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SHORT REPORT

Mycobacterium chelonae Keratitis Following Laser *in situ* Keratomileusis (LASIK) Specifically Identified by INNO-LIPA Method

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The incidence of corneal infection after laser *in situ* keratomileusis (LASIK) is appoximately 1 in 5.000 . In recent years, several unusual corneal infections have been diagnosed with increasing frequency. The keratitis caused by the non-tuberculous Mycobacterium (i.e, atypical *Mycobacterium*) is chacterized by two species: *Mycobacterium chelonae* and *Mycobacterium fortuitum* . These two species are a facultative pathogen saprophytic in the environment and may cause severe opportunistic infection in humans, including lung diseases, cutaneous abscesses, cellulitis, soft tissue infectious, as well as postoperative wound infections, and prosthetic valve endocarditis (1). Ocular infections caused by atypical mycobacteriae include keratitis, endophthalmitis, orbital granuloma, lacrimal drainage system infection and scleral abscesses (1,2). The keratitis caused by this organism occurs in association with foreign body injury , contact lens wear, or after corneal surgical procedures, including penetrating keratoplasty, radial keratotomy, suture removal, extracapsular cataract with intraocular lens implantation and posterior capsulotomy (1,3).The exact mechanism of pathogenesis of mycobacterial infections is not well understood (1). Initial inoculation of the organisms appears to result from contaminated foreign bodies or surgical instruments (1). Nontuberculous mycobacterial keratitis may be difficult to differentiate at diagnosis (4).

We report a case of keratitis after LASIK, caused by *Mycobacterium chelonae* identified by conventional methods and INNO-LIPA and discuss the difficulty and delay in identifying the organism, erroneously identified as *Nocardia* species on morphological grounds.

Case

A thirty year old female patient underwent LASIK and material obtained from the infected cornea after operation grew bacteriae in blood agar media. Our laboratory was referred to for differentation between atypical mycobacteria and *Nocardia*. Bacteriae suspension prepared from colonies were respectively stained with Gram staining and Erlich-Ziehl- Neelsen (EZN) staining and incubated in Lowenstein-Jensen (LJ) and Mycobacterium Indicator Growth Tube (MGIT) media. In Gram staining, gram positive philamentous bacilli similar to *Nocardia* were seen whereas in EZN staining acid fast bacilli with atypical appearance were seen. (Figure 1 and 2). In blood agar medium growth was observed on the second day of the incubation and MGIT medium on the 5th day of the incubation (Figure 3). From bacteria suspension, incubation was made to Mc Conkey medium, nitrate reduction and niasin tests were carried out for identification (Figure 4) The result of nitrate reduction and niasin test were found to be

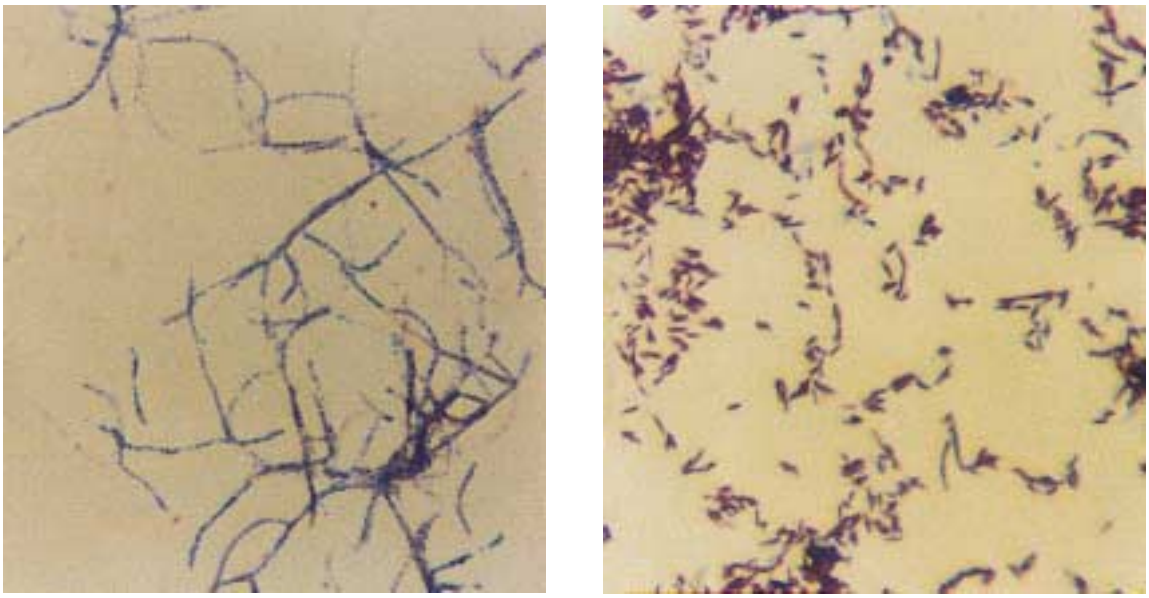

Figure 1 and 2. Colonies growing in blood base agar stained with Gram staining (on left) and EZN staining (on wright).

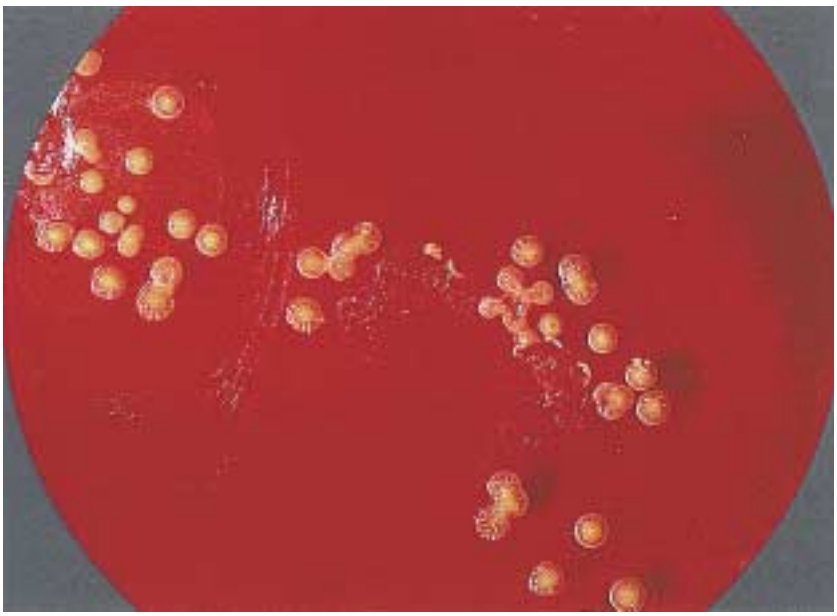

Figure 3. The appearance of colonies in blood base agar.

negative. The INNO-LIPA test was administered for specific molecular identification. After this test, it was established that the causative agent was definitely mycobacteria and that it showed a band pattern consistent with *M. chelonea* complex (group III, *M. abscessus*) (Figure 5). As to the Lowentein-Jensen medium, growth was observed only at the fourth week of incubation.

The appearance of colonies in blood base agar was shown in Figure 3.The appearance of colonies in Lowenstein-jensen and Mc Conkey media was shown in Figure 4.

Method of INNO-LIPA: The INNO-LIPA assay was performed according to the manufacturer's instructions supplied by the manufacturer (Inno-LIPA Mycobacteria; Innogenetics, Belgium).

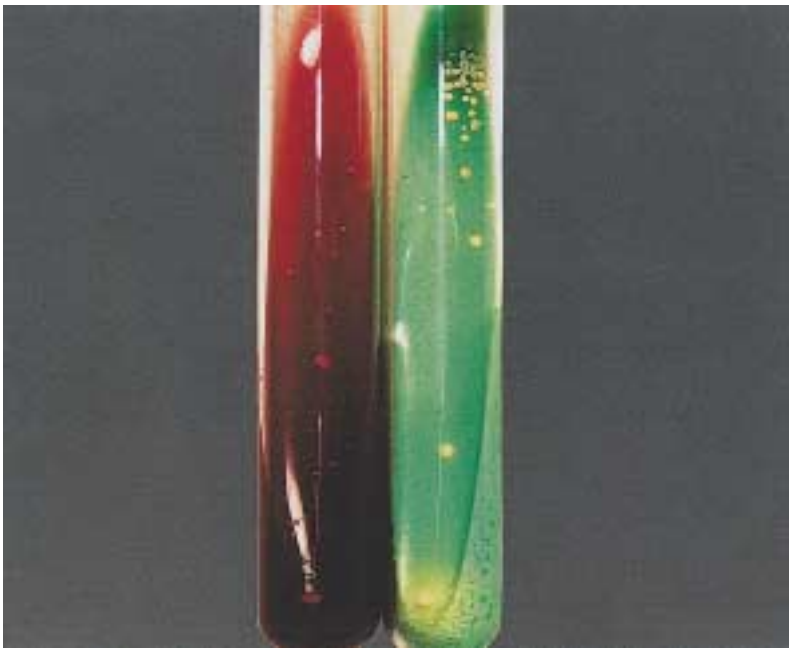

Figure 4. The appearance of colonies in Lowenstein-Jensen and Mc Conkey media.

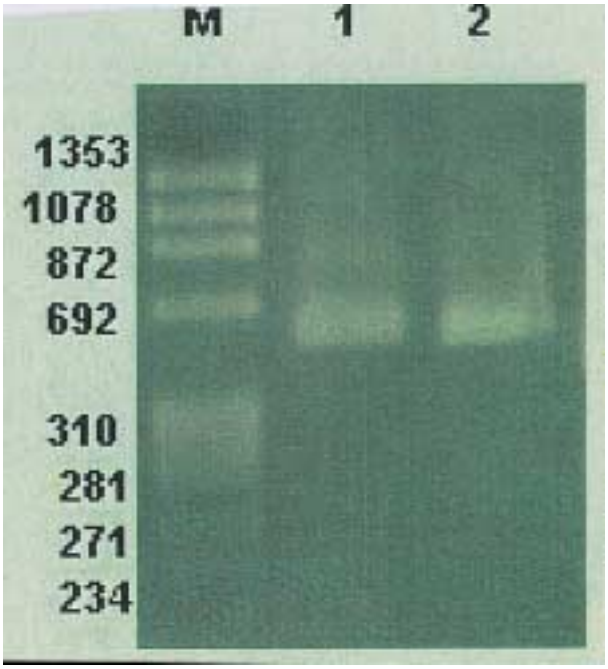

Figure 5. Band pattern consistent with *Mycobacterium chelonea* complex was determined by INNO-LIPA. An 400-550 bp amplification product determined by INNO-LIPA v2.
M: Marker (ϕX 174 Hae III), 1:Positive control, 2:Positive sample

This method is based on the reverse hybridization principle, in which the mycobacterial 16S-23S ribosomal RNA (rRNA) spacer region is amplified by polymerase chain reaction (PCR). Amplicons are subsequently hybridized with oligonucleotide probes arranged on a membrane strip and detected by a colorimetric system. The test detects the presence of *Mycobacterium* species and specifically identifies *Mycobacterium tuberculosis* complex, *Mycobacterium kansasii*, *Mycobacterium xenopi*, *Mycobacterium gordonae*, *Mycobacterium avium* complex, *Mycobacterium avium*, *Mycobacterium intracellulare*, *Mycobacterium scrofulaceum*, and *Mycobacterium chelonae - Mycobacterium abscessus* complex (5,6).

The results of LIPA were compared with the results of biochemical typing tests (nitrat reduction and niasin test). The band pattern of *M.abcessus* sub group *chelonae* determined by INNO-LIPA shown in Figure 5.

Mycobacterium fortuitum and *M.chelonae* are the two most common causes of nontubercolous mycobacterial keratitis and they may be difficult to differentiate at diagnosis (4). *Mycobacterium chelonae* is widespread in the environment. It is an acid-fast bacillus that has been classified by Runyon as group IV or rapid growing mycobacterium on the basis of its culture characteristics

(1). *Mycobacterium chelonae* is difficult to distinguish from *Nocardia* species. Encountered infrequently, this organism can be incorrectly identified as other bacteriae, including diphtheroids and *Nocardia* species. Identification and sensitivity testing are mandatory in determining appropriate treatment (7). *Mycobacterium chelonae* exist in soil, water, and the environment in a wide range of temperatures and humidities. They also colonize in body fluids such as sputum , gastric contents and skin surface of healthy individuals (1). *Mycobacterium chelonae* is an opportunistic pathogen and most patients with this infection have had predisposing injury, including surgery (1-4).

It may cause a variety of infections including lung disease, cellulitis, soft tissue abscesses, lymphoadenitis, keratitis, and scleral abscess. *Mycobacterium chelonae* is closely related to *M.fortuitum*, another agent that causes keratitis. *Mycobacterium fortuitum* can be distinguished from *M.chelonae* on the basis of positive iron uptake and nitrate reduction test (1).

Typing of atypical mycobacteria by a biochemical test (conventional methods) is difficult and takes a long time (5,6). The exact mechanism of pathogenesis is not well understood. Initial inoculation appears to result from contaminated foreign bodies or surgical instruments. In most reported cases, there was a latent period of 2 to 8 weeks after corneal insult before the keratitis was detected. Clinically, atypical mycobacterial keratitis is often characterized by a chronic, indolent course. Medical therapy is often ineffective because delayed diagnosis, slow response to therapy, inadequete drug penetration, resistance to most conventional antibiotics, and the emergence of a resistant strain, especially with single agent therapy (1-4, 8).

Rapid recognition of the causative organism and aggressive medical and surgical management of the infection is neccessary for improving clinical outcome (9).

Conventional methods are inadequate for typing of mycobacteriae and definite identification is not always possible. The INNO-LIPA test is a rapid and reliable test which can be used in the diagnosis and specific identification of mycobacteriae (5,6).

Chen et al.(10) reported a keratitis case due to *Mycobacterium chelonae* subsp abcessus. In this case rapid identification of mycobacteria to the species level was made by polymerase chain reaction and restriction enzyme analysis.

Pache et al. (11) reported a case of unilateral fungal and mycobacterial keratitis after LASIK. They emphasized the possible difficulties in diagnosing and treating both *Mycobacterium chelonae* and fungi. Malecha et al.(3) reported that *Mycobacterium chelonae* keratitis was associated with soft contact lens wear. *Mycobacterium chelonae* was cultured on Sabourard's agar. Khooshabeh et al. (12) reported a case of keratitis due to *Mycobacterium chelonae*, in a soft contact-lens wearer and discussed the difficulty and delay in identifying the organism, twice erroneously identified as *Nocardia asteroides* on morphological grounds. In our case, *Mycobacterium chelonae* keratitis was diagnosed by Gram and Ziehl-Nielsen staining, culture, biochemical test and molecular test with INNO-LIPA. *Mycobacterium chelonae* was cultured on blood base agar in 2 days and 4 weeks on Lowenstein-Jensen. In our case, *Mycobacterium chelonae* was identified by a biochemical test (i.e, nitrate reduction and niasin test), growth on Mc Conkey agar and by a molecular test (i.e, INNO-LIPA).

Nontuberculous mycobacteria such as *Mycobacterium chelonae* and *Mycobacterium fortuitum* should be considered as etiological agents in cases of infectious keratitis occuring after LASIK .Early diagnosis of causative agents is necessary for the carly institution of appropriate antibiotics, prevention of morbidity and improvement of the clinical outcome in atypical mycobacterial keratitis.

It is our conclusion that INNO-LIPA is a rapid , practical and reliable method that can be used the early diagnosis and specific identifition of mycobacterial infections such as our case.

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