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CASE REPORT

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Successful treatment of Keratitis caused by *Mycobacterium chelonae* and an overview of previous cases in Europe

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Abstract

Introduction and purpose *Mycobacterium* (*M.*) *chelonae* is responsible for a half of relatively rare nontuberculous mycobacteria (NTM) keratitis. We report a case of *M. chelonae* keratitis in a woman following sclerocorneal suture extraction after cataract surgery.

Results A 70-year-old woman presented with a red eye and corneal infiltration of her left eye six weeks following sclerocorneal suture extraction after an elective cataract surgery in another institute. She complained of a sharp, cutting pain and photophobia. Since initial corneal scrapes and conjunctival swabs proved no pathogen using culture and PCR methods, non-specific antibiotics and antifungal agents were administered. As keratitis was complicated by an inflammation in the anterior chamber and vitreous, samples of the vitreous fluid were sent for microbiologic examination. DNA of Epstein-Barr virus (EBV) was repeatedly detected. Since the intrastromal abscess had formed, corneal re-scrapings were performed and *M. chelonae* was detected using culture, MALDI-TOF MS and PCR methods. Therapy was changed to a combination of oral and topical clarithromycin, intravitreal, topical and intracameral amikacin, and oral and topical moxifloxacin. The successful therapy led to stabilization. The optical penetrating keratoplasty was performed and no signs of the infection recurrence were found.

Conclusions The diagnosis of nontuberculous mycobacterial keratitis is difficult and often delayed. An aggressive and prolonged antimicrobial therapy should include systemic and topical antibiotics. Surgical intervention in the form of corneal transplantation may be required in the active and nonresponsive infection. In the presented case this was necessary for visual rehabilitation due to scarring.

Keywords Mycobacterium chelonae, Keratitis, Antibiotic therapy, keratoplasty

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Introduction

Mycobacterium (*M.*) *chelonae* is a nontuberculous mycobacterium, which belongs among rapidly growing, nonpigmented species and, therefore, it is ranged in class IV in the Runyon classification [1, 2]. Optimal growth conditions of *M. chelonae* include 28–32 °C on most types of solid media in less than 7 days [2, 3]. *M. chelonae* occurs ubiquitously in the environment including soil and salt or fresh water and similarly to other nontuberculous mycobacteria (NTM) is relatively resistant to chlorine, organomercurials and other common disinfectants [4]. This species was first isolated by Freidmann from a sea turtle in 1903 (chelone means turtle in Greek). Contaminated water is considered as the main source of infection. Generally, immunocompromised patients including those after solid organ transplantation and those suffering from autoimmune disorders treated with corticosteroids or immunotherapy are more predisposed to *M. chelonae* infections. Similarly, the course of infections is more severe in these patients and frequently include hematogenous dissemination of the disease. However, infections in immunocompetent individuals have been more frequently reported in recent decades, mainly following surgical procedures and, thus, most probably due to an insufficient sterilization of used surgical instruments [2, 5]. *M. chelonae* is mostly present in skin and soft-tissue infections mainly of the extremities [1, 6]. *M. chelonae* infections are also associated with cosmetic surgery and tattooing [7, 8]. Osteomyelitis caused by *M. chelonae* belongs among rare but difficult to treat infections [9]. *M. chelonae* can be less commonly in bloodstream infections, catheter-related infections, or post-surgical infections. The first case of *M. chelonae* keratitis was described in 1978 by Gangadharam et al. [10]. *M. chelonae* is recognised as a cause of dacryocystitis, canaliculitis, conjunctivitis, scleritis, endophthalmitis and keratitis. The nontuberculous mycobacteria are relatively rare cause of ocular infections, accounting M. chelonae, *M. abscessus*, and *M. fortuitum* as the most common [4]. The eye is the second most common site of infection caused by *M. chelonae* in its portfolio and the cornea in particular and *M. chelonae* is responsible for half of all NTM keratitis [11–13]. Patients with interface keratitis caused by NTM including *M. chelonae* typically present with a decreased visual acuity and corneal infiltrates [13]. Infectious keratitis was also repeatedly, but still rarely described after laser in situ keratomileus (LASIK), where NTM including *M. chelonae* are leading pathogens [14–16].

Generally, treatment of NTM infections and especially those caused by rapidly growing *M. abscessus* group including *M. chelonae* is challenging due to a lack of evidence based on specific treatments, which are derived from susceptibility testing alongside with emerging resistance to the first-line antituberculosis agents. Overall there are few effective antimicrobials available for the therapy [6]. Yet, the crucial step in the successful treatment of *M. chelonae* infections is to correctly distinguish *M. chelonae* from *M. abscessus*, hence consultation with microbiologist prior of treatment is mandatory. Although the antibacterial profile of both species is not favourable, *M. chelonae* is generally more susceptible to antibiotics and the infections are potentially easier to treat. The susceptibility mostly remains preserved to macrolides, since until recently there was no documentation on presence of inducible erythromycin ribosomal methylase *erm*41 gene, which can facilitate inducible resistance to macrolides [6, 17]. On the other hand, resistance rates of *M. abscessus* are between 0 and 38% depending on the subspecies level identification and region of origin [18]. Nevertheless, clarithromycin is still the most reliable antibiotic having in vitro activity against *M. abscessus* group including *M. chelonae* with less than 10% resistant strains, but susceptibility testing is required [19].

Other drugs with low level resistance below 10% are aminoglycosides including amikacin, tobramycin or arbekacin. Some studies reported tobramycin was likely more active than amikacin or prefer arbekacin when amikacin and tobramycin are less susceptible [6, 19, 20]. However, in study by Akram et al. 2022 higher resistance of 50% to amikacin was reported. Linezolid susceptibility is also favourable with less than 20% resistance detected. Among others, fluoroquinolones, namely ciprofloxacin and moxifloxacin may be also used when the susceptibility is determined, but the resistance levels are high, between 75 and 80%. A typical feature of *M. chelonae* among other NTM is a resistance to cefoxitin reaching 90–100%. In these cases, imipenem with resistance levels of 20–40% may be used instead [1, 19].

However, the optimal therapy for *M. chelonae* infections is not precisely known. In addition, the correlation among the results of in vitro susceptibility testing and the clinical outcomes is suboptimal [21]. As *M. chelonae* may exhibit an unpredictable susceptibility profile, an antibiotic testing should be performed for each strain [2]. Recently, a case of successful treatment of a patient with disseminated cutaneous *M. chelonae* infection was reported. The approach comprised of surgical debridement, combination therapy with antibiotics and a single bacteriophage strain treatment. In that case, the surgical debridement and antibiotic therapy presented insufficient results and led to the initiation of bacteriophage therapy, which resulted in an improvement of skin lesions. Biopsies obtained at 2 and 5 months after bacteriophage treatment remained culture negative [5].

Despite advances in molecular biology, accurate species identification of *M. chelonae-M. abscessus* complex remains a challenge for diagnostic laboratories [22]. These two species are phenotypically very similar and

share almost identical 16 S rRNA sequences. Effective methods for discrimination between *M. chelonae* and *M. abscessus* and other mycobacterial species are sequencing of RNA Polymerase B (*rpo*B) gene or *hsp*65 [17, 23, 24].

Keratitis caused by *M. chelonae* is a very rare disease in Europe. According to the survey included, only 10 published cases have been reported in the last 25 years.

Case presentation

A 70-year-old woman presented with a red eye and corneal infiltration of her left eye. She complained of sharp, cutting pain and photophobia six weeks after sclerocorneal suture extraction. She underwent an elective cataract surgery in July 2018 at another clinic. There was no other ocular disease history until these procedures. She was an immunocompetent patient chronically treated for arterial hypertension and ischemic heart disease. Uncorrected visual acuity on her left eye was 0.32 (decimal value). A slit lamp examination revealed a localized nodular hypertrophy of upper perilimbal conjunctiva with significant hyperemia and whitish corneal infiltration spreading centrally. A mild anterior chamber inflammation was detected. Iris was slightly hyperemic without posterior synechiae, and the artificial intraocular lens was in the bag (Fig. 1). Fundus examination did not show any pathologies. An anterior and posterior segment of her right eye were normal.

In November 2018, the patient was admitted to the Department of Ophthalmology. Corneal swabs and conjunctival swabs were performed and sent to the Microbiology department for culture. A standard 4-day incubation under aerobic condition revealed only *Staphylococcus epidermidis* in corneal swab in negligible quantity, which was evaluated as a probable contamination from the skin during the sampling. The conjunctival swab culture provided a negative result. Treatment was commenced with topical and systemic antibiotics as follows - Tobradex (tobramycin + dexamethasone) drops hourly, Floxal (ofloxacin) drops five times a day, Tobrex (tobramycin) ointment at bedtime, and ciprofloxacin 500 mg tbl per os b.i.d. for 7 days. The effect of therapy was temporarily satisfactory, and patient was discharged to out-patient care. Unfortunately, gradual progression of keratitis occurred, and further investigation was initiated. In January 2019, a biopsy of hypertrophic bulbar conjunctiva showed only reparative inflammatory changes

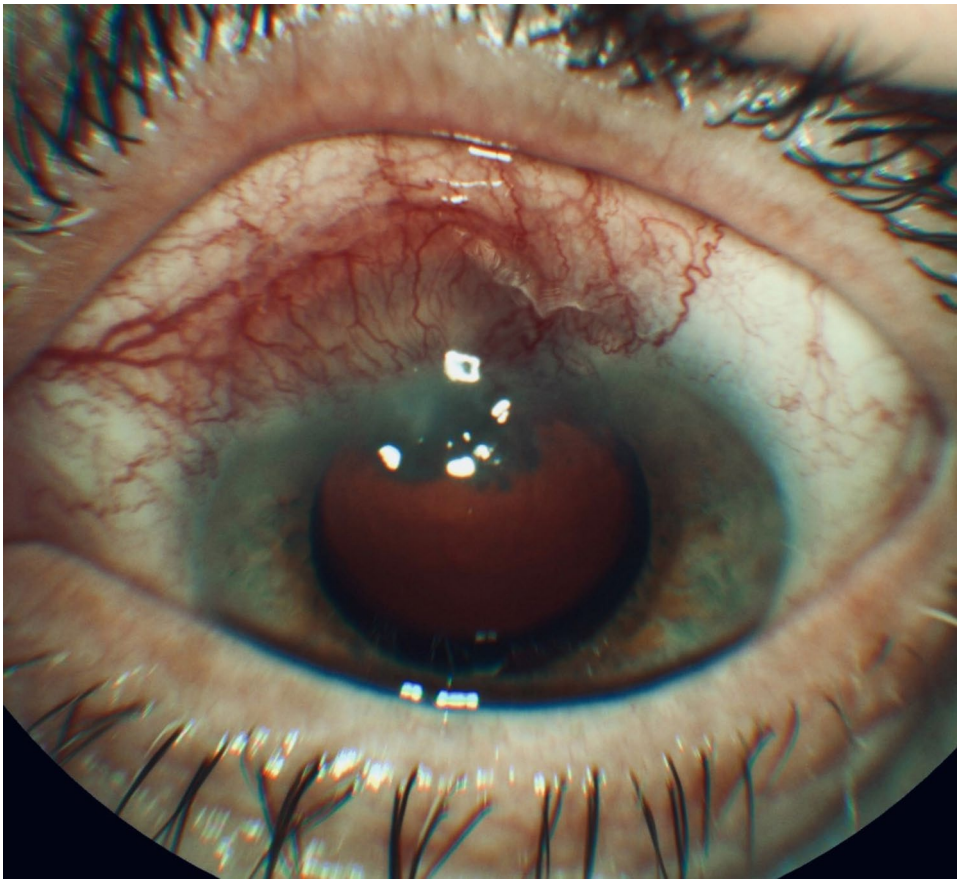

Fig. 1 Localized nodular hypertrophy of the upper perilimbal conjunctiva with a significant hyperemia and whitish corneal infiltration spreading centrally. Iris was slightly hyperemic without posterior synechiae, and an artificial intraocular lens was in the bag

and neither neoplasia nor mycotic agents were confirmed. Nevertheless, a mycotic infection was suspected, but a corneal swab sent for culturing for 7 days on Sabouraud agar was negative. The therapy was changed to Maxitrol drops (neomycin-sulphate, polymyxin-b-sulphate, dexamethasone). Because of the deterioration of corneal findings, a scraping for PCR detection of fungal DNA was obtained, again with negative results. Topical therapy was changed, where previously instilled Maxitrol drops were discontinued and only Floxal drops, and ointment were applied. Due to a nonhealing corneal ulcer, an amniotic membrane was transplanted in combined graft and patch manner. In April, another corneal scrape was sent to the laboratory for molecular identification of the broad spectrum of pathogens, including herpetic viruses (CMV, EBV, HSV, VZV), *Mycobacterium tuberculosis* complex, NTM, *Aspergillus* spp., universal fungal DNA, Mucormycetes, *Cryptococcus neoformans* and *Candida albicans* without further success. In May, an anterior chamber reaction along with hypopyon and vitreous inflammation was detected (Fig. 2).

At that time, the patient was treated with a combination of Maxitrol and Floxal drops. Simultaneously, oral prednisolone at 60 mg daily dosage had been administered for 5 days with rapid withdrawal. Samples of vitreous and aqueous humour were collected via pars plana vitrectomy. They were sent for the microbiological examination, as well as for pathology and flow cytometry to rule out the masquerade syndrome. A repeated one-week long culture again revealed negative results except for ocular swab, where *St. warneri* and *St. epidermidis* grew in insignificant quantities as a contaminating microbiota. The vitreous fluid was also examined for the DNA of herpetic viruses, bacteria, mycobacteria and fungi. Surprisingly, the DNA of EBV was repeatedly detected in vitreous fluid in the quantity of 1660 copies/mL using qPCR. Nevertheless, low levels of viremia (6680 copies/mL) supported a theory of a reactivation of latent infection rather than EBV being the causative agent. Serological markers of EBV also confirmed the EBV reactivation, since the increased levels of IgG against the viral capsid antigen (VCA) of 60.39 (index value) and IgG against the EBNA antigen of 6.98 were detected, while the IgM antibodies against these antigens were negative (Abbott Diagnostics, Wiesbaden, Germany and ELISA VIDITEST, VIDIA-DIAGNOSTIKA, CZ, respectively).

Since the strong suspicion of mycotic aetiology remained and the effect of previous medication was poor, topical voriconazole drops (VFEND 1%) were initiated hourly. After that, a temporary improvement was observed during the following month. However, in July 2019 corneal findings were progressively deteriorating, until an intrastromal abscess formed (Fig. 3).

Corneal re-scrapings (from the surface and abscess) for culture were collected by a brush and amnion membrane transplantation was performed again. On day 5 of cultivation, tiny, rough, white to grey colonies were seen on blood agar from both samples in significant quantity. The

Fig. 2 Keratitis complicated by an anterior chamber exudate (hypopyon) and vitreous inflammation

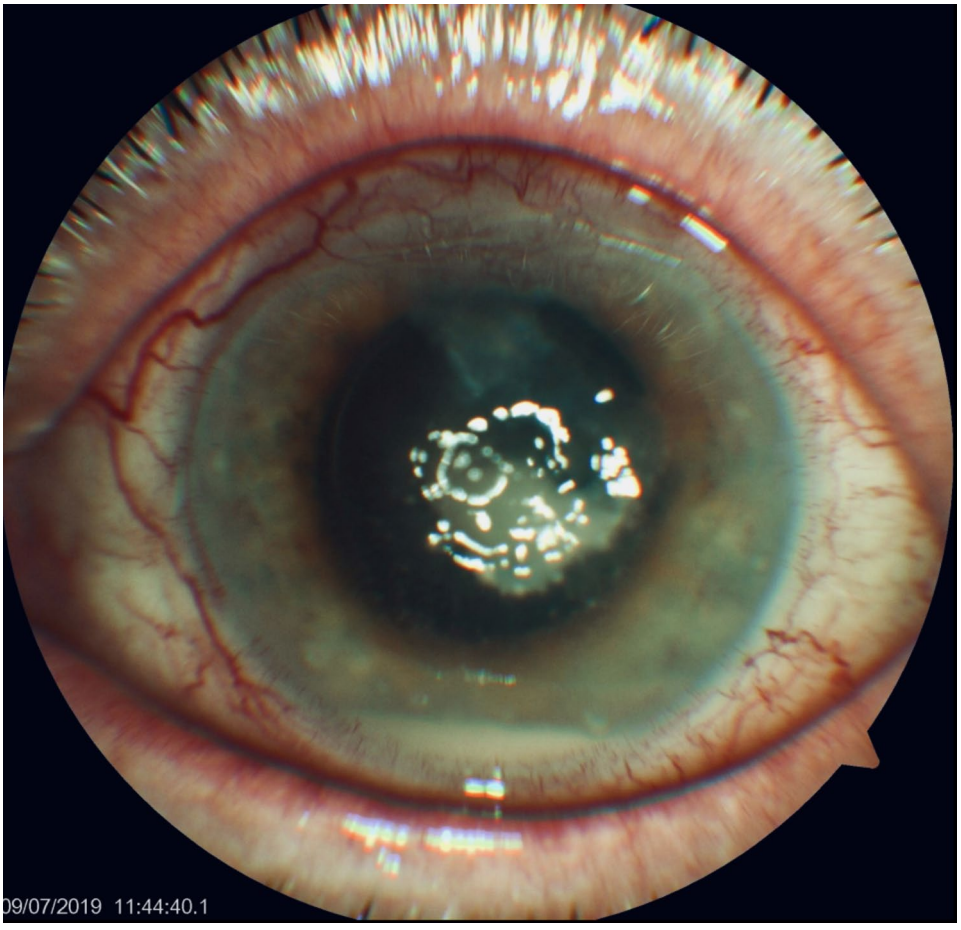

Fig. 3 The progressive deterioration of corneal findings and formation of an intrastromal abscess

Table 1 Susceptibility of *Mycobacterium chelonae* isolated from a corneal scrape to antibiotics and their potential mode of application

ATB	MIC (mg/L)	Interpretation	Routes of empiric therapy[1]	Routes of targeted therapy[2]
Clarithromycin	0.023	S	PO + AT	PO + AT
Amikacin	0.5	S	AT + AI	AT + AI + AC
Moxifloxacin	1	S	N/A	PO + AT
Ciprofloxacin	2	I	N/A	N/A
Tetracycline	0.25	S	N/A	N/A
Imipenem	1	S	IV	N/A
Linezolid	2	S	N/A	N/A

[1] Therapy adjusted after the identification of *Mycobacterium chelonae*

[2] Therapy adjusted after the completion of antibiotic susceptibility of *Mycobacterium chelonae*

AI – intravitreal, AT – topical, AC - intracameral

identification using the MALDI-TOF MS showed *M. chelonae*, but with low and unreliable identification score of 1.435. The Ziehl-Neelsen stain was carried out showing longer acid-fast rods. The isolate was therefore subjected to the *Mycobacterium* species DNA confirmation using the PCR method with a positive result for DNA of NTM (Anyplex MTB/NTM Assay, Seegene, Seoul, Korea). The identification of NTM involved PCR amplification and sequencing of *rpo*B gene using capillary electrophoresis (BI 3500, Genetic Analyzer, Applied Biosystems/Thermo Fisher Scientific, Foster City, CA, USA). The nucleotide sequence analysis showed the best BLAST identification score for *M. chelonae*. E-test susceptibility tests were performed for the MIC determination for clarithromycin, amikacin, moxifloxacin, ciprofloxacin, tetracycline, imipenem and linezolid. Testing was performed according to manufacturer´s instructions (bioMérieux, Marcy l'Etoile, France). Mueller-Hinton agar plates were inoculated by swabbing [25] with a suspension of *M. chelonae* diluted to 0.5 McFarland scale standard density. The plates were read after 3 days of incubation and the results were interpreted according to the Clinical and Laboratory Standards Institute [26] (Table 1).

Until the susceptibility to antibiotics could be determined, empirical combined therapy consisting of intravenous (IV), oral (PO), intraocular and topical (AT) antibiotics was initiated. Imipenem (1 g) was administered 3 times daily IV for 4 days and then switched to moxifloxacin 400 mg PO once a day. Clarithromycin

500 mg PO twice a day, amikacin 4 mg/1 ml, clarithromycin 10 mg/1 ml and moxifloxacin 5 mg/1 ml drops hourly and amikacin 0,4 mg/0,1 ml intravitreally (1x) and intracamerally (1x) were also included. Since the therapeutic outcome was satisfactory, intraocular treatment was applied only at the very beginning (Fig. 4). The regimen of frequent drop application was maintained for 5 weeks and then reduced to 5 times a day long-term.

Systemic treatment continued for six months and subsequently was terminated due to its adverse side effects (nausea, dyspepsia). During that period, ocular findings stabilized, and a dense corneal scar formed. In June 2021 a penetrating keratoplasty was performed. The patient is undergoing regular check-ups at another clinic and there have been no signs of any recurrence of the infection at the time of this report. The timeline of the case is summarized in Fig. 5).

Discussion and review

Keratitis is a major cause of visual morbidity worldwide, responsible for an approximately 2 million unilateral blindness cases per year. Aetiological agents of infectious keratitis can be bacterial, fungal, parasitic, and viral in nature [27]. The course of NTM keratitis can mimic fungal, herpes simplex virus or *Acanthamoeba* infection, which are often also associated with ocular trauma. Moreover, fungal and *Acanthamoeba* keratitis, as well as *M. chelonae*, are considered as pathogens involved in postoperative infections [28]. The diagnosis of NTM keratitis is, thus, often delayed, which leads to the insufficient antibiotic treatment and slow response to therapy. In the case described herein, *M. chelonae* was revealed after more than 6 months after the onset of symptoms and admission to hospital (Fig. 5). This delay could be caused by several reasons: insufficient time of culture for 4 days; poor sample collection in the beginning (conjunctival and corneal swabs); antibiotic therapy; and, in addition, late superinfection with *M. chelonae*. We can hypothesize, that it was the combination of initial poor sampling and insufficient culture time. The identification of NTM is critically dependent on correct sampling. It is generally recommended to collect corneal scraping from the leading edges and the base of the ulcer or abscess sample if formed [29]. Ocular swabs are likely insufficient for the diagnosis of NTM keratitis as showed in our report. For ophthalmologists it is generally uneasy to identify the causative infectious organism [30], which is clearly demonstrated by the case of *M. chelonae* keratitis presented here. The clinical course showed a subtle improvement at the beginning, but afterwards an obvious deterioration outweighed. This led to the overuse and frequent changing of antibiotic therapy without a proper

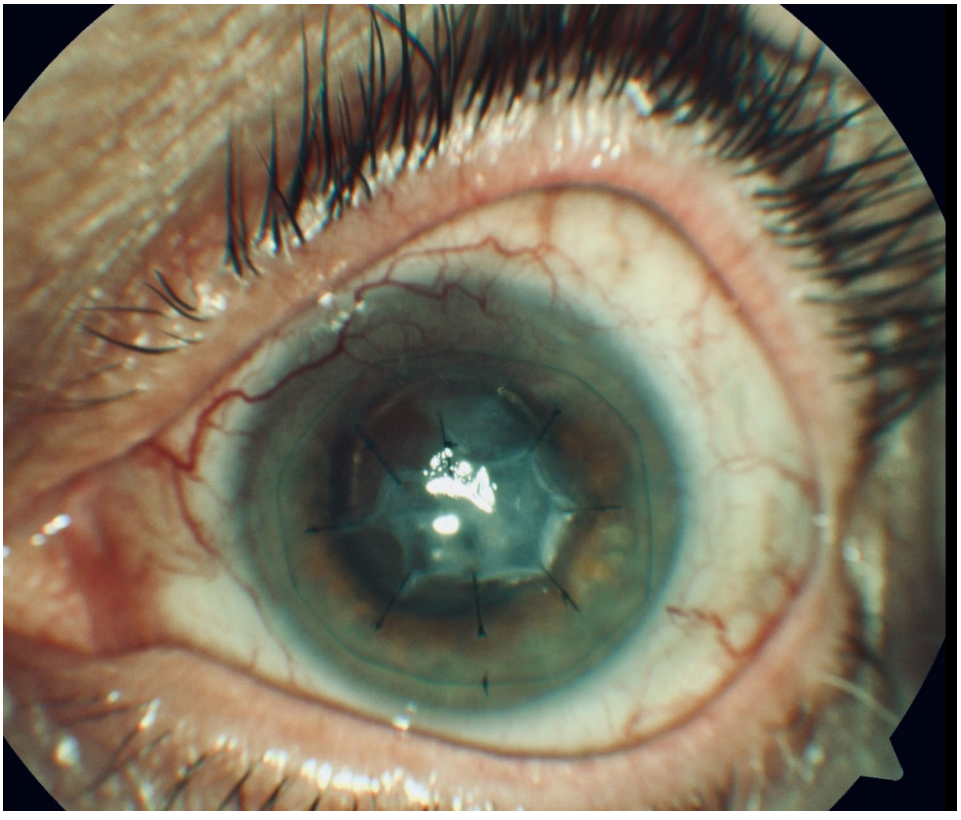

Fig. 4 The status shortly after a single administration of intraocular antibiotics

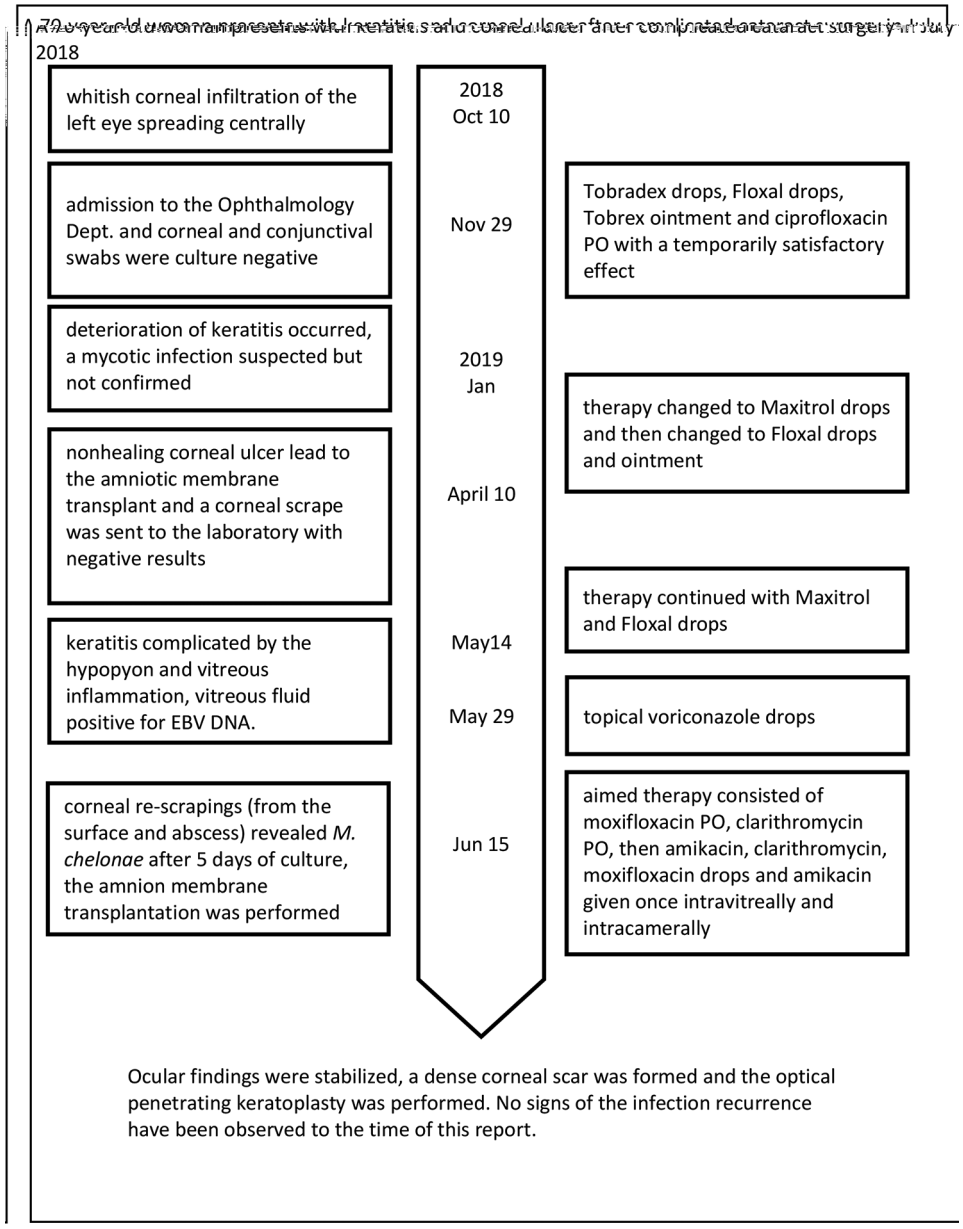

Fig. 5 The timeline of the case development

detection of the causative agent. A combination therapy with corticosteroids in order to control inflammation resulted in an escalation of the NTM infection. Until an intrastromal abscess formed, satisfactory samples to determine the aetiological agent were not obtained. Being aware of this fact, corneal biopsy should have been certainly considered. It is considered superior to the corneal scrape despite the risk of corneal perforation, especially when the therapy and pathogen identification fail [27].

Later, even if corneal scraping were performed, the unsuccessful detection of *M. chelonae* could be caused by partially effective antibiotic therapy at that point. Repeated culture of corneal re-scrapings succeeded after voriconazole monotherapy was administered. The later superinfection could not be completely excluded, but *M. chelonae* belongs among pathogens that are involved in postpoperative infections [28]. According to our analysis of previously published studies, the average length from the onset of symptoms to *M. chelonae* diagnosis is about 11 weeks, ranging from 1 week to 9 months [29].

There are several methods for NTM identification including Ziehl-Neelsen staining with lower sensitivity but with rapid results [27]. The success of culture

techniques is clearly dependent on a suspicion of NTM aetiology and therefore extension of incubation time for at least 8 weeks for slow growing species. The culture methods allow for the identification of NTM and antibiotic susceptibility and should combine both culture on solid media and in broth to enhance the yield [29]. PCR methods are used mostly for the precise identification of NTM DNA from colonies or positive Mycobacteria growth indicator tube (MGIT), and involve sequencing of the *rpo*B or *hsp*65 genes. Nevertheless, a suspicion of NTM keratitis should indicate to perform PCR detection of NTM DNA directly from clinical specimens in order to accelerate the diagnosis. The advantage of PCR methods lies mainly in their high sensitivity, but also brings a potential risk of false positive results, since NTM occurs ubiquitously. To minimize this risk, well trained and specialized laboratory technicians are required and the PCR results should always be evaluated in a clinical context [27].

The cornea is poorly vascularized leading to the generally imperfect drug distribution in the stroma and, thus, it is difficult to achieve drug concentrations exceeding minimum inhibitory concentration at the site of infection. Mycobacterial biofilms unfortunately preclude conventional antibiotic treatment and, along with diminished host immune responses, might lead to chronic infections [31]. The topical antimicrobial treatment with eye drops then usually requires a high loading dose with subsequent frequent application regimens. Topical antibiotic eye drops for severe keratitis mandate the initial loading dose of one drop every 5–15 min within the first hour followed by an intensive hourly application [32]. There is obviously a risk of patient non-adherence to the treatment plan due to drop application fatigue [30]. Antibiotic ointments represent are not suitable in these cases due to their poor solubility and low penetration into the cornea. Subconjunctival injections of antibiotics represent treatment modality of bacterial keratitis in those cases, where there is a risk of a poor effect of the topical treatment [32].

In this case, the initial empirical therapy regimen in November 2018 included tobramycin plus dexamethasone and ofloxacin eye drops with their corresponding ointments. Due to the minimal improvement after two months, the therapy was changed to neomycin, polymyxin B and dexamethasone. Poor outcome of keratitis therapy with ofloxacin and tobramycin was also reported in study by Van Der Beek et al. 2008 [33]. Subsequently, the suspicion on the mycotic infection was raised and voriconazole eye drops were applied, but without any success. Finally, in the middle of June 2019, corneal scrape and abscess samples were obtained and mycobacterial aetiology was determined. An intensive anti-mycobacterial therapy administered topically, intravitreally, and orally was initiated immediately and continued for 4–5 weeks (Fig. 5). It is therefore obvious, that the correct identification of the particular mycobacterial species and its susceptibility to antibiotics are critical for the selection of the appropriate therapy.

In this case, clarithromycin was selected, because *M. chelonae* is usually susceptible to macrolides, to which *M. abscessus* is frequently resistant [34]. When using the "classical" identification of mycobacteria and the sensitivity of NTM to clarithromycin, the culture needs to be extended to 14 days to obtain valid results. However, the susceptibility results can be obtained in an expedite way by the sequence analysis of *rrl* gene for the constitutive macrolide resistance and *erm* [41] gene for inducible macrolide resistance [35]. According to the American Thoracic Society/Infectious Diseases Society of America [20], clarithromycin alone can be used for mild localized skin infections. In cases of serious skin, bone, and soft tissue diseases this monotherapy should be combined with additional antibiotics based on the susceptibility testing for a minimum of 4 months. The survey of previous reports on therapy of *M. chelonae* linked keratitis in European countries from 1987 supports the use of combinatory antibiotic therapy (Table 2, 3, references [11, 13, 14, 16, 28, 33, 36–39]). In the majority of these studies, combination therapies consisting of three and more (7 studies) or two antibiotics (2 studies) were used. Only one study reported the successful use of antibiotic monotherapy. Macrolides were used in 9 studies, fluoroquinolones in 7 studies, aminoglycosides in 7 studies and imipenem or linezolid in two studies. Interestingly, topical linezolid was successfully used in the monotherapy of keratitis after LASIK in 33-year-old man [14]. This therapy should be accompanied with local antiseptics and surgical debridement and draining of abscesses where applicable. Oral clarithromycin is known to possess good distribution characteristics in the eye, however its bacteriostatic nature and time-dependent activity towards mycobacteria, as well as AUC/MIC dependent cure rate are the reasons, why the monotherapy for NTM keratitis should be avoided [40, 41]. In this case, once the correct diagnosis was made, clarithromycin was applied systemically with amikacin, which is the most common first-line antibiotic for the atypical mycobacterial keratitis. However, its poor penetration through epithelium of cornea and moderate activity against the clinically significant NTM species requires a combination with another antibiotic. It acts synergistically with ethambutol, clofazimine and, most importantly, with macrolides. Combinations of these antibiotics were proven to be effective in animal models. Amikacin exhibits two modes of actions - it inhibits proteosynthesis and, in the rapid growing mycobacteria it also damages bacterial cell wall [42].

Table 2 An overview of previous cases of keratitis caused by *Mycobacterium chelonae* in Europe

Patient	Clinical features, anamnestic data	Diagnosis/time to diagnosis	Initial therapy[a]	Aimed therapy[a]	Outcome	Author
56-year-old woman	painful left eye, corneal ulcer, hard contact lenses for 18 years	microscopy and culture from corneal biopsy/about 8 weeks	AT neomycin 0.5%/hydrocortisone 1.5%, chloramphenicol 0.5%, betamethasone 0.1%/neomycin 0.5%, gentamicin 0.3% and methicillin 2%, prednisolone 0.3%, acyclovir 3% 5x/day, miconazole 1% 2-hourly and atropine 1%, amikacin 2.5% hourly, dexamethasone 0.1% hourly/day, 2-hourly/night with atropine 1%	the patient was then admitted for intensive AT therapy with imipenem 0.5% hourly/day, 2-hourly/night, ciprofloxacin 0.2% hourly/day and 2-hourly/night, erythromycin 5x/day, atropine 1% 2x a day and dexamethasone 0.1% / 11 months	necessary to perform penetrating keratoplasty, two years later graft remained clear, visual acuity 6/12	Broadway et al. 1994
26-year-old man	keratitis 1 month after myopic photorefractive keratectomy (briefly after long sea swimming)	microscopy and culture from corneal scrapings/2weeks	AT tobramycin sodium, AT diclofenac sodium were applied topically 4x a day, two days later dexamethasone sodium 4x a day	eyedrops of ciprofloxacin sodium, 0.3 mg/mL/and amikacin sodium, 10 mg/mL, cyclopentolate hydrochloride and PO clarithromycin sodium, 500 mg, 2x a day/one week Then eyedrops of ciprofloxacin sodium, 0.3 mg/mL/and amikacin sodium/3 months	1 year postoperatively visual acuity 20/20, cornea had a grade 2 subepithelial haze	Brancato et al. 1997
Four, 56 to 64-year old women	all women developed keratitis after LASIK performed at the same time period with same surgeon	culture from corneal swabs and scrapes/1 week	Prednisone 60 mg a day, AT ciprofloxacin 0.3% + amikacin 50 mg/mL + clarithromycin 10 mg/mL, PO doxycycline 2 × 100 mg for all 4 patients / 6 weeks	AT ciprofloxacin 0.3% + amikacin 50 mg/mL + azithromycin 10 mg/mL, PO doxycycline 2 × 100 mg for 3 patients / 6 weeks	3 patients responded well on the therapy, fourth patient underwent additional surgery because of necrosis	Chandra et al. 2001
52-year-old woman	unilateral keratitis after LASIK	microscopy and PCR from conjunctival and corneal swabs/about 3 weeks	levofloxacin IV 500 mg 1x a day, AT ofloxacin 3x a day AT natamycin + ofloxacin and IV fluconazole	AT levofloxacin and amikacin, PO clarithromycin	despite antibiotic treatment, flap removal was necessary to control infection, visual acuity 0.2	Kohnen et al. 2003
45-year-old patient	perforating keratoplasty for alkali burn	culture of corneal biopsy/unknown	AT amphotericin B + ciprofloxacin + tobramycin + ceftazidime	PO clarithromycin 3 months, imipenem	poor response on antibiotic therapy, necessary to remove implant	Labalette et al. 2003
37-year-old man	unilateral keratitis after simultaneous LASIK	corneal tissue sample fungal filaments only microscopically, culture reveal *M. chelonae*, presence of *M. chelonae* from the beginning unclear/9 months	AT tobramycin 0.3% + amphotericin 0.5%, PO doxycycline 200 mg + prednisolone, topical 3 mg/mL gentamicin + 1 mg/mL betamethasone + and 1.8 mg/mL hyaluronic acid	AT amikacin, ciprofloxacin, PO clarithromycin / 10 months	10 months after lamellar keratoplasty and antibiotic therapy, graft remain clear	Pache et al. 2003

Table 2 (continued)

Patient	Clinical features, anamnestic data	Diagnosis/time to diagnosis	Initial therapy[a]	Aimed therapy[a]	Outcome	Author
60-year-old woman	infectious keratitis of 3 months duration, medical history included long-standing rheumatoid arthritis treated with hydroxychloroquine and secondary ocular surface syndrome, for which she was treated with topical lubricants	acid-fast rods presented in corneal scraping, PCR positive for *M. chelonae*/3 months	cefazolin 50 mg/mL hourly + tobramycin 14 mg/mL hourly and homatropine 2% 2x a day. PO ciprofloxacin 750 mg + PO clarithromycin 500 mg 2x a day	PO ciprofloxacin 750 mg + PO clarithromycin 500 mg, 2x a day and AT erythromycin 10 mg/mL + ofloxacin 3 mg/mL á 2hrs / 10 months	corneal infiltrates resolved within 5 months, after 10 months ATB therapy terminated, there was deep central corneal scarring with some los of stroma, ocular examination within normal limits, BCVA 20/40, no recurrence in following months	Van Der Beek et al. 2008
33-year-old man	keratitis - photophobia and redness of right eye with blurred vision and white corneal interface 1 month after bilateral LASIK procedure	acid-fast rods in corneal scraping, culture *M. chelonae*, multi-drug resistant (resistant to amikacin and clarithromycin)/ up to 2 weeks	AT ciprofloxacin and tobramycin, after ART revealing amikacin 0.1%, clarithromycin 1%, vancomycin 1%, moxifloxacin 0.3%, PO clarithromycin	AT linezolid 2 mg/mL / 6x a day/2 months	after 2 months detected only subtle leukoma, final visual acuity 20/30 OD, 20/40 OS	Dolz-Marco et al. 2012
71-year-old man	graft rejection after 8 months from tectonic keratoplasty complicated by painful left eye, detected white corneal infiltrate	second corneal scraping sample revealed *M. chelonae*/unknown	N/A	AT cefuroxime + amikacin + gentamicin, PO linezolid, 1 month	after 1 month of therapy, penetrating keratoplasty performed complicated by postoperative hyphema and recurrence of the endothelial	Chowdhury et al. 2016
76-year-old man	patient with Fuchs endothelial dystrophy developed infectious keratitis after DMEK (Descemet membrane endothelial keratoplasty)	culture of *M. chelonae* from the cornea bank, donor cornea transport medium and culture and PCR of *M. chelonae* from cornea graft/3 weeks	AT moxifloxacin 4x a day, tobramycin–dexamethasone 2x a day, amikacin 8x a day, and PO clarithromycin 500 mg 2x a day.	AT amikacin 8x a day, moxifloxacin 4x a day, PO clarithromycin 500 mg 2x a day, PO ciprofloxacin 500 mg 2x a day / 5 months	1 year after repeated DMEK, all precipitates resolved, no signs of active infection, visual acuity 20/25	Van Landeghem et al. 2019

[a]IV intravenous, PO peroral, AT topical

Fluoroquinolones also play an important role in the successful treatment of atypical mycobacterial keratitis [31]. Moxifloxacin, fourth-generation fluoroquinolone, is superior in ophthalmology to other fluoroquinolones, because it can achieve higher concentrations in the conjunctiva or cornea. Moreover, moxifloxacin is more effective against Gram-positive bacteria than other generations of fluoroquinolones, since it inhibits both the bacterial DNA gyrase and topoisomerase IV [43]. The antibiotics suitable for the treatment of keratitis can also possess notable side effects and good care must be taken in case of a long-term fortified eye drop usage. Thus, aminoglycosides have been reported to exert a negative effect on corneal epithelium and ciprofloxacin precipitation might impair epithelial healing. Painful sensations were reported with some topical antimicrobial eye drops [44]. Precautions should be taken during the use of topical corticosteroids in patients with corneal ulcerations, where it should be minimized due to the risk of corneal melting and perforation [30]. The outcome of therapy for this infection is often not satisfactory as many patients require corneal surgery or transplant for vision recovery [20]. Antibacterial agents utilized for the treatment of ocular infections caused by nontuberculous mycobacteria are summarized in Table 3. The most common antibiotics used for topical administration were fluoroquinolones (mostly ciprofloxacin) and aminoglycosides (mostly amikacin). Clarithromycin was the most prevalent orally used antibiotic. In other study, four patients, who developed a keratitis after LASIK, were successfully treated using a 6-week long therapy with topical ciprofloxacin, amikacin and azithromycin, with and addition of oral doxycycline [38].

However, as a result of the combination therapy administered in our patient, ocular findings stabilized and a dense corneal scar formed. There have been no signs of infection recurrence following the optical penetrating keratoplasty at the time of this report.

In the case described here, an additional interesting side finding of the repeated EBV DNA detection in vitreous fluid in the quantity of 1660 copies/mL using qPCR was made. In general, herpetic and other viruses

Table 3 Review of antibacterial agents utilized for the treatment of ocular infections caused by nontuberculous mycobacteria

ATB	Route of administration[a]	Concentration	Availability	Reference
amikacin*	AT	20–40 mg/ml	sterile compounding from IV formulation	Nixon 2018, Lin 2019
	AI	125 µg/0,1 ml or 400 µg/0,1 ml (CAVE retinal toxicity)	sterile compounding from IV formulation	Verma 2015
	SB	20 mg in 0,5 ml	sterile compounding from IV formulation	Lin, 2019
	IS	0.1 ml of 2.5 mg/ml	sterile compounding from IV formulation	Merridew, 2019
azithromycin*	AT	10 mg/ml or 15 mg/ml	commercial preparation	Lin, 2019
besifloxacin	AT	6 mg/ml	commercial preparation	Lin, 2019
ciprofloxacin	AT	3 mg/ml	commercial preparation	Lin 2019
	AI	100 µg/0,1 ml	sterile compounding from topical preparation	Verma 2015
clarithromycin*	AT	10–40 mg/ml	sterile compounding from IV formulation	Gokhale, 2008
gatifloxacin	AT	3–5 mg/ml	commercial preparation	Lin 2019
imipenem/cilastatin*	AT	10 mg/ml	sterile compounding from IV formulation	Nixon 2018
	AI	50–100 µg/0,1 ml	sterile compounding from IV formulation	Verma 2015
levofloxacin*	AT	15 mg/ml	commercial preparation	Lin, 2019
linezolid	AT	2 mg/ml	sterile IV formulation	Nixon 2018, Lin 2019
moxifloxacin*	AT	5 mg/ml	commercial preparation	Lin, 2019
	AI	200 µg/0,1 ml	sterile compounding from topical preparation (preservative free)	Verma, 2015

[a] IV intravenous, AI intravitreal, SB subconjunctival, IS intrastromal, AT topical

∗) systemic use of antimicrobials possible [49, 50]

Intensive and prolonged antibacterial treatment of keratitis can impair wound healing by delayed epithelization and stromal corneal thinning even if infection receded [30]. Preservative free formulations are recommended to avoid corneal toxicity

represent significant causative agents of endophthalmitis, mostly of an endogenous origin. However, other EBV diagnostic markers in this case support this finding as a sign reactivation of the latent EBV infection. These included serological markers of EBV reactivation and were further supported by the finding of EBV viremia of 6680 copies/mL. Previous reports have established, that EBV spreading by the hematogenous route within lymphocytes may cause although rarely ocular inflammation, mostly necrotizing retinitis in patients with B and NK/T-cell lymphoid tumors, other malignancies, or possibly HIV [45–47]. Nevertheless, in such cases, the quantity of EBV DNA is significantly higher in ocular samples than in blood [47]. In addition, among herpetic viruses HSV is the more typical causative agent of keratitis [46].

Taken together, the treatment of eye infections caused by NTM tends to be cumbersome and requires an aggressive approach. Despite the fact, that *M. chelonae* accounts for about half of all NTM keratitis cases described, the management of such eye infections is poorly established [12]. Therefore, all clinical isolates should be tested for their antimicrobial susceptibility. Subsequently, a combination therapy is recommended in order to prevent bacterial resistance [3]. Thus, based on our experience, we can recommend to treat complicated keratitis with abscess formation using the combination of oral and topical clarithromycin, intravitreal, topical and intracameral amikacin, and oral and topical moxifloxacin. Moreover, such combination approach for NTM ocular infections along with discontinuation of corticosteroids is strongly recommended by Kheir et al. 2015 [48].

Conclusion

Diagnosis of nontuberculous mycobacterial keratitis is difficult and often causes a delay in the initiation of treatment. An aggressive and prolonged antimicrobial therapy should include systemic and topical antibiotics. Surgical intervention in the form of corneal transplantation may be required in active and nonresponsive infections.

Despite the fact that *M. chelonae* is responsible for a half of all NTM keratitis reported, it is still a rare disease with only several well described cases in Europe. In our case the infection was well managed, however the unambiguous procedure for the antibiotic therapy of keratitis caused by NTM including *M. chelonae* still has to be established. Moreover, the close cooperation between ophthalmologists, microbiologists, antibiotic centres, and pharmacists has proven to be essential to successfully manage such complicated cases.

Author contributions

Investigation and data analysis, L.R., K.N., P.R. and R.K.; writing—original draft preparation, R.K., L.R. and L.N.; writing—review and editing, P.B.; supervision, L.R. and P.B.; funding acquisition, I.P. and P.B. All authors have read and agreed to the published version of the manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Consent to Publish
Not applicable.

Disclosure of interests
The authors have no relevant financial or non-financial interests to disclose.

Competing interests
The authors declare no competing interests.

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