Suture-related keratitis caused by *Corynebacterium macginleyi*

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Running title: Keratitis caused by *Corynebacterium macginleyi*

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Abstract

We report two cases of suture-related keratitis following penetrating keratoplasty. *Corynebacterium macginleyi* was isolated from both corneal specimens. Scanning electron microscopy revealed that corynebacteria could aggregate and form a biofilm. Both isolates had high minimum inhibitory concentrations to sulbenicillin and fluoroquinolones. *C. macginleyi* can cause keratitis with biofilm formation.
Case reports

Case 1. A 74-year-old woman underwent penetrating keratoplasty (PK) for a corneal opacity. Postoperatively, she was treated with topical corticosteroids (0.1% dexamethasone) and 0.3% gatifloxacin four times daily, and her recovery was uneventful. Four months later, she visited us with a complaint of blurred vision in her right eye. Slit-lamp biomicroscopy revealed an epithelial defect and a moderate degree of stromal infiltration, along with a loose corneal suture thread. We scraped over the surface of the suppurative area of cornea and removed the loose corneal suture thread. Direct microscopy and bacterial culture of the corneal scraping were performed. Direct microscopy of the corneal scraping demonstrated Gram-positive rods, and confluent growth of corynebacteria occurred after 48 h incubation at 37°C in a 5% CO₂-enriched atmosphere on Columbia agar plates supplemented with 5% sheep blood (SBA). Colonies are grayish translucent and less than 0.5 mm in diameter. We considered corynebacteria to be the causative agent of the keratitis. We stopped the topical corticosteroids and 0.3% gatifloxacin, and started treatment with topical 0.3% tobramycin and 0.5% cefmenoxim every hour. The corneal lesion responded to these agents promptly, and the corneal infiltration healed within 1 week.

Postoperatively, he was treated with topical corticosteroids (0.1% dexamethasone) and 0.5% levofloxacin four times daily, and his recovery was uneventful. The antibiotic eye drops were stopped 1 year after surgery. When he visited us 3 years after the surgery, slit-lamp biomicroscopy revealed an epithelial defect and a corneal plaque with a loose corneal suture thread (Fig. 2A). We removed the loose corneal suture thread, and performed direct microscopy and bacterial culture of the removed corneal plaque. Direct microscopy demonstrated numerous Gram-positive rods (Fig. 2B), and a large number of small colonies (<0.5 mm in diameter after 48 h of incubation) was observed on SBA. We diagnosed keratitis caused by corynebacteria, and stopped the topical corticosteroids and initiated treatment with topical 0.3% tobramycin and 0.3% gatifloxacin every hour. The epithelial defect and a corneal plaque were disappeared within 1 week.

**Bacteriological findings**

The isolates (EC009 from case 1, and EC010 from case 2) were suspected of lipophilic corynebacteria because small-colony-forming (<0.5 mm in diameter) were found after 48 h of incubation on SBA. In order to identify corynebacteria, biochemical testing and molecular genetic methods were performed. The commercial API Coryne system (bioMerieux, Marcy l’Etoile, France) was used...
sed together with the API Coryne database 2.0 (6) according to the manufacturer’s instructions. In
the API Coryne system, both EC009 and EC010 produced the numerical pattern 5-1-0-0-3-0-5,
which were identified by the API Coryne database as *C. macginleyi* with 99.5% probability. Furth-
more the complete 16SrRNA (~1.5kb) and the partial *rpoB* gene were amplified with previously
described primers (11) (12).

Primer sequences were as follows: 16S rRNA (8UA: 5’-AGAGTTTGATCMTGGCTCAG-3’ and 1485B: 5’-TACGGTTACCTTGTTACGAC-3’), *rpoB* (C2700F: 5’-CGWATGAACATYGGBCAGGT-3’ and C3130R: 5’-TCCATYTCRCCRAARCGCTG-3’). The DNA sequences were compared to published sequences searched in the GenBank database (National Center for Biotechnology Information, National Library of Medicine, Bethesda, MD, USA). CLUSTAL W software, originally described by Thompson et al. (20), was used to align the sequences, calculate percent sequence similarity and construct a phylogenetic tree. The 16SrRNA gene sequence obtained for both clinical isolates had 99.2% similarity with both *C. macginleyi* CIP 104099 (accession number X80499) and *C. accolens* CIP 104783 (accession number AJ439346). While the *rpoB* sequence of both isolates show 97.3% and 91.6%
similarity with *C. macginleyi* CIP 104099 (accession number AY492276) and *C. accolens* CIP 104783 (accession number AY492242), respectively. Our clinical isolates were identified *C. macginleyi* by the 16SrRNA and *rpoB* sequence along with the result of API Coryne system.

The minimum inhibitory concentrations (MICs) of various antimicrobial agents used in ophthalmic solutions for these isolates and the type strain (Gifu Type Culture Collection 3120^T^) were determined by the microtiter broth dilution method following specific guidelines from the CLSI (Clinical and Laboratory Standards Institute) (1). All samples were cultured with Mueller-Hinton medium containing 3% lysed horse blood and incubated at 35°C in ambient atmosphere for 24 h and 48 h. We estimated the MICs for both isolates and the type strain because breakpoints have not been established for corynebacteria (1). The MICs of most antimicrobial agents for the type strain were low, while the clinical isolates had high MICs for sulbenicillin and fluoroquinolones (Table 1).

**Scanning electron microscopy of the suture threads**

The suture threads removed in cases 1 and 2 were pre-fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 2 h, washed with cacodylate buffer, immersed in 1% tannic acid in
aqueous solution for 1 h, washed with cacodylate buffer, post-fixed with 2% osmium tetroxide in cacodylate buffer for 2 h, washed again with distilled water, dehydrated in a graded ethanol series, and dried using the critical point drying method. The sutures were observed under a scanning electron microscope (Hitachi S-800, Tokyo, Japan). In case 1, the scanning electron microscopy (SEM) findings showed numerous bacilli on the suture knot, with a matrix connecting the bacilli, and the bacilli appeared to form a biofilm on the surface (Fig. 1A, B).

In case 2, the SEM findings showed some agents covering the suture, and high magnification confirmed that these agents were a substantial gathering of bacilli (Fig. 1C, D).

**Nucleotide sequence accession numbers**

The nucleotide sequences of the 16SrRNA genes of EC009 and EC010 have been given Genbank accession numbers AB354691 and AB354692. The sequences of EC009 and EC010 were deposited under accession no. AB354693 and AB354694 for the *rpoB* gene.

**Discussion**

Corynebacteria, along with *Staphylococcus epidermidis* and *Propionibacterium acnes*, comprise the major colonizers of the conjunctival sac, eyelids, and meibomian glands (8). Corynebacteria other than *C. diphtheriae* seem to have low virulence against the cornea (18). Therefore,
corynebacteria are considered microflora if they are isolated in infectious keratitis. However, several studies have found that some strains of corynebacteria cause keratitis (9, 14). *C. macginleyi* was recently uniquely isolated from the ocular site and found to cause conjunctivitis and endophthalmitis (4, 5, 7, 10). *C. macginleyi* was first identified in 1995 by Riegel et al. during investigations of lipophilic corynebacteria (17). However, it was not clear whether *C. macginleyi* could cause keratitis, and the factors contributing to the virulence of *C. macginleyi* are not well understood. In our cases, corynebacteria were considered causative agents because confluent growth occurred at the site of inoculation on culture plates and the results of the cultures were consistent with direct microscopy, showing Gram-positive pleomorphic rods.

The keratitis in both cases might have been triggered by a loose suture thread adhered by *C. macginleyi*. The loose suture thread seems to be risk factor of microbial keratitis following keratoplasty because organisms are easily to attach on the suture thread and migrate in the cornea.

Previous report also described that 35% of infectious keratitis following keratoplasty were related to sutures (2). Thus biofilm formation on the suture seems to be one of pathogenicity in the infectious keratitis. In our cases the SEM findings revealed *C. macginleyi* strongly attached to the nylon suture and an extracellular matrix appeared to be on the surface of the organisms.
Furthermore, case 2 had a plaque consisting of an aggregation of *C. macginleyi*. These facts imply that *C. macginleyi* can form a biofilm and aggregate, and thereby cause keratitis. Mihara *et al.* reported that corynebacteria, which could not be identified to species, formed a biofilm on the cornea (16). In previous case reports, *C. macginleyi* was the pathogen causing infections of intravenous and bladder catheters (3, 21), and SEM demonstrated that corynebacteria could form a biofilm on catheters (15). Kwaszewska *et al.* showed that 75.6% of lipophilic corynebacteria isolated as natural flora from human skin were able to form biofilms (13). Therefore, biofilm formation seems to be a factor contributing to the virulence of corynebacteria, especially *C. macginleyi*. However, because little is known about the mechanism of biofilm formation in corynebacteria, further investigation is required.

A previous study demonstrated that *C. macginleyi* isolated from cases of conjunctivitis were sensitive to fluoroquinolones (10). However, the two isolates in our cases had high levels of resistance to the fluoroquinolones levofloxacin and gatifloxacin, which are used in ophthalmology. It was likely that the long-term use of topical fluoroquinolones for prophylaxis against infection led to the appearance of fluoroquinolone-resistant *C. macginleyi*. Along with topical antibiotics, we applied topical corticosteroids for prophylaxis against corneal rejection. Therefore, steroids can
immunocompromise the cornea and lead to infections. When the mechanism of resistance to
fluoroquinolones in corynebacteria was investigated in a previous study, a mutation in the \textit{gyrA}
gene of corynebacteria resulted in high MICs for the fluoroquinolones ciprofloxacin, levofloxacin,
and moxifloxacin (19). Therefore, the high concentrations of fluoroquinolones in ophthalmic
solutions might create an environment that selects isolates carrying a mutation of \textit{gyrA}. To prevent
the appearance of fluoroquinolone-resistant strains, we should avoid the unnecessary use of
ophthalmic solutions containing fluoroquinolones.

In conclusion, we found that \textit{C. macginleyi} can cause keratitis and that biofilm formation seems
to contribute to their virulence. The use of topical fluoroquinolones and steroids might facilitate
keratitis caused by \textit{C. macginleyi}.

**Acknowledgements**

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References

1. **Clinical and Laboratory Standards Institute.** 2006. Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria. M-45A. Clinical and Laboratory Standards Institute, Wayne, PA.


Figure legends

Figure 1. Case 1 (A) Photograph of a scanning electron micrograph (low magnification) of the suture showing organisms attached to the suture knot. (B) Photograph of a scanning electron micrograph (high magnification) of the suture showing bacilli with the matrix. Case 2 (C) Photograph of a scanning electron micrograph (low magnification) of the suture showing agents surrounding the suture. (D) Photograph of a scanning electron micrograph (high magnification) of the suture showing the aggregation of numerous bacilli.

Figure 2. Case 2 (A) Photograph of the cornea showing corneal plaque with the loose suture thread. (B) Photograph of a Gram-stained specimen of the corneal scraping showing an aggregation of Gram-positive rods.
### Table 1. Antibiotic susceptibilities of *Corynebacterium macginleyi* strains

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC (µg/ml)&lt;sup&gt;a&lt;/sup&gt; for strain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EC009&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sulbenicillin</td>
<td>16</td>
</tr>
<tr>
<td>Cefmenoxime</td>
<td>0.5</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>≦ 0.13</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>2</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>0.5</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>&gt;128</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>128</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>128</td>
</tr>
<tr>
<td>Levofoxacin</td>
<td>&gt;128</td>
</tr>
<tr>
<td>Gatifloxacin</td>
<td>32</td>
</tr>
</tbody>
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<sup>a</sup>Determined using the microbroth dilution method with Mueller–Hinton medium containing 3% lysed horse blood

<sup>b</sup>EC009 isolated from case 1; <sup>c</sup>EC010 isolated from case 2

<sup>d</sup>Type strain obtained from the Gifu Type Culture Collection (GTC3120<sup>T</sup>)