Corynebacterium macginleyi Conjunctivitis in Canada

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Sequence for NML 080212 has been deposited under Genbank accession numbers FJ960444 (16S rRNA gene) and FJ960445 (partial rpoB gene) respectively.
Abstract

This report describes for the first time *Corynebacterium macginleyi* as a cause of conjunctivitis in Canada, and where menaquinone analysis was done as part of strain characterization. This species is typically isolated from ocular surfaces of patients from Europe and Japan. The isolate was resistant to erythromycin and clindamycin.

Case Report

A 54-year-old microbiologist was seen by an ophthalmologist in Winnipeg, Manitoba Canada for evaluation of bilateral conjunctivitis. She had no history of eye injury or prior eye complaints, nor was a laboratory-acquired infection suspected. None of her family members had similar symptoms. She had no recent travel outside North America. Swabs from each eye were sent within several hours to a hospital laboratory for direct microscopy and bacterial culture. Until culture results were known, the patient was told to apply an over-the-counter ‘tear refresher’ to the eyes, to provide some relief. Approximately 5 days later, when microbiological results became apparent, the patient was prescribed 1 drop of 0.5% moxifloxacin hydrochloride in each eye 3 times per day for 7 days. The infection by that point had begun to resolve, but the patient did comply with the entire treatment course and since has had no recurrence. No follow up culture was done to see if the organism had been eradicated from the eyes.

At the hospital laboratory, after ~48-h of incubation under aerobic and facultatively anaerobic conditions at 35°C on 5% sheep blood agar, the culture from each sample grew fine (~1-mm diameter), round and convex colonies that were slightly α-hemolytic.
Growth in broth was enhanced by the addition of ~1% v/v sterile Tween 80 and so a lipophilic corynebacterium was suspected. Staining revealed Gram-positive coccobacilli in short chains and clusters. A few larger Gram-positive bacillary forms (thicker at one end) were also found, interspersing among coccobacilli. No other bacterial types were recovered. Isolates from each eye were deemed to be identical to each other and both strains were catalase-positive, oxidase-negative and facultatively anaerobic. The API Coryne system (bioMérieux, Montreal QC) was used for identification by the testing laboratory, generating API code 5100305 after 24-h incubation, which could be identified as *Corynebacterium macginleyi* with a 99.5% probability by that system.

One strain was referred to the Canadian National Microbiology Laboratory (NML) for confirmation of identification (NML 080212). Because such isolates have not been previously described as being recovered in Canada, the strain was extensively characterized. Conventional carbohydrate broth sugars enhanced with ~1% serum (v/v) and other biochemical testing procedures were used as reviewed previously (2). This strain slowly fermented glucose, sucrose, and ribose, but, contrary to Riegel et al (14) also slowly fermented glycogen, maltose as well as mannose, glycerol and fructose, but not galactose, lactose, mannitol, raffinose, trehalose, and xylose. When tested using the API Coryne strip, only glucose, ribose and sucrose were reactive, similar to findings by Riegel et al. By conventional methods, the strain reduced nitrate to nitrite, but did not degrade urea, casein, tyrosine, starch, or gelatin. Using the API Coryne strip, the strain produced alkaline phosphatase and pyrrolidonyl arylamidase but not pyrazinamidase, *β*-galactosidase, *β*-glucuronidase, *α*-glucosidase, or *N*-acetyl-*β*-
glucosaminidase. By the API ZYM strip (BioMérieux), the isolate was only reactive with alkaline phosphatase, acid phosphatase and naphthol-AS-BL-phosphohydrolase.

Cellular fatty acid composition analysis was done using the Sherlock system (MIDI, Newark De, with operating system ver 4.5), whereby predominately straight-chain and mono-unsaturated CFAs, but not tuberculostearic acid, were detected, similar to those described for other members of the genus (1). Menaquinones for *C. macginleyi* were not elucidated as part of the species novum description (14) and so this assay was undertaken for NML 080212 and analyzed by LCMS, as described previously (4). Minor amounts of MK-8 (H2) were detected, which is consistent for those of the genus *Corynebacterium* (6). Small volumes of lactic, succinic and acetic but not propionic acid were detected as metabolic products, as found previously for this species (2).

The 16S rRNA gene was amplified as described (3), aligned using Clustal W and compared using Neighbour-Joining software found in MEGA4. The resulting 1483 bp sequence was found to be 100% homologous to *C. macginleyi* CIP 104099T accession no. AJ439345 (Fig. 1). However, as *C. accolens* and *C. macginleyi* can not be adequately resolved by 16S rRNA gene sequencing alone (12), partial *rpoB* gene sequencing was also done. *rpoB* sequences were first aligned using Clustal W, then the full (~3100 bps) of *rpoB* sequences for *C. accolens* AY492242, *C. macginleyi* AY492276 and *C. camporealensis* AY492246 were trimmed to ~400bps, prior to comparison using Neighbour-Joining software found in MEGA 4, with the partial sequence (403 bp) generated for NML 080212. This strain had 98.5% identity with *C. macginleyi* CIP 104099T accession no. AY492276 but only 92.7% identity with *C. accolens*.
accolens CIP104783\textsuperscript{T} accession no. AY492242, thus providing definitive identification between these otherwise closely-related lipophilic species (Fig. 2).

Antimicrobial susceptibility and minimum inhibitory concentrations were determined by the broth microdilution method using Mueller-Hinton medium containing 2.5% v/v lysed horse blood using CLSI methods and breakpoint standards (5) with commercial Sensititre plates and antibiotics as described previously (4). The strain was resistant to erythromycin and clindamycin. \textit{ErmX}, the gene associated with this phenotype, was detected using methods outlined by Rosato (15). Otherwise the strain was susceptible to ampicillin, cefepime, cefotaxime, ceftriaxone, cefuroxime, chloramphenicol, ciprofloxacin, daptomycin, ertapenam, gatifloxacin, gentamicin, levofloxacin, linezolid, meropenem, moxifloxacin, penicillin, quinupristin/dalfopristin, rifampin, telithromycin, tetracycline, tigecycline, trimethoprim/sulfamethoxazole and vancomycin.

Discussion

Riegel \textit{et al}. were the first to describe \textit{Corynebacterium macginleyi} among the lipophilic corynebacteria genomospecies (14). Since then, several clinically-relevant isolates have been detected, almost exclusively from ocular surfaces of symptomatic patients in Europe (e.g., Switzerland, Germany and Italy) (9-11). This species was only very rarely recovered from a study of healthy eyes (20). Extra-ocular involvements have also been reported, such as urinary tract infection, central venous catheter infection, endocarditis and septicemia (7, 13, 18, 19). In 2007, suture-related keratitis caused by this agent was described by Suzuki \textit{et al} (17) and in 2008, Eguchi \textit{et al}. described \textit{C. macginleyi}
isolates from among ocular infections studied in Japan (8). In this instance, the patient was clearly symptomatic with a bilateral ocular infection and the same bacterium was recovered from both eyes with no other aerobic or facultatively anaerobic organism being found. Although no followup culture of the eyes was done after the antibiotic course was completed, these observations suggested that the *C. macginleyi* isolates recovered were causative agent of infection. This would be the first such case of conjunctivitis reported in Canada, and second in North America, as a case involving *C. macginleyi* recovered from a corneal ulcer scraping has been recently described (16), suggesting a worldwide distribution of this organism. The strain described here was consistent with the description of *C. macginleyi* using a polyphasic rather than single (biochemical, genetic and chemotaxonomic (including first description of menaquinones for this species)) identification approach.

Joussen *et al.* reported *C. macginleyi* in 13 of 107 (12%) culture-positive conjunctivitis, indicating its importance in ocular surface infections, especially in immunocompromised hosts (11). Eguchi *et al.* showed *C. macginleyi* being the only bacterium recovered from their cultured conjunctivitis cases (8).

The initial reports by Funke *et al.* and Joussen *et al.* showed almost uniform susceptibility of *C. macginleyi* to commonly-used topical antibiotics, with some resistance to erythromycin and clindamycin being observed (9, 11). Additional resistant phenotypes however are emerging, including to fluoroquinolone, co-trimoxazole, clindamycin, tobramycin and erythromycin (8, 18). Our isolate was resistant to erythromycin and clindamycin, supported by *ermX* gene expression.
It was unexpected that reports describing recovery of *C. macginleyi* from ocular infections from North American sources did not exist prior to 2010 (this present study, (16)). This could be attributed to the frequent practice of treating acute conjunctivitis without obtaining cultures. Our findings, along with cited literature make culture and susceptibility testing highly advisable. Secondly, clinicians empirically prescribing topical antibiotics without performing culture may encounter resistant strains (8).

References cited


Figure 1. Neighbour-Joining Phylogenetic tree based on 16SrRNA gene sequences, showing the relationship of NML 080212 to closest *Corynebacterium* species

Footnotes for Figure 1. Percentages at nodes are bootstrap values based on 1000 replicates. Sequence from the type strain of the type species, *C. diphtheriae* used as outgroup. Bar, 0.005 substitutions per nucleotide position.
Figure 2. Neighbour-Joining Phylogenetic tree based on partial rpoB gene sequences, showing the relationship of NML 080212 to closest Corynebacterium species

Footnotes for Figure 2. Percentages at nodes are bootstrap values based on 1000 replications. Two sequences derived from Japanese outbreak involving C. macginleyi (8) also included as close relatives, with sequence from distantly-related C. camporealensis used as outgroup. Bar, 0.01 substitutions per nucleotide position.