Identification of *Actinomyces meyeri* actinomycosis in middle ear and mastoid by 16S rRNA analysis

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Actinomycosis is a rare, chronic and slowly progressive granulomatous disease caused by filamentous, Gram-positive, anaerobic, non-acid-fast bacteria of the *Actinomycetaceae* family (genus *Actinomyces*) (Wong et al., 2011). Pathogenic *Actinomyces* species do not exist freely in nature, but are commensals that inhabit the oropharynx, gastrointestinal tract and female genital tract of healthy humans (Mehta et al., 2007). The most common site of *Actinomyces* infection is the cervicofacial region (about 60%) (Oostman & Smego, 2005), whereas actinomycosis of the middle ear and mastoid is a rare entity. Difficulty in diagnosing actinomycosis by bacterial cultures of clinical specimens has been attributed to the fact that most species are hard to grow on agar plates (Tarabichi & Schloss, 1993; Olson et al., 1989). To date, almost all reported cases of actinomycosis of the middle ear and mastoid have been diagnosed by examination of histopathological features (Budenz et al., 2010; Mehta et al., 2007; Shishegar et al., 2009; Sivarajasingam & Rajan, 2007; Sobol et al., 2004; Subha et al., 2004).

Bacterial identification using 16S ribosomal RNA (16S rRNA) sequencing is particularly important when dealing with unusual phenotypic profiles, rare, slow-growing or uncultivable bacteria, and in patients with culture-negative infections. Not only has use of this method provided insight into aetiologies of various infectious diseases, but it facilitates choice of appropriate antibiotics and infection control procedures, as well as determination of treatment duration (Woo et al., 2008). This report describes a unique case of middle ear actinomycosis caused by *Actinomyces meyeri* diagnosed using bacterial 16S rRNA gene sequence analysis. To the best of our knowledge, this is the first report of actinomycosis of the middle ear and mastoid caused by *A. meyeri*.

Case report

A 55-year-old male presented to another institution’s ENT (ear, nose and throat) clinic in January 2011 with chief complaints of left otorrhoea and otalgia. The patient was receiving medical treatment for diabetes. There had been dental therapy a few years before and persistent otitis media since childhood. On physical examination, the right ear had no abnormalities, but in the left ear, a polypoid mass that completely occluded the external auditory canal was found. Histopathological examination of the polypoid mass revealed inflammatory granulation. Bacterial cultures of middle ear secretions were negative. Pure tone audiometry demonstrated conductive hearing loss on the left side and a normal-hearing right ear. The patient’s symptoms failed to respond to non-invasive treatment, and a postauricular abscess developed. Pathological analysis of a biopsy specimen from the abscess raised the suspicion of actinomycosis; therefore, in July 2011, he was referred to the ENT department of Tohoku University Hospital.

Physical examination at this institution revealed purulent discharge from the left ear, bulging of the posterior wall of the posterior left external auditory canal, and a thickened left tympanic membrane containing a large perforation. Inflammatory granulation tissue extending from the middle ear cavity was seen. There was no nystagmus, fistula symptom or facial palsy. Bacterial cultures of middle ear secretions were negative. Immediate surgery was initially refused, and it was decided that intravenous ampicillin 2000 mg daily would be administered. However, dizziness and left hearing loss occurred in August, and the patient was admitted in September 2011 to undergo surgery. Pure tone audiometry demonstrated profound hearing loss and a postauricular abscess was noted. The patient initially refused, and it was decided that a transcanal myringotomy with removal of the granulation tissue would be performed. Histopathological examination of the polypoid mass revealed inflammatory granulation. Bacterial cultures were negative. A 16S rRNA analysis of a biopsy specimen from the abscess raised the suspicion of actinomycosis; therefore, in October 2011, he was referred to the ENT department of Tohoku University Hospital.

On admission, the patient was in pain and dysfunction of the left ear was noted. He also had a postauricular abscess. He initially refused, and it was decided that a transcanal myringotomy and removal of the granulation tissue would be performed. Histopathological examination of the polypoid mass revealed inflammatory granulation. Bacterial cultures were negative. A 16S rRNA analysis of a biopsy specimen from the abscess raised the suspicion of actinomycosis; therefore, in November 2011, he was referred to the ENT department of Tohoku University Hospital.

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Actinomycosis of the middle ear and mastoid is extremely rare. Here, we report a unique case of actinomycosis of the middle ear and mastoid caused by *Actinomyces meyeri* diagnosed by 16S rRNA gene sequence analysis.
deafness on the left side. Computed tomography (CT) of the temporal bone revealed complete opacification of the left middle ear and mastoid. The incus seemed to have disappeared and a bony defect of the left lateral semicircular canal was suspected.

The patient underwent left intact canal wall tympanomastoidectomy, which revealed that the tympanum and mastoid were occupied by granulation tissue. The incus had disappeared and a fistula was found at the lateral semicircular canal. Although there was no lymphorrhoea, the interior of the semicircular canal was also invaded by granulation tissue. Presumably, inflammation could have spread to the inner ear from this fistula. Histopathological examination of the surgical specimen revealed Gram-positive, non-acid-fast bacterial colonies surrounded by inflammatory cells, consistent with a diagnosis of actinomycosis (Fig. 1).

Pus from the external auditory canal, middle ear cavity and mastoid, as well as granulation tissue from the mastoid were obtained intraoperatively; DNA from these samples was extracted using the QIAamp DNA Mini kit (Qiagen). PCR amplifications were performed using universal (Baker et al., 2003; Johansson et al., 2004) and Actinomyces-specific primers (Xia & Baumgartner, 2003) of 16S rRNA. PCR amplicons were purified with a QIA quick PCR Purification kit (Qiagen), followed by DNA sequencing using the ABI BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems) and the ABI3730xl Analyser (Applied Biosystems). BLAST was used for sequence analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

DNA sequencing revealed the presence of Fusobacterium spp., detected by the universal primers. The amplicons detected by the Actinomyces-specific primers from all four samples were 100% identical with those of A. meyeri.

The patient was treated with intravenous ampicillin 3000 mg daily for 7 days, followed by oral amoxicillin 1500 mg daily for 5 weeks. At the 18 month follow-up examination, the left ear was dry and the patient was asymptomatic, except for having permanent hearing loss in that ear.

Discussion

Actinomycosis usually occurs in immunocompetent persons, but may also afflict persons with diminished host defences. Actinomyces species are generally of low pathogenicity, and usually cause disease only in the setting of antecedent tissue injury (Oostman & Smego, 2005). In the current case, dental therapy might have triggered the development of actinomycosis. The route of spread was believed to be from the oropharynx via the Eustachian tube to the middle ear and mastoid (Mehta et al., 2007). Of 30 known Actinomyces species, A. israelii and A. gerencseriae

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Fig. 1. Histopathological findings. Actinomyces colony surrounded by inflammatory cells (arrows). Bar, 50 μm (haematoxylin–eosin stain).
have been found in almost 70% of orocervicofacial actinomycosis (Wong et al., 2011). The diagnosis of actinomycosis is most accurately made by isolation of *Actinomyces* species in cultures of clinical specimens or in histopathological tissue sections.

For cervicofacial infection, parenteral administration of penicillin G may be followed by oral penicillin V. Adequate drainage is indicated if abscesses are present. The prognosis for cervicofacial actinomycosis is generally good; full recovery occurs in more than 90% of patients managed by antimicrobial treatment alone (Oostman & Smego, 2005). In the current case, prior to surgery, hearing loss had progressed severely and dizziness had developed, presumably secondary to the lateral semicircular canal fistula. Symptoms progressed in spite of antibiotic therapy, so earlier surgical treatment might have resulted in a better hearing outcome.

To the best of our knowledge, only 17 cases of middle ear actinomycosis have previously been reported in the English literature during the modern antibiotic era (Ajal et al., 1997; Böör et al., 1998; Budenz et al., 2010; Fletcher, 1956; Hoshino et al., 1996; Leek, 1974; Lester & Juhasz, 1990; Mehta et al., 2007; Miglets & Branson, 1983; Olson et al., 1989; Shelton & Brackmann, 1988; Shishegar et al., 2009; Sivarajasingam & Rajan, 2007; Sobol et al., 2004; Subha et al., 2004; Tarabichi & Schloss, 1993). The diagnosis of middle ear actinomycosis in all previously reported cases was confirmed by histopathological features. In the current case, actinomycosis was first suspected after pathological analysis of a biopsy specimen obtained 5 months after initial presentation to the first clinic. Among the 17 previously reported cases, only two (Hoshino et al., 1996; Lester & Juhasz, 1990) were diagnosed as actinomycosis by biopsy extraction, which preceded surgical extraction. While one case (Lester & Juhasz, 1990) suffered from purulent discharge and mastoid opacification like the others, the infection resolved without surgery. It is possible that early diagnosis may facilitate successful non-surgical treatment of actinomycosis.

Cultures are negative in up to 70% of actinomycosis infections (Mehta et al., 2007); only 10% are correctly diagnosed at initial presentation (Oostman & Smego, 2005). Therefore, 16S rRNA gene sequence analysis for genetic identification of bacterial pathogens is a useful diagnostic tool, as illustrated in the current report. In this study, we used two pairs of universal primers, one universal for bacteria (Baker et al., 2003; Johansson et al., 2004) and the other specific for *Actinomyces* (Xia & Baumgartner, 2003). The former detected *Fusobacterium* and the latter *A. meyeri*. The current patient’s course suggests that chronic *A. meyeri* infection was followed by secondary infection with *Fusobacterium* in the anaerobic environment of the middle ear cavity, where an acute inflammatory reaction subsequently developed. *A. meyeri* is rarely isolated in patients with actinomycosis. Previously reported sites of infection, in order of frequency, include lung, skin, long bones, liver, brain and muscle (Apothéloz & Regamey, 1996). To the best of our knowledge, this is the first report of a case of middle ear actinomycosis caused by *A. meyeri* being diagnosed using 16S rRNA gene sequence analysis. It is hoped that widespread use of this diagnostic tool for patients with culture-negative infections might lead to earlier diagnosis and more success with non-surgical treatment regimens.

References


