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Case Report

Mycobacterium aurum keratitis: an unusual etiology of a sight-threatening infection

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ABSTRACT

Atypical fast-growing *Mycobacterium* species are usually identified after laser-assisted in situ keratomileusis, cosmetic surgeries, and catheter-related, pulmonary or soft tissue infections. We herein present the case of a 56-year-old man with purulent discharge, redness, and foreign body sensation in his left eye. He underwent two surgeries that partially controlled the infection but were not curative. Corneal transplantation was performed, and a biopsy of the excised cornea indicated *Mycobacterium aurum* infection, which was confirmed by polymerase chain reaction-restriction fragment length polymorphism analysis. This appears to be the first documented case of keratitis attributable to the non-tuberculous mycobacteria *M. aurum*. The intractable extra-ocular progression of the disease in the absence of general signs or symptoms was notable. We suggest considering non-tuberculous mycobacteria among the probable causes of complicated keratitis or keratitis that does not respond to drug treatment, especially in regions where tuberculosis is endemic.

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Introduction

Ocular tuberculosis (TB) is a complex clinical entity that presents diagnostic and management challenges for both ophthalmologists and infectious disease specialists, as well as for public health workers. Depending on its clinical manifestations, ocular TB can involve intraocular or extraocular tissues and structures. Intraocular TB is more common than the extraocular form,¹ and its diagnosis is more complicated.² Interstitial keratitis, which indicates the spread of infection to the corneal stroma, is one of the miscellaneous presentations

of extraocular TB. Scleritis (necrotizing or non-necrotizing) can also occur simultaneously.³

Mycobacterium aurum is a Gram-positive, acid-fast bacterium which is fast-growing and mostly non-infectious.⁴ It belongs to the atypical category of mycobacteria that do not cause TB or Hansen's disease. This species, also called non-tuberculous mycobacteria (NTM), is also known as environmental mycobacteria or mycobacteria other than tuberculosis-causing. In contrast to typical TB infections, this atypical mycobacterial infection is non-communicable. The species is present in the environment, especially in wet soils, rivers, lakes, and

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swamps. Rapidly growing mycobacteria other than *M. aurum* include *Mycobacterium fortuitum*, *Mycobacterium chelonae*, *Mycobacterium abscessus* and *Mycobacterium neoaurum*. Except for *M. aurum*, which is usually commensal, other species are pathogenic in humans, and can cause cellulitis, osteomyelitis, wound infections, and central catheter-related infections.^{5,6}

Non-tuberculous mycobacterial keratitis was first reported by Turner and Stinson in 1965.⁷ Since then, the number of cases recorded has increased. In 2002, there was an outbreak of *M. chelonae* keratitis associated with laser-assisted *in situ* keratomileusis. Further research showed that this type of surgery could be a risk factor for the development of NTM keratitis.⁸ Despite the increase in the number of documented cases since the first description in 1965, NTM keratitis continues to pose challenges in terms of diagnosis and management.

To the best of our knowledge, this is the first documented case of keratitis caused by the NTM *M. aurum*. The bacterium was responsible for keratitis as well as the troublesomely silent extra-ocular progression of the disease in the absence of general signs or symptoms.

The Institutional Review Board and the Ethics Committee of Shiraz University of Medical Sciences approved the decision to report this novel case. The patient also gave his written informed consent.

Case presentation

The patient was a 56-year-old man who presented with a chief complaint of left eye redness, foreign body sensation and tearing. Based on an initial diagnosis of viral conjunctivitis, a topical corticosteroid was prescribed. One month later, the patient returned with aggravated symptoms and purulent discharge, eyelid swelling, photophobia, and corneal infiltration. He was diagnosed as having secondary bacterial keratitis and was treated with moxifloxacin and sulfacetamide. In the course of follow-up, during the subsequent two months, the cornea did not heal and the patient developed progressively blurred vision.

At this time a confocal scan was negative for fungal or acanthamoeba infection. Superficial keratectomy was done for diagnostic and therapeutic purposes. The pathology and microbiology reports were negative. The complete preoperative laboratory work-up included complete blood cell count, fasting blood sugar, blood urea nitrogen, serum creatinine, sodium, potassium, phosphate, and calcium, liver function tests, lipid profiles, urinalysis, and stool analysis for occult blood, ova, and parasites. All results were normal except for an elevated lymphocyte count (45.8% whole blood cells, equivalent to 4000/ μ L) slightly elevated uric acid (8.9 mg/dL), and low-density lipoprotein (133 mg/dL). Blood cultures were performed with blood samples from three different sites, but no organisms grew. Plain chest X-ray revealed no abnormal findings.

Because of worsening infective keratitis and invasion of deeper layers of the cornea (Fig. 1A), a conjunctival flap procedure was done. However, after one month the flap became necrotic and retracted, and was therefore debrided. A second corneal biopsy was obtained, and this time the pathology report indicated mycobacterial keratitis with positive acid-fast staining. As a result of this diagnosis, topical and oral moxifloxacin and azithromycin were started. However, because the infection progressed and early corneal melting appeared, we decided to perform corneal collagen cross-linking (CXL) with riboflavin and ultraviolet light. Curative CXL was done with two major goals: to stop and resolve the infective process, and to slow corneal melting. After 1-2 weeks we observed partial resolution of the infection. However, due to progression of the residual infection and dense opacification of the cornea, we decided to perform corneal graft transplantation with the penetrating keratoplasty method.

A final biopsy of the excised cornea was sent to the national TB reference center in Tehran, Iran for polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) assay, which confirmed *M. aurum* as the causal agent. Throughout the course of the infection, the patient was afebrile and physical examination was normal. At the time of writing, the patient is well (Fig. 1B) with satisfactory slit lamp findings, and a single-eye visual acuity of 20/400.



Fig. 1 - Slit lamp findings. (A) One month after initial presentation, worsening infective keratitis and invasion of deeper layers of the cornea were accompanied by dense infiltration. (B) After corneal transplantation, the clear corneal graft was accompanied by a nuclear cataract due to chronic inflammation.

Identification of non-tuberculosis mycobacteria by PCR-RFLP

The mycobacterial species was identified by molecular methods. DNA was extracted from the eye biopsy sample with a standard method.⁹ Briefly, 1 mL of decontaminated sample was heat-killed at 90°C for 30 min. The sample was lysed in digestion buffer (500 mM Tris-HCl, pH 9.0, 20 mM EDTA, 10 mM NaCl, 1% SDS). The DNA was purified by phenol and chloroform/isoamyl alcohol (24:1 vol/vol) and was precipitated by isopropanol. The pellet was washed in 70% ethanol and dried at room temperature. It was then resuspended in TE buffer (50 mM Tris, 100 mM EDTA, pH 8.0) and stored at 4°C.

A nested PCR method was used to amplify a 440-bp fragment of the 65-kDa heat shock protein gene. The first PCR reactions (50 µL) contained target DNA (10 ng), 16 pmol of the primers tb15 (5'-CGT AYG ACG AAG AGG CCCGT-3') and tb17 (5'-WAS GGR TCC TCS AGG ACSGC-3'), 0.2 mM dNTP (Fermantase), 2.5 U Taq DNA polymerase (Cinagene), 2 mM MgCl₂, and 5 µL 10× buffer. The reaction was subjected to amplification for 5 min at 95°C for initial denaturation, 20 s at 95°C, 1 min at 60°C, 40 s at 72°C for 30 cycles, followed by 5 min of extension at 72°C.

For the second round of PCR, the reaction mixture (50 µL) contained 2 µL of the target PCR product, 16 pmol of the primers Tb11 (5-ACC AAC GAT GGT GTG TCC AT) and Tb12 (5-CTT GTC GAA CCG CAT ACC CT), 0.2 mM dNTP (Fermantase), 2.5 U Taq DNA polymerase (Cinagene), 2 mM MgCl₂, and 5 µL 10× buffer. After 5 min at 95°C for initial denaturation, the reaction was subjected to 40 cycles of amplification for 30 s at 94°C, 1 min at 56°C, 40 s at 72°C, followed by 5 min of extension at 72°C. The PCR products were then run on 2% agarose gels made up in 0.5× Tris-Borate-EDTA buffer with ethidium bromide, and examined for the presence of the bands of interest.

The amplified fragment was digested by two endonuclease enzymes (*Hae*III and *Bst*EII). For *Bst*EII digestion, 10 µL of the PCR product were added directly to a mixture containing 1 µL of enzyme, 2 µL of restriction buffer (5× buffer B), and 18 µL water, and the mixture is incubated for 16 h at 37°C. Similarly, 10 µL of the product was digested at 37°C in a solution containing *Hae*III enzyme, the corresponding buffer (5× buffer R), and water.¹⁰

To evaluate the restriction patterns, after digestion, 5 µL of gel loading buffer (0.25% bromophenol blue, 40% sucrose in water) were added to 15 µL of the digested samples. The mixture was loaded onto a 10% acrylamide-bisacrylamide gel. Fragments were visualized by ethidium bromide staining under ultraviolet light. The patterns found after digestion with *Hae*III were four bands of 135, 115, 70, and 60 bp. The digestion product pattern with *Bst*EII was a single 440-bp band. Comparison of the patterns with standard strains from the Pasteur Institute of France (<http://app.chuv.ch/prasite/index.html>) confirmed that the strain isolated was *M. aurum* type 2 (Fig. 2).

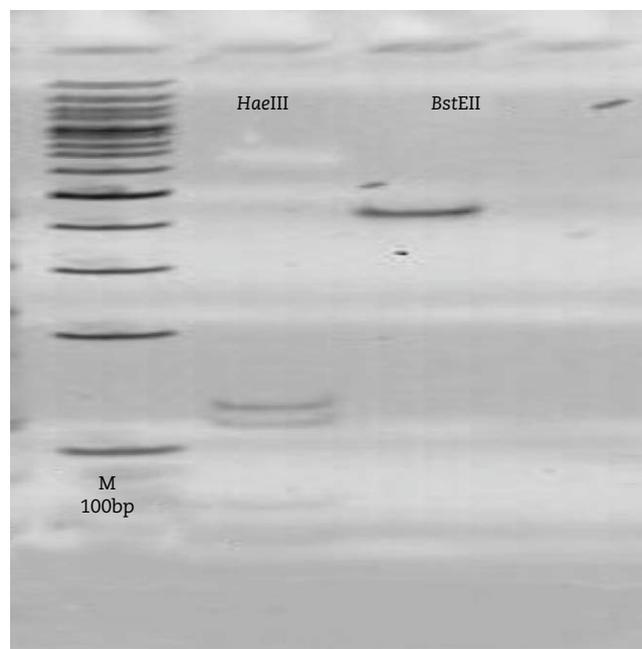


Fig. 2 - The result of PCR restriction enzyme analysis for the 65-kDa heat shock protein gene.

Lane 1: 100-bp marker (M). Lane 2: Digestion product with *Hae*III: four bands of 135, 115, 70 and 60 bp.

Lane 3: Digestion product with *Bst*EII: a single 440-bp band was seen. Comparison of the bands with standard strains from the Pasteur Institute of France (<http://app.chuv.ch/prasite/index.html>) confirmed that the strain isolated from our patients was *Mycobacterium aurum* type 2.

Discussion

Mycobacterium aurum was first described by Tsukamura in 1996.¹¹ Since then, only three cases of infection by this type of *Mycobacterium* have been published. The first one (1998) was a 60-year-old man being treated for T-cell non-Hodgkin's lymphoma. He presented with fever, chills and weight loss of three weeks' duration, and catheter-related bacteraemia by *M. aurum* was found to be responsible.¹² The second one (2003) was a 5-year-old boy with metastatic Wilms' tumor of the right kidney, who was admitted with a 1-day history of fever, preceded by a 27-week course of chemotherapy. After a prolonged search, the source of infection was found to be a Broviac catheter.⁵ The third case (2008) reported to date was a 25-year-old man with idiopathic bilateral uveitis treated with intravenous corticosteroids and infliximab. A few days after the treatment started, he developed fever and weakness. In the two previous cases, *M. aurum* was found in blood cultures, but in the last case blood cultures were negative and *M. aurum* was detected by solid-organ lung biopsy.¹³

The bacterium can be identified via two methods: biochemical analysis and molecular-based methods. The first, which is conventionally used, depends on the diagnosis and isolation of mycobacteria and subsequent phenotypic or whole-cell fatty acid analysis. Molecular-based analysis is the gold standard method, particularly in unidentified cases.¹⁴ Gene sequencing is the best molecular-based method, which in some cases leads to reclassification of previously diagnosed *M. aurum* to other mycobacterial species.^{15,16} But PRA (PCR-RFLP Analysis) method is also one of the most reliable techniques, especially for identification of mycobacterial species, and its efficacy has been well-established and well-proven.¹⁷⁻²¹ We used this method by means of 65-kDa heat shock protein on two samples and both results definitively confirmed the presence of *M. aurum*.

In comparison to previous cases, our patient's general condition was good and no signs or symptoms were detected, even on chest X-ray. He was not immunocompromised in any way, but was well and had no underlying disease (including diabetes or malignancy). He was not taking immunosuppressants, and did not have an arterial or venous catheter. The only factor that may have made our patient prone to this infection was occupational exposure, since the air at his workplace contained large amounts of dust and soil particles in suspension. The patient also mentioned a minor trauma to his eye.

Infection by *M. aurum* is a potential threat in the management of critical cases of ocular TB even in patients with no predisposing factors. Early diagnosis and prompt treatment may be sight-saving. We suggest considering NTM among the probable causes of complicated keratitis, and as a possible cause in patients who fail to respond to drug treatment, especially in regions where TB is endemic.^{22,23} We also recommend additional laboratory tests when the type of *Mycobacterium* remains unidentified.

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Conflict of interest

All authors declare to have no conflict of interest.

REFERENCES

- Alvarez GG, Roth VR, Hodge W. Ocular tuberculosis: diagnostic and treatment challenges. *Int J Infect Dis.* 2009;13(4):432-435.
- Bodaghi B, LeHoang P. Ocular tuberculosis. *Curr Opin Ophthalmol.* 2000;11(6):443-438.
- Chang CH, Sheu MM, Wang HZ. Intractable nontuberculous mycobacterial keratitis successfully treated by penetrating keratoplasty-3 cases report. *Gaoxiong Yi Xue Ke Xue Za Zhi.* 1994;10(2):106-14.
- Gupta A, Bhakta S, Kundu S, et al. Fast-growing, non-infectious and intracellularly surviving drug-resistant *Mycobacterium aurum*: a model for high-throughput antituberculosis drug screening. *J Antimicrob Chemother.* 2009;64(4):774-781.
- Koranyi KI, Ranalli MA. *Mycobacterium aurum* bacteremia in an immunocompromised child. *Pediatr Infect Dis J.* 2003;22(12):1108-1109.
- Wallace RJ Jr. Nontuberculous mycobacteria and water: a love affair with increasing clinical importance. *Infect Dis Clin North Am.* 1987;1:677-686.
- Turner L, Stinson I. *Mycobacterium fortuitum* as a cause of corneal ulcer. *Am J Ophthalmol.* 1965;60:329-331.
- Winthrop KL, Steinberg EB, Holmes G, et al. Epidemic and sporadic cases of nontuberculous mycobacterial keratitis associated with laser in situ keratomileusis. *Am J Ophthalmol.* 2003;135(2):223-224.
- Van Soolingen D, Hermans PWM, Van Embden JDA. Analysis of mycobacteria. National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands. 1995.
- Brunello F, Ligozzi M, Cristelli E, et al. Identification of 54 mycobacterial species by PCR-restriction fragment length polymorphism analysis of the hsp65 gene. *J Clin Microb.* 2001;39:2799-2806.
- Tsukamura M, Tsukamura S. *Mycobacterium aurum*, a new species. *Igaku To Seibutsugaku.* 1966;72:270-273.
- Esteban J, Fernandez-Roblas R, Roman A, et al. Catheter-related bacteremia due to *Mycobacterium aurum* in an immunocompromised host. *Clin Infect Dis.* 1998;26(2):496-497.
- Martin-Aspas A, Guerrero-Sanchez F, Garcia-Martos P, et al. Bilateral pneumonia by *Mycobacterium aurum* in a patient receiving infliximab therapy. *J Infect.* 2008;57(2):167-169.
- Bramante CT, Talbot EA, Rathinam SR, et al. Diagnosis of ocular tuberculosis: a role for new testing modalities? *Int Ophthalmol Clin.* 2007;47(3):45-62.
- Simmon KE, Low YY, Brown-Elliott BA, et al. Phylogenetic analysis of *Mycobacterium aurum* and *Mycobacterium neoaurum* with redescription of *M. aurum* culture collection strains. *Int J Syst Evol Microbiol.* 2009;59(Pt 6):1371-1375.
- Brown-Elliott BA, Wallace RJ Jr., Petti CA, et al. *Mycobacterium neoaurum* and *Mycobacterium bacteremicum* sp. nov. as causes of mycobacteremia. *J Clin Microbiol.* 2010;48(12):4377-4385.
- da Costa AR, Lopes ML, Furlaneto IP, et al. Molecular identification of nontuberculous mycobacteria isolates in a Brazilian mycobacteria reference laboratory. *Diagn Microbiol Infect Dis.* 2010;68 (4):390-394.
- da Silva Rocha A, Werneck Barreto AM, Dias Campos CE, et al. Novel allelic variants of mycobacteria isolated in Brazil as determined by PCR-restriction enzyme analysis of hsp65. *J Clin Microbiol.* 2002;40(11):4191-4196.

19. Martin A, Uwizeye C, Fissette K, et al. Application of the hsp65 PRA method for the rapid identification of mycobacteria isolated from clinical samples in Belgium. *J Microbiol Methods*. 2007;71(1):39-43.
20. McNabb A, Eisler D, Adie K, et al. Assessment of partial sequencing of the 65-kilodalton heat shock protein gene (hsp65) for routine identification of *Mycobacterium* species isolated from clinical sources. *J Clin Microbiol*. 2004;42(7):3000-3011.
21. Chimara E, Ferrazoli L, Ueky SY, et al. Reliable identification of mycobacterial species by PCR-restriction enzyme analysis (PRA)-hsp65 in a reference laboratory and elaboration of a sequence-based extended algorithm of PRA-hsp65 patterns. *BMC Microbiol*. 2008;8:48.
22. Shenai S, Rodrigues C, Mehta A. Time to identify and define non-tuberculous mycobacteria in a tuberculosis-endemic region. *Int J Tuberc Lung Dis*. 2010;14(8):1001-1008.
23. Ryoo SW, Shin S, Shim MS, et al. Spread of nontuberculous mycobacteria from 1993 to 2006 in Koreans. *J Clin Lab Anal*. 2008;22(6):415-420.