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CASE REPORT

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Tsukamurella pulmonis conjunctivitis in patients with an underlying nasolacrimal duct obstruction – report of two cases

Peter Kechker[1], Yigal Senderovich[1], Shifra Ken-Dror[1], Sivan Laviad-Shitrit[2] and Malka Halpern[2,3,*]

Abstract

Tsukamurella pulmonis (*Actinobacteria*), a Gram-positive, obligate aerobic and weakly or variably acid-fast bacterium, is an opportunistic pathogen. Here we report two cases of conjunctivitis caused by *T. pulmonis*. Both patients had a previous history of nasolacrimal duct obstruction (NLDO). Isolation of *T. pulmonis* was performed on chocolate, tryptic soy blood and Columbia nalidixic agars. After 24 h of incubation, odourless, white-greyish, membrane-like colonies were observed. The VITEK-2 bacterial identifier system failed to identify the species, while Vitek-MS matrix-assisted laser desorption ionization time-of-flight technology, successfully identified the isolate from case 2 but not from case 1. Final identification was verified using 16S rRNA gene sequencing. An antibiogram was performed and according to the results cefazoline in addition to vancomycin eye drops for 5 days, were suggested as a treatment in case 1. In case 2 the infection was ended without treatment. This is the first report of *Tsukamurella* as a pathogen that causes conjunctivitis in patients with NLDO.

INTRODUCTION

Species of the genus *Tsukamurella* are Gram-positive, weakly or variably acid-fast, non-motile, rod-shaped, obligate aerobic *Actinomycetes* [1]. The phylogenetic identification of this genus has had a complex history. The first member of this genus was isolated by Steinhaus [2] from the mycetoma and ovaries of bedbugs (*Cimex lectularius*) and mistakenly identified as a *Corynebacterium paurometabolum*. Tsukamura and Mizuno identified a similar species as *Gordonia aurantiaca* [3]. Goodfellow and Kumar [4] showed that *Tsukamurella* is closely related to *Mycobacterium* and *Nocardia*, but does not belong to these genera [5]. Finally, based on 16S rRNA gene sequence analyses, Collins *et al.* [6] reclassified the isolates that were mentioned above within a new genus, *Tsukamurella*. The reason for the complexity of the phylogenetic classification history of *Tsukamurella* stems from the fact that traditional phenotypic methods and commercial kits that allow identification of most commonly encountered bacterial species in clinical microbiology laboratories, often fail to differentiate *Tsukamurella* from related genera of the order *Corynebacteriales*, such as *Nocardia*, *Rhodococcus* and *Gordonia* [7, 8]. The increasing availability of PCR amplification, and sequencing of universal gene targets in clinical laboratories, has enabled unambiguous identification results, especially in cases where bacterial isolates could not be identified by phenotypic tests [9].

Matrix-assisted laser desorption ionization time-of-flight mass-spectrometry (MALDI-TOF MS) has emerged in recent years as a revolutionary technique for the identification of bacterial and fungal pathogens, yielding rapid, accurate and highly reproducible results [10–12]. Nonetheless, the application of MALDI-TOF MS technology for the identification of *Tsukamurella* species, using the original device databases, has not been fully explored to date [13, 14].

At the time of writing, the genus *Tsukamurella* comprises 16 species with validly published names. Among them, 11 are known to be associated with human infections [15]. However, *Tsukamurella* infections are not routine, and can be regarded as a kind of nosocomial and sporadic infection [5]. The most common infections are indwelling device-related infections, for example due to infected catheters [16] or a knee prosthesis [17]. However, the spectra of *Tsukamurella* infections comprise pulmonary and cutaneous infections, bacteraemia, meningitis, peritonitis, brain abscess, acute otitis media,

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Author affiliations: [1]W. Hirsch Regional Microbiology Laboratory, Clalit Health Services, Haifa, Israel; [2]Department of Evolutionary and Environmental Biology, Faculty of Natural Sciences, University of Haifa, Haifa, Israel; [3]Department of Biology and Environment, Faculty of Natural Sciences, University of Haifa, Oranim, Tivon, Israel.

***Correspondence:** Malka Halpern, mhalpern@research.haifa.ac.il

Keywords: opportunistic pathogen; *Tsukamurella pulmonis*; acid-fast; conjunctivitis; eye infection; nasolacrimal duct obstruction.

Abbreviations: AST, antibiogram susceptibility testing; BMD, broth microdilution; MALDI-TOF, matrix-assisted laser desorption ionization time-of-flight; NLDO, nasolacrimal duct obstruction.

Accession numbers of the isolates: MT032343 and MT032344.

keratitis and conjunctivitis [5]. In ophthalmic infections, prolonged use of contact lenses may constitute the indwelling device risk factor [18].

Here we report two cases of *Tsukamurella*-related conjunctivitis in patients who had a previous history of nasolacrimal duct obstruction (NLDO), and were identified in January 2018 and May 2019 (case 1 and 2, respectively) in our institute, the Clalit Health Services of Haifa and Western Galilee district, Israel. This is the first report of *Tsukamurella* as a pathogen that causes conjunctivitis in patients with NLDO.

CASE 1

A patient over the age of 70 years with a history of bilateral NLDO, wet age-related macular degeneration, diabetes mellitus with diabetic nephropathy, chronic heart failure and ischaemic heart disease presented with a history of prolonged bilateral conjunctivitis and mucopurulent discharge. Initial empirical treatment with chloramphenicol ointment provided no significant improvement, and thus ofloxacin eye drops were also added. This treatment again provided no significant improvement, and thus ciprofloxacin was continued. After only minimal improvement, neomycin-dexamethasone in addition to gentamicin were prescribed. Again, this treatment provided no significant improvement, and thus an eye swab (Copan) was used to sample the exudate, and was sent to our laboratory.

At 24 h after the initial incubation time, odourless, white-greyish, membrane-like colonies were noticed as an absolute bacterial culture on chocolate agar, tryptic soy blood agar, and Columbia nalidixic agar (Hylabs) (Fig. 1a). Six days after inoculation, the colony morphology changed drastically to a dry-yellow crumble texture (Fig. 1b). The suspected bacterium failed to be identified by means of the VITEK-2 bacterial identifier system (bioMérieux) and by using Vitek-MS MALDI-TOF technology (bioMérieux), and therefore was suspected as a rapidly growing *Actinobacteria*. This bacterium was subjected to 16S rRNA gene sequencing in accordance with Senderovich *et al.* [19] and was finally identified as *Tsukamurella pulmonis*. The *T. pulmonis* 16S rRNA gene sequence was submitted to GenBank with the accession number MT032343 (Fig. 2).

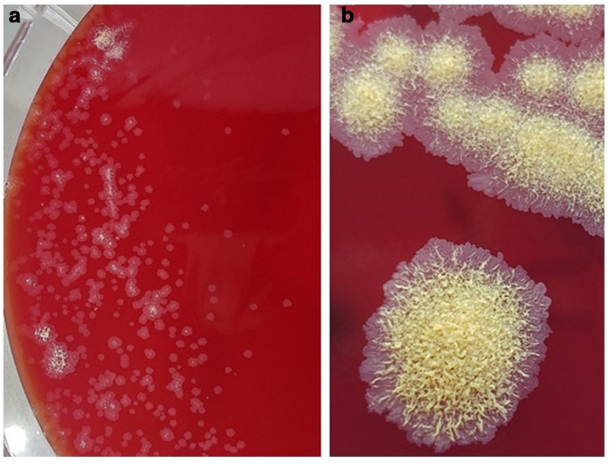

Fig. 1. Colonial appearance of *Tsukamurella pulmonis* on a Columbia nalidixic agar plate (a) 24 h and (b) 6 days after inoculation.

The isolate underwent complete antibiogram susceptibility testing (AST) by the E-test technique (bioMérieux) with interpretation according to the *Corynebacterium* spp. CLSI guidelines [Clinical and Laboratory Standards Institute (CLSI), M45, 2015] (Table 1). According to the antibiogram results, all medications were stopped and cefazoline in addition to vancomycin eye drops for 5 days, were started. On follow-up, the conjunctivitis had resolved, and the patient became symptom-free.

CASE 2

An infant under 1 year old with NLDO presented with right eye conjunctivitis and mucopurulent discharge. A diagnosis of bacterial conjunctivitis was made, and dexamethasone–neomycin–polymyxin eye drops treatment was started empirically. On follow-up, the patient displayed minimal improvement, and thus an eye swab (Copan) was used to sample the exudate and was sent to our laboratory.

At 24 h after initial incubation, a similar absolute bacterial culture, as seen in case 1, was noticed on the same agars as mentioned above. This time, the suspected bacteria were successfully identified as *Tsukamurella* species by using Vitek-MS MALDI-TOF technology. This bacterium was also subjected to 16S rRNA gene sequencing, as described in case 1. The 16S rRNA gene sequence (accession number MT032344), showed a high sequence identity to *T. pulmonis* DSM 44142^{T} (Fig. 2).

The isolate in the current case underwent complete AST as described in case 1 (Table 1). By the time the identification of the bacteria and the AST were completed, the conjunctivitis of the infant had resolved without any additional antibiotic treatment.

DISCUSSION

Tsukamurella species are environmental saprophytes and can be found in different environmental aquatic and terrestrial sources (soil, water, sludge and foam) [4, 20–27]. The type species, *Tsukamurella paurometabola,* was first isolated from mycetoma and ovaries of bed bugs [6]. *Tsukamurella* species can cause a variety of infections in the human body: meningitis, peritonitis, cutaneous infections, lung infections, cardioverter-defibrillator infections, knee prosthesis infections, ocular infections and device-related infections, such as catheter-related bacteraemia [16, 17, 28–32].

The most common manifestations of ocular infections due to *Tsukamurella* are conjunctivitis and keratitis. Eleven cases of culture-positive *Tsukamurella* ocular infection were identified retrospectively from 2005 to 2018 by Leung *et al.* [33]. Of

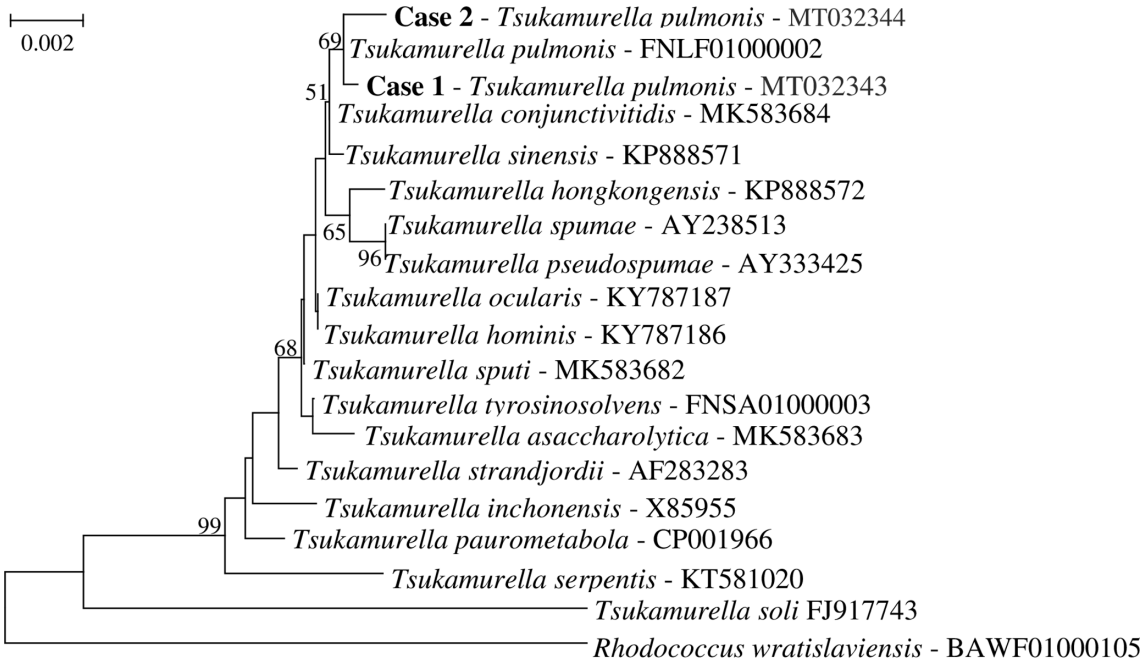

Fig. 2. Phylogenetic tree showing the relationship of the isolates from case 1 and case 2 with other *Tsukamurella* species. Sequence alignment was performed using the CLUSTAL W program, and the tree was generated using the neighbour-joining method with Kimura two-parameter distances in MEGA 4.1 software. Bootstrap values (from 1000 replicates) greater than 50% are shown at branch points. Bar, 0.2% sequence divergence.

these, six cases (54.5%) resulted in conjunctivitis, two (18%) in keratitis, and one of each kind (9%) resulted in blepharitis, canaliculitis, and postenucleation ocular implant-related infection [33]. In the two cases reported in the present article, *Tsukamurella* caused the most prevalent kind of ocular infection – conjunctivitis.

The most common isolates of ocular infections due to *Tsukamurella* belong to *T. tyrosinosolvens* and *T. pulmonis* [8, 18, 34–36]. Rarely, *T. spumae* has also been isolated from patients with keratitis [8]. In the current case report, *T. pulmonis* was identified from two conjunctivitis cases. Table 2 summarizes medical cases where *T. pulmonis* was reported as the causative agent of conjunctivitis infection in humans.

Table 1. MICs (μg ml^{-1}) of *Tsukamurella pulmonis* strains

Antibiotic	Case 1	Case 2
Ceftriaxone	2.0	12.0
Meropenem	2.0	0.38
Gentamycin	12.0	4.0
Ciprofloxacin	0.38	0.38
Tetracycline	0.5	12.0
Clarithromycin	0.75	2.0
Vancomycin	4.0	4.0

Tsukamurella-related infections are rare and sporadic, and thus they can be misidentified or misdiagnosed because of the difficulty with their isolation and identification. Thus, it is vitally important for medical laboratories to recognize the unique colony morphology of *Tsukamurella* (Fig. 1). In addition, differential colony morphological diagnosis of *Gordonia* species, *Williamsia* species, and rapid growing *Mycobacteria* (e.g. *M. abscessus*, *M. chelonae*, and, *M. fortuitum*) should be performed using MS [9] and 16S rRNA gene sequencing.

Broth microdilution (BMD) is the gold standard technique of AST for *Tsukamurella* species. Nevertheless, BMD is not available in most medical laboratories, and hence, the acceptable method of *Tsukamurella* species AST is the E-test technique. However, currently there are no available E-test MIC criteria for *Tsukamurella* species, and the majority of AST information is obtained from case reports. Several antibiotic combinations have been proposed for the treatment of *Tsukamurella*-related infections, such as the combination of β-lactams and aminoglycosides.

To date there is no information about any specific virulence factor for *Tsukamurella* species, but it was previously speculated that the genomes of *T. tyrosinosolvens* and *T. pulmonis* may encode adhesins for binding to unique receptors 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 [18].

Table 2. *Tsukamurella pulmonis* conjunctivitis infection in humans

Age (years)	Sex	Background	Antibiotic eye drop	Oral antibiotics	Duration of treatment	Outcome	Reference
>70	M	Nasolacrimal duct obstruction (NLDO), diabetes mellitus with diabetic nephropathy, chronic heart failure and ischaemic heart disease	Cefazoline and vancomycin	None	1 week	Resolved	Current study
0.5	M	NLDO	Dexamethasone–neomycin–polymyxin (given before species identification)	None	1 week	Resolved	Current study
50	F	Ocular implant infection after enucleation	Fusidic acid and gentamicin	Clarithromycin; doxycycline	22 weeks	Ocular implant removal	[33]
75	M	Ocular cicatricial pemphigoid; bullous pemphigoid; hypertension	Chloramphenicol and fusidic acid	Doxycycline	2 weeks	Resolved	[33]
44	F	–	Chloramphenicol	None	1 week	Resolved	[33]
50	F	Blepharoconjunctivitis, non-insulin-dependent diabetes mellitus, hypertension and systemic lupus erythematosus	Gentamicin	None	2 weeks	Resolved	[34]
81	F	Posterior blepharitis, hypertension, non-insulin-dependent diabetes mellitus and successful cataract extraction in the right eye	Tobramycin and dexamethasone (Tobradex)	None	2 weeks	Resolved	[34]
69	F	Hypertension and bronchogenic carcinoma with right upper lobectomy	Polymyxin B–neomycin	None	10 days	Resolved	[36]

Tsukamurella is an opportunistic pathogen and, in many cases, its infection occurs in the presence of background diseases or risk factors. For example, although *T. serpentis* was isolated from the oral cavity of two venomous snakes (*Naja atra*) in China, there is no report of infection by this species in healthy humans after being bitten by the snake [37]. The background diseases and risk factors that increase the risk of *Tsukamurella* infection can be immunodeficiency, underlying malignancy, organ transplant, systemic lupus erythematosus, diabetes [36], and prolonged contact lens wear [18]. The patient described in case 1 had underlying diabetes mellitus and chronic heart failure. In case 2, the infant was treated with dexamethasone–neomycin–polymyxin eye drops before approaching the diagnostic laboratory. The conjunctivitis infection of this infant resolved while the species causing the infection was identified and without any further antibiotic treatment, probably due to the initial treatment and because he was otherwise a healthy baby with a normal functioning immune system.

Both patients in the present study had NLDO. In NLDO the nasolacrimal duct, which is supposed to carry tears from the lacrimal sac of the eyes into the nasal cavity, is obstructed. When the flow of tears, which should clean the eyes and remove various potential pathogens from the eyes, is obstructed, this can lead to eye infections. Furthermore, obstruction of the nasolacrimal duct leads to excessive tearing and ocular discharge. Irritation and rubbing produced by dripping of tears and discharge due to inadequate drainage can cause erythema of the periorbital skin, and upper and lower eyelids, leading to conjunctivitis [38].

In conclusion, here we report two cases of conjunctivitis caused by *T. pulmonis* in patients who had a previous history of NLDO. In case 1, the patient was treated with cefazoline in addition to vancomycin eye drops and the treatment eliminated the eye infection. In case 2, the conjunctivitis of the infant was resolved without antibiotic treatment. *Tsukamurella* is an opportunistic pathogen and can exploit the pathological condition of NLDO, and cause an eye infection, such as conjunctivitis. This is the first report of *Tsukamurella* as a pathogen that causes conjunctivitis in patients with NLDO.

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Author contributions
P.K., Y.S., S.L.S., identified the strains; P.K., Y.S., performed the antibiogram tests; P.K., Y.S., wrote the manuscript, S.K.D., M.H., reviewed the manuscript; M.H., S.L.S., edited the manuscript; S.K.D., M.H., contributed reagents/materials/publication fees.

Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
This study was carried out in accordance with the recommendations of the guidelines of the Helsinki Committee and was approved by the Helsinki Committee of Clalit Health Services, Israel (approval no. 0192-15-COM1). Consent to publish has been obtained.

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