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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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ABSTRACT

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.

1. Introduction

Campylobacter rectus is a Gram-negative, anaerobic, motile bacterium that is known to reside in the human oral cavity and is a causative agent of periodontitis [1,2]. *C. rectus* is also detected gastrointestinal tract with ulcerative colitis or Crohn's disease [3–5]. However, little is known regarding clinical relevance and pathogenic potential about this microorganism. In addition, extraoral infections caused by *C. rectus* have rarely been reported.

Acute otitis media (AOM) is one of the most common diseases in pediatric practice. Its pathogenesis involves complex interactions between bacteria, viruses, and the host inflammatory response. *Streptococcus pneumoniae* and *Haemophilus influenzae* colonize the nasopharynx, and are the most frequent causative pathogens of AOM [6]. Conversely, AOM caused by *C. rectus* has not been reported before. To the best of our knowledge, we are reporting the first case of culture negative severe AOM caused by *C. rectus* in a previously healthy adolescent.

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

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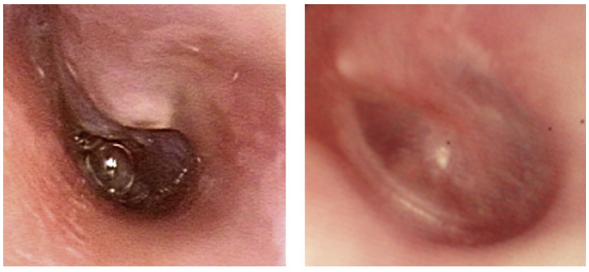

Fig. 1. Tympanic membrane at the time of admission (a) and 12 days after discharge from our hospital (b). Pulsating serous discharge from the left ear, along with circumferential bulging of the posterior wall of the left external auditory canal and thickening of the left tympanic membrane (a). The left ear returned to normal (b).

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% CO_2, on bromothymol blue lactose agar (Nippon Becton Dickinson. Co. Ltd., Tokyo, Japan) and chromagar candida (Kanto Chemical Co., Inc., Tokyo, Japan) at 35 °C under aerobic conditions, on brucella HK agar (Kyokuto Pharmaceutical Co.) at 35 °C under anaerobic conditions (10% H_2, 10% CO_2, with 80% N_2) 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 99.06% (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]. Mahlene 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,