What are the Most Responsible Pathogenic Bacteria in the Adenoid for Intractable Acute Otitis Media in Japanese Children?

Masashi Hamada[*,1,3], Miyako Sekiguchi[1], Kazuhiro Yamakawa[2] and Kahori Hirose[3]

[1]*Department of Otolaryngology Tokai University School of Medicine, Japan*

[2]*Department of Otolaryngology Fukui Red Cross Hospital, Japan*

[3]*Department of Otolaryngology, Kochi Medical School, Japan*

Abstract: *Introduction*: Although *Streptococcus pneumoniae (SP)*, *Haemophilus influenzae (HI)*, and *Moraxella catarrhalis (MC)* are major pathogenic bacteria of acute otitis media (AOM) in children, responsibility of their resistance to antimicrobial agents for intractable AOM has not been cleared. In this study, cultured bacteria from the adenoid of otitis-prone children were compared with those of children who had no apparent episodes of AOM to know the most responsible pathogens for intractable AOM.

Methods: Sixty-eight children who had episodes of recurrent or persistent AOM were subjected to this study and 19 children without apparent episodes of AOM but with obstructive sleep apnea were taken as controls. Nasopharyngeal swab specimens were obtained from the adenoid transorally during the adenoidectomy, instead of conventional transnasal harvesting, to avoid contamination. Prevalence of *SP*, *HI*, and *MC* in each group was compared using the chi-squared or Fischer's exact test, and p-values <0.05 were considered significant.

Results: *SP* was identified in 60.3% of otitis-prone children and in 52.6% of control children, and this difference indicated no statistically significance (p=0.54). *HI* was isolated from 77.9% of subjects and from 47.4% of controls, and the difference revealed significant (p=0.009). Above all, beta-lactamase negative *HI* (*BLNAR*) was caught in 39.7% of the study group, but in none of the control group (p=0.002). *MC* was identified in 32.4% and in 5.3%, individually, with significant difference (p=0.04).

Conclusion: *HI* was more frequently isolated from otitis-prone children, and was considered to make AOM more intractable. The pathogenic role of *MC* for AOM may be evident.

Keywords: Otitis-prone children, adenoidectomy, nasopharyngeal culture, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*.

INTRODUCTION

Acute otitis media (AOM) is the most common disease in children over the world, especially in infants and toddlers. *Streptococcus pneumoniae (SP)*, non-typeable *Haemophilus influenzae (HI)*, and *Moraxella catarrhalis (MC)* are well known to be major bacteria which cause AOM. *SP* has long been the leading pathogen of AOM [1-3], but *HI* was recently reported to increase its frequency [3, 4]. In addition, pathogenesis of *MC* has not yet been sufficiently elucidated [5]. Furthermore, bacterial resistance to antimicrobial agents has become an increasing problem in the treatment of AOM, and thereby has induced AOM to be more intractable in many countries [6-10].

On the other hand, adenoidectomy is generally accepted to be an effective treatment for otitis media with effusion (OME) persisted in rather older children [11, 12]. It nowadays becomes more difficult to distinguish persistent otitis media in infants and toddlers from classical OME, since resistant bacteria has been proved in the middle ear effusion [13, 14]. In addition, adenoid has been recently closed up as a reservoir of antibiotics-resistant bacteria [13, 14]. However, the role of adenoid and bacteria on site for intractable AOM is still unclear.

In this paper, we aimed to clarify what are the most responsible bacteria in the adenoid of otitis-prone (OP) Japanese children for intractability of AOM in the present time and to discuss about the role of adenoid as a reservoir of those bacteria. To achieve this purpose, nasopharyngeal swab specimens taken transorally from the inside of adenoid of OP children were compared with those of children who had no apparent episodes of AOM.

SUBJECTS AND METHODS

Sixty-eight children (43 boys and 25 girls) who had episodes of recurrent AOM or persistent otitis media were subjected to this study. They underwent adenoidectomy and/or tympanostomy tube insertion under general anesthesia in Kochi Medical School hospital from 2002 through 2007. Recurrent AOM means 4 episodes of AOM or more during the last 6 months and persistent otitis media has fluid collection in the middle ear observed over 3 months. Nineteen children (10 boys and 9 girls) without apparent

*Address correspondence to this author at the Department of Otolaryngology, Tokai University, School of Medicine, 143 Shimokasuya, Isehara, 259-1193, Japan; Tel: +81-463-93-1121; Fax: +81-463-94-1611; E-mails: mhamada@is.icc.u-tokai.ac.jp, mhenttusm@yahoo.co.jp

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episodes of AOM but with obstructive sleep apnea (OSA), in whom adenotonsillectomy were indicated during the same period, were taken as controls. Age distribution was from 11 months to 3 years (mean 1.8 years) in the study (OP) group and from 1 to 3 years (mean 2.5 years) in the control (OSA) group. In the present study, we focused on the intractable AOM in infants and toddlers, and thereby limited the age distribution up to 3 years. Written informed consent was taken from guardians of all children. This study conformed to the principles outlined in the Declaration of Helsinki, and was approved by the ethical committee of Kochi Medical School.

The nasopharyngeal swab specimens were obtained transorally from the adenoid during the surgery, instead of conventional transnasal harvest, in order to eliminate the contamination (Fig. **1**). Nasopharyngeal samples were inoculated on blood agar, chocolate agar, and other appropriate plates, if applicable. The plates were incubated for 24-48 hours at 37°C with 5% carbon dioxide. Colonies were identified based on the generally accepted methods.

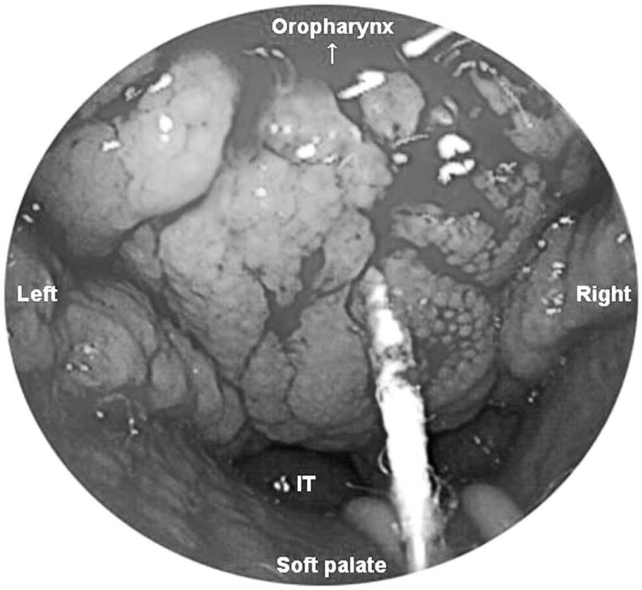

Fig. (1). Endoscopic view for transoral harvest of nasopharyngeal specimen by the use of swab. Soft palate was elevated by transnasal catheter and the swab was put deeply inside the adenoid without oral contamination. IT: inferior turbinate of left nose.

The minimum inhibitory concentrations (MICs) of the isolates to antibiotics were determined according to the standard methods of the Clinical Laboratory Standards Institute (CLSI). *SP* with MICs ≦0.06 ug/ml for penicillin G was defined as penicillin-susceptible *SP (PSSP)*, that with MICs of 0.125-1.0 ug/ml as penicillin-intermediate susceptible *SP (PISP),* and that with MICs ≧2 ug/ml as penicillin-resistant *SP (PRSP)*. Beta-lactamase negative *HI* with MICs ≧1 ug/ml for ampicillin was defined as *BLNAR*.

Prevalence of *SP*, *HI*, and *MC* in each group was compared using the Fischer's exact test and *p*-values <0.05 were considered significant.

RESULTS

Bacteria isolated from nasopharyngeal swab of the study OP group and the control OSA group were shown in Figs. (**2**, **3**), respectively. One hundred and fifty-seven isolates were obtained from 68 children with intractable AOM, whereas 44 were cultured from 19 children without AOM but with OSA. *SP, HI,* and *MC* altogether accounted for more than 70% of all isolates in the OP group and for 45% in the OSA group.

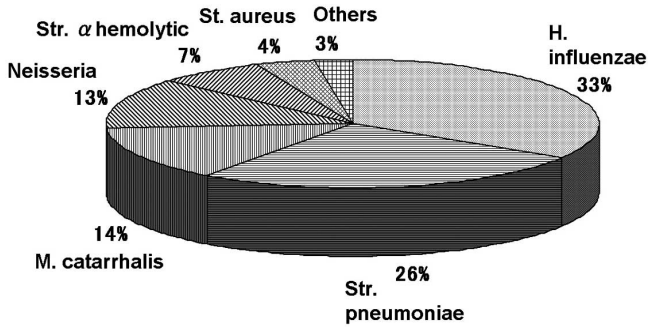

Fig. (2). Bacteria isolated from the adenoid of otitis-prone children. One hundred and fifty-seven isolates were cultured from 68 children, and 3 major pathogens (*Streptococcus pneumoniae, Haemophilus influenzae*, and *Moraxella catarrhalis*) together reached above 70% of all isolates.

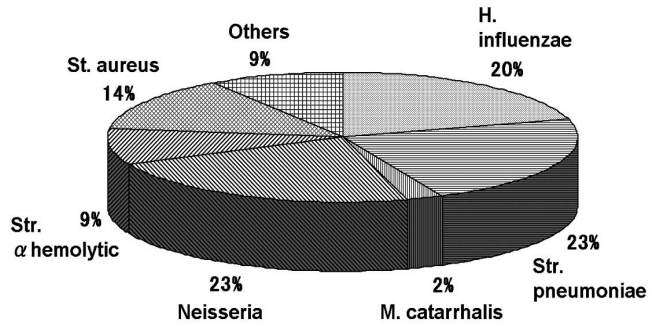

Fig. (3). Bacteria isolated from the adenoid of children with obstructive sleep apnea. Forty-four isolates were cultured from 19 children, and 3 major pathogens accounted for 45% of all isolates.

The prevalence of *SP, HI*, and *MC* were presented in Fig. (**4**). *SP* was identified in 60.3% of samples from OP children and in 52.6% from OSA children, and this indicated no statistically significant difference (p=0.54). Regarding the antibiotics-resistance, *PISP* or *PRSP* was found in 52.9% of the OP group and 42.1% of the OSA group (p=0.4). *HI* was isolated from 77.9% of the study group and from 47.4% of the control group, and the difference between two groups revealed statistically significant (p=0.009). *BLNAR* was caught in 39.7% of the study group and in none of the control group. This difference was significant statistically (p=0.002). *MC*, all of which had beta-lactamase, was identified in 32.4% and in 5.3%, individually, with showing a significant difference (p=0.04).

DISCUSSION

Bacteria cultured from middle ear fluid, regardless it seems purulent or not, are thought to be more specific pathogens to AOM. However, tympanocentesis for drainage and diagnosis is not always carried out in the clinic. In addition, the detection rate by the standard culture is not high enough to know the real pathogens of AOM even if middle ear fluids are harvested [15]. Polymerase chain reaction (PCR) may be more sensitive to prove the existence of

bacteria [16, 17], but this technique has not yet been established as clinical use and still has a question if DNA of bacteria detected really means pathognomonic. On the other hand, nasopharyngeal culture by the use of transnasal swab is more acknowledged to represent the pathogenic bacteria of AOM than that from middle ears. This method is still a gold standard, but may have a chance of contamination and thereby can lead us to misunderstanding. That is a reason that we took the nasopharyngeal swab transorally without touching any place in the nose and mouth in order to know the true nasopharyngeal colonization and further real pathogens of intractable AOM.

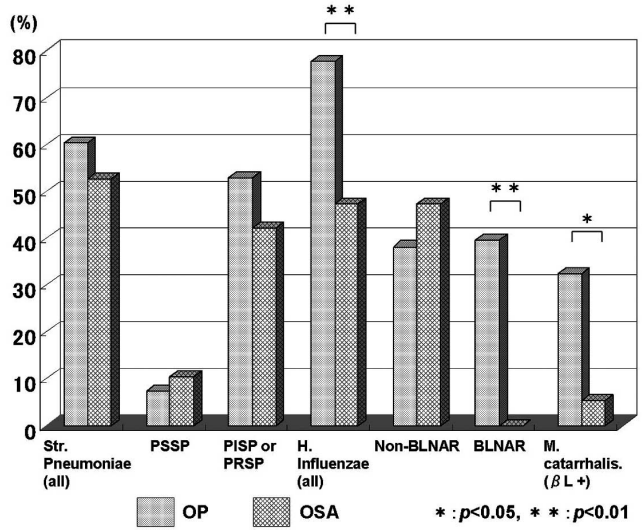

Fig. (4). Comparison of prevalence of 3 major pathogenic bacteria between otitis-prone (OP) group and obstructive sleep apnea (OSA) group. There was significant difference between two groups in the prevalence of total *Haemophilus influenzae*, *BLNAR*, and *Moraxella catarrhalis*.

As a result, *SP, HI,* and *MC* were still considered to be 3 major pathogens of AOM, because their total percentage to all isolates reached over 70% from the pure nasopharyngeal specimens of the OP children and this proportion was far higher compared to OSA group. Above all, *HI* was the most frequently cultured in the OP group, and its prevalence was significantly higher than that of the control OSA children. These results may indicate that *HI* resides in the adenoid tissue of the OP children and that *HI* is the most convincing candidate to cause AOM intractable. Hotomi *et al.* [18] have recently suggested that *HI* may act a role of intracellular pathogens to contribute to persistent existence and intractable course of otitis media. In addition, further more evident difference was obtained between OP group and OSA group in the positivity of *BLNAR*. *HI* likely survives even after an attempt of decolonization by repetitive administration of antibiotics [19]. *BLNAR* may be induced by such an inappropriate management of recurrent AOM, and thus never be isolated in OSA children who had no episodes of AOM in this study. Furthermore, the biofilm formation of *HI* was clearly shown by Moriyama *et al.* [20] and this may result in persistent course of otitis media.

On the other hand, *HI* was positively isolated as well in the control OSA children in the present study. *HI* has been highly proved to be resident in the adenoid of the classical OME children either by PCR or by the standard culture [21]. Furthermore, Brook *et al.* [22] reported that *HI* was isolated even from the healthy children and was thereby thought to play some role for adenoid growth. The relationship between *HI* and adenoids remains so unclear that significance of the difference in the prevalence between the OP and OSA children shown in the present study should be further discussed.

Surprisingly, *SP* is highly proved in the adenoid of the control children as well as of OP children. *PISP* and *PRSP* were majority of *SP* in both groups, but there seems no difference in the prevalence of these resistant *SP* between two groups. *SP* is a component of normal flora in children and can be easily spread among children through daycare centers [23, 24]. *PISP* and *PRSP* are supposed to replace the susceptible organisms after initiation of antibiotics treatment [25]. In Japan, antibiotics has been likely used even for mild acute upper respiratory tract infections in the pediatric primary care, and this trend may be one of the reasons that the significant difference was not obtained in the resistant *SP* prevalence between the OP group and the OSA group. Furthermore, since even *PISP* and *PRSP* are thought to be controlled by the high dose of amoxicillin administration [26] which is currently prevailed in Japan, *SP* may exist in the adenoid neither with presenting with active AOM nor with being completely eradicated.

Although *MC* has been generally accepted as one of 3 major bacteria responsible for AOM, its pathogenesis and virulence have been long on the argument [5]. Current researches have led to a better understanding of the molecular mechanisms involved in *MC* pathogenesis [27]. Broides *et al.* [28] showed *MC* was more associated to rather clinically mild AOM. The present study suggested that *MC* had some pathogenic contribution to intractable AOM.

Conclusively, *HI* was the most frequently isolated from the adenoid of OP children, and its prevalence was significantly higher in the OP children than in the OSA children. These findings may indicate that *HI* is the most responsible pathogen for intractable AOM in the present days. Many of latest reports showed *HI* prevalence had increased up to the highest frequency among otopathogenic bacteria over the world [3, 4, 29]. Furthermore, *HI* was recently proved to have close relation to persistent and recurrent morbidity of AOM [3, 4, 30]. Results of the present study seem in agreement with those reports.

CONCLUSION

Transoral swab was attempted to know the real pathogenic bacteria in the adenoids of otitis-prone (OP) Japanese children. Non-typeable *Haemophilus influenzae (HI)* was the most frequently isolated from the OP children, and *BLNAR* was currently considered to have a significant role for intractability of acute otitis media.

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CONFLICT OF INTEREST

None declared.

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