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Case report

First report of severe acute otitis media caused by *Campylobacter rectus* and review of the literature

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1. Introduction

*Campylobacter rectus* is a member of the human oral flora and is associated with periodontal disease. We report the first case of severe acute otitis media (AOM) due to *C. rectus* in a previous healthy 15-year-old boy, which was confirmed by 16S ribosomal RNA gene sequencing. *C. rectus* is a possible causative pathogen of AOM.

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2. Case report

In January 2014, a previously healthy 15-year-old boy presented to an ear, nose and throat (ENT) clinic with left-sided hearing loss. AOM was diagnosed and was treated with oral cefditoren-pivoxil for 7 days, followed by prulifloxacin for 4 days. He was then referred to the pediatrics department of a general hospital with worsening left otalgia accompanied by headache and hearing loss. After treatment with intravenous ceftriaxone for two days, he was transferred to the ENT department of Tohoku University Hospital for further evaluation.

On examination, his temperature was 37.0 °C. Otoscopic examination revealed pulsating serous discharge from the left ear, along with circumferential bulging of the posterior wall of the left external auditory canal and thickening of the left tympanic membrane (Fig. 1a). The right ear was normal and there was no nystagmus or facial palsy. Neurological examination was unremarkable, and nuchal rigidity was absent. He had no history of previous ear discharge or significant illnesses. There was no evidence of periodontal disease or other active dental problems. He kept dogs and cats as pets.

Pure tone audiometry demonstrated conductive hearing loss on the left side (pure tone average: 47.5 dB) and normal hearing on the...
right side. Laboratory tests revealed a white blood cell count of $10.0 \times 10^3/\mu l$ with 79.1% neutrophils. C-reactive protein was increased to 2.2 mg/dl. Results of liver and renal function tests were within normal limits. Cerebrospinal fluid examination and magnetic resonance imaging revealed no evidence of meningitis at the previous hospital. In addition, aerobic culture of middle ear fluid performed at the previous hospital yielded negative results. Because Gram staining of middle ear fluid was negative, anaerobic culture had not been done in previous hospital.

Sampling of middle ear fluid for bacterial culture was done before the initiation of treatment at our hospital. Antimicrobial therapy was commenced with intravenous ampicillin (3.0 g/day). After treatment for 7 days, his symptoms resolved other than slight persistent left otorrhea. Oral amoxicillin (1000 mg/day) therapy was provided for 5 days after discharge. Twelve days after discharge from hospital, the left ear was normal (Fig. 1b) and the patient’s symptoms had resolved (pure tone average: 6.3 dB).

Culture of middle ear secretions was done on sheep blood agar plates (Nissui Pharmaceutical Co., Tokyo, Japan) and chocolate agar (Kyokuto Pharmaceutical Co., Tokyo, Japan) under 5% CO2, on brucella HK agar (Kyokuto Pharmaceutical Co.) at 35 °C under aerobic conditions, on brucella HK agar (Kyokuto Pharmaceutical Co.) at 35 °C under anaerobic conditions (10% H2, 10% CO2, with 80% N2) for 48 h, and on HK semisolid agar (Kyokuto Pharmaceutical Co.) at 35 °C under aerobic conditions for 7 days. All of these cultures were negative.

3. Microbiological studies

3.1. 16S ribosomal RNA gene sequencing and bacterial studies

To identify the causative organism, 16S ribosomal RNA (16S rRNA) gene sequencing was performed. Bacterial DNA was extracted from middle ear fluid using the QiAamp DNA MiniKit (Qiagen, Hilden, Germany), after which polymerase chain reaction (PCR) amplification and 16S rRNA gene sequencing were performed as described previously [7]. We used the EzTaxon-e Database for sequence analysis (http://eztaxon-e.ezbiocloud.net). The bacterial 16S rRNA gene sequence detected in middle ear fluid was 99.6% (1371/1385 bp) identical to that of the type strain C. rectus (ATCC33238, accession number: L04317), while concordance with other Campylobacter species was less than 97.27%. Thus, identification of C. rectus was confident. For investigation of the major pathogens of AOM, PCR was also performed for S. pneumoniae, Haemophilus spp., and Streptococcus pyogenes [8–10]. Results for all of these bacteria were negative.

3.2. Viral studies

To determine the association of AOM with respiratory viral infection in this patient, viral studies were performed for the following viruses: influenza A/B virus, parainfluenza virus, adenovirus, cytomegalovirus, respiratory syncytial virus, enterovirus, rhinovirus, coronavirus, herpes simplex virus, human herpesvirus 6, human herpesvirus 7, varicella zoster virus, Epstein–Barr virus, human metapneumovirus, human bocavirus, WU virus, and KI virus. Viral nucleic acids were extracted from samples using the PureLink Viral RNA/DNA MiniKit (Invitrogen, Carlsbad, USA) according to the manufacturer’s instructions. To synthesize complementary DNA, the final extract was used as the template with Moloney Murine Leukemia Virus and random hexamers (Invitrogen, Carlsbad, USA). Next, PCR or real-time PCR was performed as described previously with partial modification [11–16]. All of the viruses investigated were negative.

4. Discussion

To our knowledge, this is the first report of AOM caused by C. rectus. AOM is often considered to be a bacterial infection, but its pathogenesis actually involves complex interactions among viruses and bacteria [6]. In most children who develop AOM, viral infection of the upper respiratory tract initiates the cascade of events that finally leads to this condition [17]. Inflammation of the nasopharynx and Eustachian tube caused by viruses may allow bacteria to infect the middle ear. In the present patient, C. rectus could not be isolated by conventional culture at clinical laboratory in our hospital. However, we identified C. rectus by 16S rRNA gene sequencing and excluded the major bacterial and viral pathogens of AOM by PCR, RT-PCR, or real-time PCR. As a result, C. rectus was found to be the causative organism of severe AOM in this patient. C. rectus is difficult to culture and identify. It requires anaerobic conditions for optimal isolation, although the reported composition of the atmosphere needed for successful culture varies among authors [1]. Mahlone et al. obtained the isolate at the same culture conditions with us after 7 days incubation [1]. The supposable reasons why we could not obtain the isolate in spite of the same culture condition of the literature might be attributed to the prior administration of antimicrobial or short time cultivation time. Currently, identification of C. rectus is based on analysis of 16S rRNA. For clinical diagnosis of C. rectus infections, routine methods allowing accurate identification by microbiological laboratories are needed.

It has been reported that C. rectus is typically isolated from the oral cavity, and it was found in 50–94.6% of oral samples from children [2,18]. Familial transmission of oral bacteria is generally accepted [18] and it had been recently suggested that oral bacteria could also be transmitted between humans and their companion dogs. Kato et al. reported that 92.3% of pet dogs analyzed in Japan had C. rectus in their oral flora [19]. In addition, Yamasaki et al. found that 66.7% of companion dogs and 21.0% of their owners were positive for C. rectus in oral samples [20]. C. rectus was detected in fecal samples of healthy or diarrheic pet dogs [21], and the fecal-oral transmission might be one of the possible rote. Because the present patient had dogs, it may be suggested that the origin of C. rectus infection was his companion pets. The route of spread was suspected to be from the oral cavity via the Eustachian tube to the middle ear. To clarify the relationship between C. rectus and AOM, further clinical and epidemiological studies among this organism, human and pet animals are needed.

Only seven cases of C. rectus infection have been reported in the English literature (Table 1). The present patient had several different characteristics from the previous 7 cases. First, all of the previous patients were adults, while this patient was an adolescent. Second,
the previous patients had underlying diseases, while this patient had no predisposing factors except for keeping pets. Third, culture was positive in all of the previous patients, while it was negative in this case. Although 7/8 cases, including this patient, responded to surgery and/or antimicrobial therapy, it is noteworthy that one patient died of ruptured intracranial mycotic aneurysm. Thus, C. rectus infections can occur in previously healthy young people and are occasionally fatal, and C. rectus is a potential causative pathogen of culture negative AOM. Therefore, further investigation of this microorganism in patients with AOM seems to be warranted.

Treatment of complicated infections due to C. rectus usually requires surgical drainage with prolonged antibiotic therapy [22]. There are limited antimicrobial susceptibility data for C. rectus, and there is no standard antibiotic regimen for infections due to this bacterium. One study showed that C. rectus was sensitive to imipenem, levofloxacin, amoxicillin-clavulanic acid, cefoxitin, clindamycin, and metronidazole, with beta-lactamase activity not being detected [22]. The present patient recovered after receiving intra-venous ampicillin and oral amoxicillin, so C. rectus may have been susceptible to penicillin in this case.

In conclusion, we described the first patient with AOM due to C. rectus infection detected by 16S rRNA gene analysis. This case suggests that C. rectus may be a possible causative pathogen of AOM. Accordingly, we need to perform culture while taking C. rectus into consideration in patients with AOM, and methods for its accurate identification by clinical microbiological laboratories are required. For adequate treatment of C. rectus infection, antibiotic selection guidelines and surveillance of its antimicrobial susceptibility profile are also required. Further clinical and epidemiological studies will be needed to clarify the relationship between C. rectus and AOM.

Nucleotide sequence accession number. The obtained sequence of the Campylobacter rectus isolate was deposited as DDBJ accession number LC147456.

All parts of the present study were performed in accordance with the guidance of the Declaration of Helsinki. Informed consent was obtained from the patient, and the review board of Tohoku University approved publication of this case report.

Conflict of interest

There is no conflict of interest.

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