

## Three cases of *Actinomyces* isolation from the eye lesions of patients with a chronic and recurrent ophthalmic infection

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### ABSTRACT

Actinomycosis is an indolent, slowly progressive infection caused by Gram-positive facultative anaerobic bacteria from the genus *Actinomyces*. These bacteria have been reported as a cause of ophthalmic infections such as endophthalmitis, keratitis, and canaliculitis. The objective of the present study was to investigate the pattern and antibiotic sensitivity profile of anaerobes isolated from lesions in the medial canthus of the eye.

Three pus aspirate samples (from eye) were delivered to a microbiology laboratory in a strict anaerobic condition in Robertson Cooked Meat media (RCM). The samples were inoculated on Blood Agar and incubated anaerobically in a Gas Pack Jar incubator at 5%-10% CO<sub>2</sub> and in aerobic condition at 37°C. Pure colonies isolated on anaerobically incubated plates were identified by the VITEK® 2 COMPACT system. Antibiotic sensitivity testing was conducted using an Epsilometer-strip test.

Three isolates identified and confirmed with the help of VITEK® 2 were *Actinomyces israelii*, *Actinomyces odontolyticus*, and *Actinomyces meyeri*. All three species of *Actinomyces* were sensitive to Vancomycin, Moxifloxacin, and Imipenem, but they were resistant to Metronidazole.

Since there have been several cases of anaerobic ophthalmic infections reported to date, samples from patients with chronic eye infections should be analyzed for anaerobic culture for correct diagnosis and proper treatment. Moxifloxacin but not Metronidazole is a suitable drug for the treatment of anaerobic eye infection.

**Keywords:** eye infection, anaerobic infection, *Actinomyces* species

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### INTRODUCTION

Actinomycosis is a chronic infection caused by Gram-positive, facultative anaerobic bacteria from the genus *Actinomyces* [1]. *Actinomyces* has also been reported as a causative agent of such ophthalmic infections as endophthalmitis, keratitis, and canaliculitis [2]. It was reported that *Actinomyces israelii* is responsible for 13%-25% of the cases of chronic lacrimal canaliculitis [3].

The severity of anaerobic ophthalmic infections can range from self-limiting to sight-threatening blindness. The emerging trend of antibiotic resistance further

contributes to the development of this infection into a more dangerous and serious health threat in the future [4]. Here, we present three cases of an eye infection caused by the *Actinomyces* species.

### MATERIALS AND METHODS

All patients participated in the study signed an informed consent. The aspirates from the eye lesions of patients with chronic and recurring ophthalmic infections were sent to a microbiology laboratory in Robertson's Cooked

Meat (RCM) media for the identification of the anaerobic culture and analysis of its sensitivity to antibiotics. The RCM was incubated for 48 h and then Gram staining was performed. Then, the blood agar plates were inoculated with the substructure from RCM in a triplicate manner – one plate was incubated at aerobic conditions for 24 h, another plate – in atmosphere containing 5%-10% CO<sub>2</sub> for 24 h, and the third plate – at anaerobic conditions (sealed with parafilm) for 72 h in a Gas Pack Jar at 37°C (Fig. 1). The growth of bacteria in anaerobic conditions was confirmed by a VITEK® 2 test. The antibiotic sensitivity tests were performed using the Epsilon strips (E-test) [5, 6, 7].

## RESULTS

### Participants

#### Case 1

In July 2021, a 45-year-old female patient visited the eye outpatient department (OPD) with complaints of severe itching, burning sensation, watering, and pain in the left eye lasting for six months. This patient has no diagnosed comorbidities other than hypertension for the past year. On examination, a small white lesion was found in the medial canthus of the left eye (Fig. 2). The vision was 20/20 in both eyes, with all other ophthalmic parameters being normal. The right eye was normal; no complaints were noted at all from the patient. The pus from the lesion of the left eye was aspirated after local anesthesia under sterile conditions. Then, the testing of the pus sample in order to check for the presence of anaerobic bacteria and their sensitivity to antibiotics has been suggested. To do this, an aspirate taken from the lesion was sent to a microbiological laboratory for analysis.

#### Case 2

The same patient as in the first case came again to the eye OPD after 30 days since the treatment of the first *Actinomyces* infection with the same complaints as during the first visit. The examination revealed one new lesion in the medial canthus of the same left eye. The analogous tests were performed as during the first visit.

#### Case 3

In September 2021, a 52-year-old female patient complained of ocular hypertension with pain and burning for 4.5 months in the left eye. She had diabetes for the past 4 years and had been taking anti-diabetic drugs (her sugar was under control). Visual acuity test showed a 20/20 score. The external examination revealed mild redness in the left eye and a grayish to white color lesion with a white pinhead in the lacrimal sac. An aspirate from the lesion was sent to a microbiology laboratory for analysis.

Both patients recovered after the treatment with Moxifloxacin eye drops three times a day for 10 days.

### Microbiological analysis of the obtained samples

The plates inoculated with aspirates collected from the eyes of the patients were incubated at different conditions. The plates incubated under aerobic conditions for 24 h showed no culture growth in all three cases. On the contrary, plates incubated in a CO<sub>2</sub> atmosphere for 24 h showed bacterial growth (Fig. 3A). The colonies growing at anaerobic conditions in a Gas Pack Jar were gray in color, 2-3 mm in size, low convex, flat with irregular margin, and non-hemolytic. Thin filamentous bacilli showed a positive result in Gram stain procedure (Fig. 3B). The anaerobic growth was confirmed by VITEK® 2. The isolated bacteria were identified as *Actinomyces israelii* (Case 1), *Actinomyces odontolyticus* (Case 2), and *Actinomyces meyeri* (Case 3).

According to the antibiotic susceptibility tests, all three species of *Actinomyces* showed sensitivity to Vancomycin, Moxifloxacin, and Imipenem but resistance to Metronidazole. The results are summarized in Table 1.

## DISCUSSION

Ocular infections caused by *Actinomyces* species are uncommon. Unilateral chronic conjunctivitis is usually caused by *Actinomyces* which might be associated with canaliculitis [8]. In the above-mentioned case series, we have isolated three species of *Actinomyces*, namely *A. israelii* and *A. odontolyticus* from the first patient and *A. meyeri* from the second one. Anaerobic bacteria like *Actinomyces* species have been reported as a causative organism in cases of canaliculitis. *Propionibacterium acnes* have been isolated from the lacrimal gland in 29 out of 55 healthy and diseased eyes, which show an anaerobic environment in eye, whereas *Actinomyces meyeri* has been reported as a causative organism of canaliculitis [9,10,11]. *Actinomyces* species have been commonly reported in cases of canaliculitis [12]. In addition, *Actinomyces* species has been associated with other ophthalmic infections, such as keratitis, dacryocystitis, conjunctivitis, and post operative endophthalmitis. Usually, no generalized systemic invasion is observed in these cases [13]. An incidence of bilateral blepharoconjunctivitis in the absence of canaliculitis due to *Actinomyces* species have been reported [14]. Nair et al. [15] also reported *Actinomyces* canaliculitis complicating congenital nasolacrimal duct obstruction in an infant showing the diversity of population amenable to this disease. An ocular actinomycosis mimicking meningioma caused by *Actinomyces* species in a 67-year-old female patient was described by Kobayashi et al. [1]. The case of chronic lacrimal



Fig. 1. Gas Pack Jar



Fig. 2. White color lesion in the eye of the patient (Case 1)



A



B

Fig. 3. *Actinomyces odontolyticus* growth from the aspirate sample (Case 2) (A); bacteria stained according to the Gram procedure (B).

Table 1. Antimicrobial susceptibility testing of clinical *Actinomyces* isolates

Antibiotic	Minimum inhibitory concentration (MIC)		
	<i>A. israelii</i> Case 1	<i>A. odontolyticus</i> Case 2	<i>A. meyeri</i> Case 3
Penicillin	0.2 µg/ml (S)	>256 µg/ml (R)	>256 µg/ml (R)
Ampicillin	0.25 µg/ml (S)	>256 µg/ml (R)	0.25 µg/ml (S)
Vancomycin	1.5 µg/ml (S)	1.5 µg/ml (S)	1.25 µg/ml (S)
Moxifloxacin	2 µg/ml (S)	2 µg/ml (S)	2 µg/ml (S)
Tetracycline	8 µg/ml (S)	>256 µg/ml (R)	>256 µg/ml (R)
Imipenem	0.5 µg/ml (S)	2 µg/ml (S)	3 µg/ml (S)
Metronidazole	>256 µg/ml (R)	>256 µg/ml (R)	>256 µg/ml (R)

S – sensitive, R – resistant



canaliculitis caused by *A. israelii* in a 70-year-old retired male patient was described by Mohanty et al. [16]. Bhole et al. [17] described a case of keratoactinomycosis in a 50-year-old female farmer caused by *Actinomyces* species after vegetative trauma. The case of Actinomycotic lacrimal canaliculitis was described in a 60-year-old male patient [18]. Hickman et al. [19] also presented a case of lacrimal canaliculitis with bleeding from the eye caused by the *Actinomyces* species. The above-mentioned studies prove the significant role of *Actinomyces* species as a causative agent of ocular infections worldwide. To the best of our knowledge, there have been no reports published on cases with the infections caused by *Actinomyces israelii* and *Actinomyces odontolyticus* in the same patient

in a 30-day gap. All of the above studies showed that the different species of *Actinomyces* cause ophthalmic infection in a broad diversity of patients.

## CONCLUSION

Anaerobic bacteria should be considered as a possible causative agent of every chronic ocular infection, and the corresponding laboratory tests should be performed. Since we found the resistance to Metronidazole in all three reported here cases, antibiotic susceptibility tests should always be performed before any antibiotic treatment. Proper susceptibility testing before the treatment decreases the chance of the development of an antibiotic resistance pattern.

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