High Incidence of *Alloiococcus otitidis* in Children with Otitis Media, Despite Treatment with Antibiotics

Atsushi Harimaya,1* Ryuta Takada 1 Panu H. Hendolin,2 Nobuhiro Fujii,3 Jukka Ylikoski,4 and Tetsuo Himi1

Department of Otolaryngology, Sapporo Medical University School of Medicine, Sapporo, Japan;1 Institute of Biotechnology, University of Helsinki, Helsinki, Finland;2 Department of Microbiology, Sapporo Medical University School of Medicine, Sapporo, Japan;3 and Department of Otorhinolaryngology, Helsinki University Central Hospital, Helsinki, Finland4

Received 13 October 2005/Returned for modification 12 December 2005/Accepted 22 December 2005

Acute otitis media (AOM) and otitis media with effusion (OME) are common diseases in childhood. *Alloiococcus otitidis* is a newly recognized species of gram-positive bacterium which was recently discovered as a pathogen associated with OME. Although some studies show that *A. otitidis* is frequently detected in children with OME, no study is available concerning the clinical efficiency of antibiotics against this organism. The prevalence of *A. otitidis* in 116 middle ear effusion specimens from 36 AOM and 52 OME patients was examined by culture and PCR. In addition, the prevalence of the bacterium was retrospectively investigated in relation to antibiotic use. *A. otitidis* was detected in 20 (50%) AOM and 47 (61%) OME specimens. The organism was the most frequent bacterium in AOM as well as in OME and was highly detected even in patients who had been treated with antibiotics, such as beta-lactams or erythromycin. The incidence of *A. otitidis* in our study was higher than that in Western countries, and our results suggest that drug-resistant strains of *A. otitidis* may be frequently spread in Japanese children. Our study suggests that antibiotics such as beta-lactams or erythromycin may not be sufficiently effective to eliminate this organism. Further investigation is expected to reveal the clinical role of the organism in otitis media.

Acute otitis media (AOM) and otitis media with effusion (OME) are common diseases in childhood (3, 4). *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* are the three major pathogens in AOM as well as in OME (4, 20).

In 1989, an unknown gram-positive coccus was recovered from middle ear effusions of children with OME (6). This organism was determined to be a new species of bacterium by 16S rRNA analysis and was named *Alloiococcus otitidis* (1); later the name was revised to *Alloiococcus otitis* (9). This organism is difficult to detect in middle ear effusions by conventional culture, because it shows slow growth in vitro and could hinder recovery of the organism from clinical specimens (6). On the other hand, by PCR, *A. otitidis* was detected in about 50% of OME patients, a higher rate than for the three major pathogens (2, 14). These studies suggest that *A. otitidis* is one of the major pathogens of OME.

However, only a limited number of studies of *A. otitidis* have been conducted, and no clinical study of *A. otitidis* is available, although a few studies are available concerning the prevalence or the bacteriological character of this organism. Studies concerning the prevalence of *A. otitidis* in OME have been performed only in Finland (13, 14, 18) and in the United Kingdom (2). Other than in these two countries, only a few clinical strains of *A. otitidis* have been isolated in the United States (5, 6), Turkey (16), Spain (8), and Brazil (5). In Asian countries, even the isolation of *A. otitidis* has not been reported yet. In addition, as regards the detection of *A. otitidis* in AOM patients, only the study by Leskinen et al. (19) is available. Because almost all cases of OME originate after episodes of AOM (7), it is necessary to investigate the prevalence of *A. otitidis* not only in OME but also in AOM as well.

In the present study, to clarify the prevalence of *A. otitidis* in Japanese children with AOM and OME, we assayed for *A. otitidis* by culture and by PCR and compared its incidence with those of the three major middle ear pathogens. We also retrospectively investigated the prevalence of *A. otitidis* in patients who had been treated with antibiotics. As far as we know, this is the first report about the prevalence of *A. otitidis* in Asian countries and is also the first report about association between antibiotic use and the frequency of *A. otitidis*.

**MATERIALS AND METHODS**

**Patient population.** A total of 116 specimens of middle ear effusions were obtained from 88 children (39 females and 49 males). Forty (34.5%) of the 116 specimens were from 36 children with AOM (16 females and 20 males, from 9 months to 8 years, median age of 3.5 years), and 76 samples (65.5%) were from 52 children with OME (23 females and 29 males, from 6 months to 12 years, median age of 4 years). For the children from whom more than one specimen was obtained, these were multiple specimens from different ears during the same episode. In addition, some pathogens were double counted when they were detected from both ears. Patient information was extracted from the medical records, and the medical histories were reviewed. All of these children were Japanese, and non-Japanese children were not enrolled in this study. Written informed consent was obtained from the parents of all children. All of the specimens were obtained during myringotomy, performed as the treatment for AOM or OME. In cases of OME, myringotomy was performed before insertion of a ventilation tube. AOM was diagnosed based on signs of inflammation of the tympanic membrane, the presence of middle ear effusion, and symptoms of otalgia, tugging at or rubbing of the ear, fever, or irritability. For OME, diagnosis of the presence of middle ear effusion was based on the yellow or opaque appearance of eardrums, the presence of conductive hearing loss, and low compliance of the tympanic membrane with pneumatic otoscopy (B curve or C curve
patients. In specimens 1, 2, and 4, lanes 1 to 3 are from AOM patients, and 4 to 6 are from OME of bacterial DNA. Lanes 1 to 6 show the PCR products of the specimens. Lane n shows the negative control, which contains water instead of bacterial DNA. Lanes 1 to 6 show the PCR products of the specimens. Lane n shows the negative control, which contains water instead of bacterial DNA. Lanes 1 to 6 show the PCR products of the specimen was used for culture to detect bacterial pathogens. Culture for *S. pneumoniae* was performed using sheep blood agar; culture for *H. influenzae* was performed using chocolate agar; culture for *M. catarrhalis* was performed using blood and chocolate agar; and culture for *A. otitidis* was performed using sheep blood agar, chocolate agar, and brain heart infusion agar with 5% rabbit blood as recommended in the literature (5, 6, 8). The incubation was extended to 14 days because *A. otitidis* shows very slow growth (5, 6).

Detection of the four middle ear pathogens by multiplex PCR. The other half of each specimen was used for multiplex PCR, which was set up in order to simultaneously detect *A. otitidis* and the three major pathogens *S. pneumoniae, H. influenzae,* and *M. catarrhalis,* as in previous studies by Hendolin et al. (13, 14), using a modification of the multiplex PCR method of Post et al. (21). After boiling the specimens for 10 min, extraction and purification of DNA were performed using SepaGene (Sanke Junyaku Co., Ltd., Tokyo, Japan) in accordance with the manufacturer’s instructions. Precipitated DNA was washed with 70% ethanol, dissolved with 20 μl of sterile water, and used for multiplex PCR. The multiplex PCR was performed as previously reported (13, 14). PCR products were separated in 3% NuSieve 3:1 agarose containing 3 μg/ml of ethidium bromide per ml at 6.5 V/cm for 2 h and visualized by UV light illumination (Fig. 1).

**RESULTS**

Detection of bacterial pathogens by culture and PCR. Of the 40 AOM specimens, bacterial pathogens were detected in 10 (25%) by culture. By PCR, a total of 29 (72.5%) of the 40 AOM specimens were positive for *A. otitidis* or one of the three major pathogens. By the combination of culture and PCR, 31 (77.5%) of the 40 AOM specimens were positive for bacterial pathogens. As shown in Table 1, by culture and PCR, *A. otitidis* was the most frequently detected pathogen in AOM specimens. There was a significant difference between the detection of *A. otitidis* by culture and that by PCR (*P < 0.001, chi-square test).

Of the 76 OME specimens, bacterial pathogens were detected in 16 (21.1%) by culture. By PCR, altogether 53 (69.7%) of the 76 OME specimens were positive for *A. otitidis* or one of the three major pathogens. By combination of culture and PCR, 57 (75%) of the 76 OME specimens were positive for bacterial pathogens. As shown in Table 1, by culture and PCR, *A. otitidis* was the most frequent pathogen as well in OME specimens. There was a significant difference between the detection of *A. otitidis* by culture and that by PCR (*P < 0.001, chi-square test).

**Antibiotic use and pathogen detection.** Because the frequency of pathogens may be affected by antibiotic use, we next investigated the frequency of the pathogens in relation to antibiotic use.

Of the 40 AOM specimens, 19 (47.5%) were from 17 patients without antibiotic treatment within 30 days before specimen collection and 15 (37.5%) were from 14 patients who had been treated with antibiotics for 3 days or more until the day before specimen collection. Of the 15 AOM specimens from patients with a history of antibiotic use, 12 (80%) were from 11 patients who had been treated with beta-lactams (penicillins in 2 patients and cephalosporins in 9 patients) for 3 to 7 days with a median of 5 days until the day before specimen collection. When the frequency of the pathogens was investigated in relation to the antibiotic use mentioned above, there was no difference in the frequency of *A. otitidis* between the groups with and those without beta-lactam treatment (*P = 0.498, chi-square test*) (Table 2). For other pathogens, there was no difference in the frequencies between the groups with antibiotic treatment and those without, but the number was too small to evaluate statistical significance.

Of the 76 OME specimens, 27 (35.5%) were from 17 patients without antibiotic treatment within 30 days before specimen collection and 34 (44.7%) were from 25 patients who had been treated with antibiotics for 3 days or more until the day before specimen collection. Of the 34 OME specimens from patients with a history of antibiotic use, 13 (38.2%) were from 12 patients who had been treated with beta-lactams (penicillins in 2 patients and cephalosporins in 10 patients) for 3 to 10 days, with a median of 5 days until the day before specimen collec-

![Figure 1. Detection of *A. otitidis*, *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* by multiplex PCR. The figure shows representative agarose gel electrophoresis of PCR products. The sizes of PCR products are 237 bp for *M. catarrhalis*, 264 bp for *A. otitidis*, 484 bp for *S. pneumoniae*, and 525 bp for *H. influenzae*. Lane M shows the 100-bp DNA marker. Lanes Ao, Mc, Sp, and Hi show the positive controls for *A. otitidis*, *M. catarrhalis*, *S. pneumoniae*, and *H. influenzae*, respectively. Lane n shows the negative control, which contains water instead of bacterial DNA. Lanes 1 to 6 show the PCR products of the specimens: lanes 1 to 3 are from AOM patients, and 4 to 6 are from OME patients. In specimens 1, 2, and 4, lanes 1 to 3 are from AOM patients, and 4 to 6 are from OME patients. In specimens 1, 2, and 4, lanes 1 to 3 are from AOM patients, and 4 to 6 are from OME patients.

![Table 1. Frequency of *A. otitidis*, *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* in 116 specimens of middle ear effusions from otitis media patients.](http://jcm.asm.org/)

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>AOM (n = 40)</th>
<th>OME (n = 76)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Culture or PCR</td>
<td>Culture or PCR</td>
</tr>
<tr>
<td><em>A. otitidis</em></td>
<td>0 (0)</td>
<td>20 (50)</td>
</tr>
<tr>
<td><em>S. pneumoniae</em></td>
<td>5 (12.5)</td>
<td>5 (12.5)</td>
</tr>
<tr>
<td><em>H. influenzae</em></td>
<td>2 (5)</td>
<td>3 (7.5)</td>
</tr>
<tr>
<td><em>M. catarrhalis</em></td>
<td>4 (10)</td>
<td>8 (20)</td>
</tr>
</tbody>
</table>

*The other pathogens which were detected by culture were Staphylococcus aureus in 1 (2.5%) specimen and Streptococcus pyogenes in 1 (2.5%) specimen.*

*The other pathogens which were detected by culture were Staphylococcus aureus in 4 (5.3%) specimens.*
tion. In addition, 21 (61.8%) specimens were from 13 patients who had been treated with erythromycin for 14 to 84 days, with a median of 28 days until the day before specimen collection. When the frequency of the pathogens was investigated in relation to antibiotic use, there was no difference in the frequency between the groups with and without antibiotic treatment, but the number was too small to evaluate statistical significance.

### DISCUSSION

*A. otitidis* is a bacterium which requires special growth medium, and it is very difficult to culture (5, 6). So far, only a few clinical strains of *A. otitidis* has been isolated (5, 6, 8, 16). However, previous studies by Hendolin et al. showed that *A. otitidis* was never detected by culture, but it was detected by PCR more frequently than other middle ear pathogens (13, 14). Also in other reports in the literature, *A. otitidis* was frequently detected by PCR, whereas it was never detected by culture (2, 18, 19). Although it had been unknown whether a PCR product from bacterial DNA represents viable organisms or not, M. G. Rayner et al. demonstrated clear evidence that the presence of bacterial DNA suggested the presence of viable, metabolically active, intact organisms even in culture-negative otitis media (22). In this study, we also showed that PCR was more effective than culture in detecting *A. otitidis*. In addition, our data showed that the bacterium was very frequently detected in Japan. In OME, the frequency of *A. otitidis* in this study (60.5%) was higher than that in Finland (20% in reference 14 and 46.3% in reference 13) or in the United Kingdom (50% in reference 2). Also in AOM, the frequency of *A. otitidis* in this study (50%) was higher than that in Finland (25% in reference 19). These studies and ours suggest that the organism may have already spread with a high prevalence over almost all the world. Although Leskinen et al. suggested that *A. otitidis* may have no clinical significance in AOM itself (19), they also showed that the presence of *A. otitidis* was associated with a more prolonged course of OME (18). So, the organism may not be associated with the pathology of AOM; however, it may have influence on the condition of OME, which occurs after AOM.

The use of antibiotics is one of the choices for initial treatment of AOM and OME. However, drug-resistant strains of the three major middle ear pathogens (*S. pneumoniae, H. influenzae*, and *M. catarrhalis*) have been increasing rapidly in recent years (4). Concerning *A. otitidis*, Bosley et al. showed that some clinical isolates were drug resistant (5). As far as we know, nothing is known about the efficacy of antibiotics against *A. otitidis* in clinical cases. Our data revealed that *A. otitidis* was frequently detected even in patients who had been treated with beta-lactams or erythromycin. Our results suggest that drug-resistant strains of *A. otitidis* may frequently be spread in Japanese children with otitis media and also suggest that antibiotics such as beta-lactams or erythromycin, which are frequently used for otitis media, may not be sufficiently effective to eliminate this new organism. Our findings are consistent with the in vitro study by Bosley et al., which shows that isolates of *A. otitidis* have resistance to beta-lactams and erythromycin (5).

Although *A. otitidis* is frequently detected in otitis media patients, it is questionable whether this organism has enough pathogenic potential to induce otitis media. Even if *A. otitidis* is frequently detected in middle ear effusion, the bacterium may be one of the normal flora in the middle ear cavity or it may just be a factor contributing to otitis media in a polymicrobial environment. To clarify this point, we have studied the immunogenicity of *A. otitidis* and host response against *A. otitidis*. Our previous studies suggest that *A. otitidis* may have enough immunogenic potential to modulate a host immune response, as well as the three major middle ear pathogens, and also be able to contribute singly to an inflammatory reaction in the middle ear cavity (10, 11, 15, 17, 24). In addition, our clinical study showed that nasopharyngeal colonization of *A. otitidis* is enhanced in otitis-prone children compared to that in non-otitis-prone children (12, 23).

In the present study, we showed that *A. otitidis* was frequently detected in Japanese children with otitis media. In addition, the organism was frequently detected even in patients who had been treated with beta-lactams or erythromycin. Our results suggest that *A. otitidis* is the most frequent bacterium in both AOM and OME and also suggest that *A. otitidis* may not be eliminated in many patients despite such antibiotic treatment. Even if a culture shows negative for *A. otitidis* in patients with antibiotic treatment, many of these cases of infection may be associated with this new organism. We recommend the use of PCR, in order to ensure that the organism is detected when present. Further investigation is expected to reveal the clinical role of *A. otitidis* in otitis media.

### ACKNOWLEDGMENT

This work was funded by the Sapporo Medical University Foundation for the Promotion of Medical Science.

### REFERENCES