection identified using 16S ribosomal DNA sequencing. Clin. Infect. Dis. **36:**e69–e70.
20. **Stanley, T., L. Crothers, M. McCalmont, J. Xu, B. C. Millar, C. E. Goldsmith, and J. E. Moore.** 2006. The potential misidentification of *Tsukamurella pulmonis* as an atypical *Mycobacterium* species: a cautionary tale. J. Med. Microbiol. **55:**475–478.
21. **Thompson, J. D., T. J. Gibson, F. Plewniak, F. Jeanmougin, and D. G. Higgins.** 1997. The CLUSTAL_X Windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. **25:**4876–4882.
22. **Tsukamura, M., and K. Kawakami.** 1982. Lung infection caused by *Gordona aurantiaca* (*Rhodococcus aurantiacus*). J. Clin. Microbiol. **16:**604–607.
23. **Tsukamura, M., K. Hikosaka, K. Nishimura, and S. Hara.** 1988. Severe progressive subcutaneous abscesses and necrotizing tenosynovitis caused by *Rhodococcus aurantiacus*. J. Clin. Microbiol. **26:**201–205.
24. **Woo, P. C., A. H. Ngan, S. K. Lau, and K. Y. Yuen.** 2003. *Tsukamurella* conjunctivitis: a novel clinical syndrome. J. Clin. Microbiol. **41:**3368–3371.
25. **Yassin, A. F., F. A. Rainey, H. Brzezinka, J. Burghardt, H. J. Lee, and K. P. Schaal.** 1995. *Tsukamurella inchonensis* sp. nov. Int. J. Syst. Bacteriol. **45:** 522–527.
26. **Yassin, A. F., F. A. Rainey, H. Brzezinka, J. Burghardt, M. Rifai, P. Seifert, K. Feldmann, and K. P. Schaal.** 1996. *Tsukamurella pulmonis* sp. nov. Int. J. Syst. Bacteriol. **46:**429–436.
27. **Yassin, A. F., F. A. Rainey, J. Burghardt, H. Brzezinka, S. Schmitt, P. Seifert, O. Zimmermann, H. Mauch, D. Gierth, I. Lux, and K. P. Schaal.** 1997. *Tsukamurella tyrosinosolvens* sp. nov. Int. J. Syst. Bacteriol. **47:**607–614.