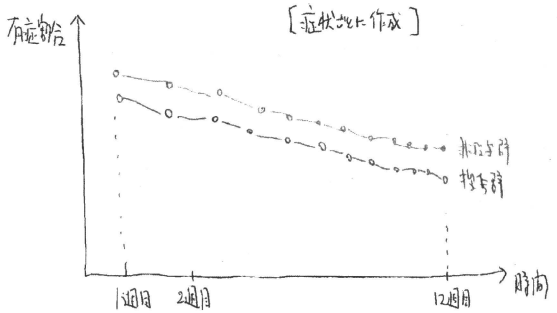
付録D



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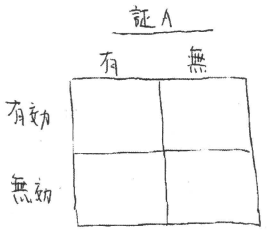

Acta Oto-Laryngologica, 2009; 129: 14–18

informa
healthcare

ORIGINAL ARTICLE

Effects of Japanese herbal medicine, Juzen-taiho-to, in otitis-prone children – a preliminary study

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Abstract

Conclusion: Juzen-taiho-to (JTT, TJ-48), a Japanese herbal medicine that improves immune function, was found to be effective in otitis-prone in children. *Objective:* To evaluate the efficacy of JTT against intractable and recurrent infections in immature immune systems, we administered JTT to otitis-prone infants and investigated clinical changes before and during JTT administration. *Subjects and methods:* Twenty-four otitis-prone infants were administered JTT at 0.10–0.14 g/kg/day twice a day for 3 months. We compared clinical course, such as frequency of acute otitis media (AOM), duration of fever and antibiotics administration, and hospital visits for the periods before and during JTT administration. *Results:* Medication compliance rate was 87.5%, and administration of JTT led to remission in 95.2% patients. No apparent side effects were observed. The frequency of AOM decreased significantly (Wilcoxon signed rank test, $p = 0.000$) with JTT. The duration of fever ($p = 0.000$) and administration of antibiotics ($p = 0.001$), as well as the number of hospital visits ($p = 0.001$) and emergent hospital visits ($p = 0.000$) showed significant decreases after JTT administration. After the end of the JTT period, 14 of 21 (66.7%) patients started to take it again, as they experienced purulent otitis media and/or other infections after discontinuation. The frequency of AOM increased significantly after stopping JTT ($p = 0.004$) and decreased again with JTT resumption ($p = 0.005$).

Keywords: *JTT, TJ-48, immune function, drug-resistant bacteria, acute otitis media, immune immaturity*

Introduction

The unique role played by traditional Japanese herbal medicines (Kampo) is gradually attracting worldwide attention. Juzen-taiho-to (JTT, JT-48) is a nourishing agent that is used to improve disturbances and imbalances in homeostasis, and it is known to increase immune function by enhancing phagocytosis [1], cytokine production [2], antibody production [3], and the mitogenic activity of spleen cells [4]. Recent investigations have also reported that JTT has anti-tumor effects, such as suppression of tumor metastasis [5] and prolongation of survival [6], and that it protects against the adverse effects of chemotherapy [7] and radiotherapy [8] by influencing the immune response. JTT has also been reported to have protective effects against Candida albicans infection [9].

Acute otitis media (AOM) is one of the most common diseases in early infancy and childhood. However, some children experience more frequent and recurrent bouts of AOM. The increase in the number of otitis-prone infants and the rapid emergence of drug-resistant bacteria associated with AOM are now generating increasing concern [10–12]. Although antibacterial medication is generally effective against bacterial infection, overuse of antibiotics has led to the increased emergence of drug-resistant bacteria. In addition, otitis-prone patients are sometimes unable to recover from intractable inflammation of the middle ear, which can progress to more serious conditions, such as mastoiditis, meningitis, cerebritis, otitis interna, and sigmoid sinus thrombosis.

Onset and progression of infection are determined by the relative balance between the reproductive

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power of microbes and host defense, and immune immaturity is one of the reasons why the majority of otitis-prone patients are under 3 years of age. It has also been reported that response and development of immune function are poor in otitis-prone children [13–15]. In this study, we investigated the effects of JTT in otitis-prone children.

Subjects and methods

We examined 24 otitis-prone infants who experienced repeated purulent otitis media, despite conventional management. Otitis-prone children were defined as children who had more than three episodes in 6 months, more than four episodes in 1 year, or more than four episodes by the age of 2 years [14]. After obtaining informed consent, JTT at 0.10–0.14 g/kg per day was administered twice a day before milk or food for 3 months. No restrictions were imposed on conventional treatments for otitis media or any other disease while JTT was administered. We investigated the frequency of AOM, duration of fever and antibiotics administration, and number of hospital visits and emergent hospital visits in the periods before and during JTT administration. AOM was diagnosed by otolaryngologists based on examination of general and tympanic findings. Unilateral AOM was counted as a single occurrence, while bilateral AOM simultaneously diagnosed in both ears was counted as two occurrences. Recurrent ear discharge and exacerbation during treatment were regarded as different episodes of AOM. Fever was defined as a body temperature of more than 37.0°C in patients whose normal temperature was under 37.0°C, or as a rise of more than 1°C in patients whose normal temperature was more than 37.0°C. Hospital visits included otolaryngology, pediatrics, and emergency room visits for any reason related to patient conditions. Total hospital visits were counted as 'hospital visits,' and unscheduled hospital visits due to unexpected circumstances, such as sudden fever, infectious symptoms or other diseases, were counted as 'emergent hospital visits.' The number of days before and during administration of JTT was also counted for each patient, and each parameter was calculated as an average number per 30 days (number per month). The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and complied with the Helsinki Declaration of 1975, as revised in 1983.

Statistical parameters were ascertained using SPSS software (13.0J for Windows; SPSS Inc., IL, Chicago, USA). The statistical significance of differences between the groups was determined by Wilcoxon signed rank test. $p < 0.05$ was considered significant.

Results

JTT was given to 24 otitis-prone infants (15 males, 9 females; median age at the start of JTT administration, 14 months; range 6–33 months). Observational time before starting JTT administration was 20–214 days (mean $\pm$ SD = 86.0 $\pm$ 46.7 days). While three infants were unable to continue taking JTT, 21 infants had no difficulties. The medication compliance rate was 87.5% and no apparent side effects were observed during JTT treatment.

The frequency of AOM decreased significantly (Wilcoxon signed rank test, $p = 0.000$) with oral administration of JTT (Figure 1). Mean and standard deviation of the frequency of AOM before the JTT administration period was 3.41 $\pm$ 2.00 (times/month), which decreased to 0.53 $\pm$ 0.75 (times/month) during the JTT administration period. In the most effective case, a 13-month-old female, the frequency of AOM decreased from 8.28 times/month to 0 times/month. Duration of fever

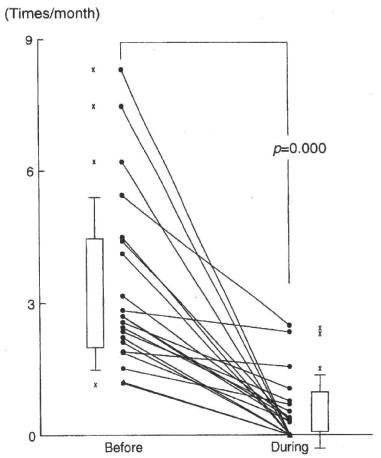


Figure 1. Frequency of AOM before and during JTT administration. Number of occurrences of AOM were counted before and during JTT administration in 21 patients. The average frequency of AOM per month (30 days) was calculated and compared before and during JTT administration. The frequency of AOM decreased significantly with JTT (Wilcoxon signed rank test, $p = 0.000$) (mean $\pm$ SD; before, 3.41 $\pm$ 2.00; during, 0.53 $\pm$ 0.75).

($p=0.000$) (Figure 2) and antibiotics administration ($p=0.001$) (Figure 3), as well as the number of hospital visits ($p=0.001$) (Figure 4) and emergent hospital visits ($p=0.000$) (Figure 5) decreased significantly during the JTT administration period.

Two of three patients who could not continue taking JTT required the surgical insertion of a ventilation tube due to recurrent otitis media. In addition, 1 of the 21 cases (1 of 42 ears) taking JTT also needed this surgery, as recurrence of otitis media continued, albeit at a lower frequency than before JTT administration. The other 20 patients (95.2%) showed remission and had no need of the surgical insertion of a ventilation tube.

After the end of the JTT administration period, 14 of 21 (66.7%) patients started to take JTT again, as they experienced purulent otitis media and/or other infections after discontinuation of JTT, and their parents decided to resume JTT. Intermission times before resumption of JTT in these 14 patients ranged from 4 to 166 days (mean $\pm$ SD $=41.2\pm$ 51.3 days). Administration periods of JTT after resumption were decided as part of the patients' clinical course. Twelve of 14 children have already finished JTT due to spontaneous recovery from recurrent AOM. We compared the frequency of AOM among the following periods: first administration, during intermission, and after resumption.

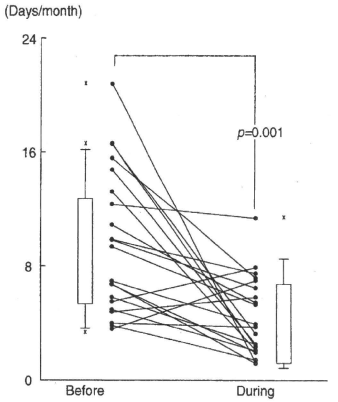


Figure 3. Administration of antibiotics before and during JTT administration. Average number of days of antibiotic administration per month decreased significantly after JTT administration ($p=0.001$) (mean $\pm$ SD; before, 9.62 ± 5.02; during, 4.36 ± 2.77).

With regard to patients taking JTT for more than 3 months after resumption, we investigated the frequency of AOM during the 3 months after restart

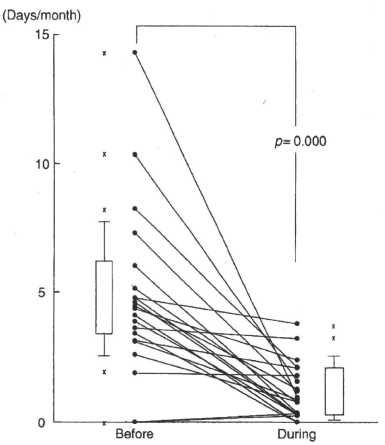


Figure 2. Duration of fever before and during JTT administration. Average duration of fever per month during JTT administration showed a significant decrease vs before JTT administration ($p=0.000$) (mean $\pm$ SD; before, 4.74 ± 3.25; during, 1.15 ± 1.04).

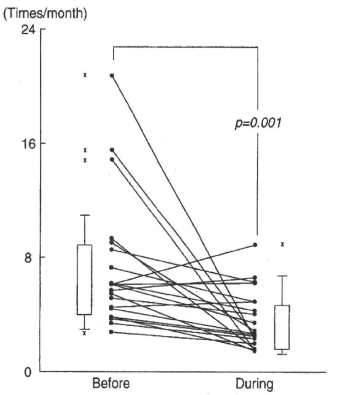


Figure 4. Total hospital visits before and during JTT administration. Total scheduled and unscheduled hospital visits to otolaryngology, pediatrics, and emergency departments for any reasons related to patient condition were counted and calculated as per month (30 days). Significant decreases in number of hospital visits were seen with oral administration of JTT ($p=0.001$) (mean $\pm$ SD; before, 7.28 ± 4.57; during, 3.59 ± 2.05).

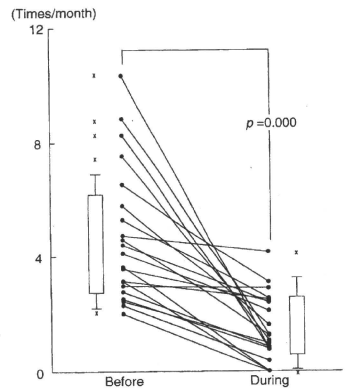


Figure 5. Emergent hospital visits before and during JTT administration. Unscheduled hospital visits due to unexpected circumstances, such as sudden fever, infectious symptoms, and other diseases, were defined as emergent hospital visits. Emergent hospital visits per month decreased significantly with JTT administration ($p=0.000$) (mean±SD; before, 4.69±2.39; during, 1.49±1.13).

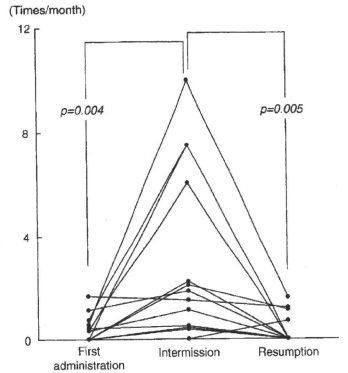


Figure 6. Frequency of AOM in 14 cases resuming JTT; comparison between first administration, during intermission, and resumption. After the 3 month JTT period, 14 of 21 (66.7%) patients resumed JTT administration, as they experienced purulent otitis media and/or other infections after discontinuation. Comparison of the frequency of AOM showed significant increases after stopping JTT ($p=0.004$), followed by decreases after JTT resumption ($p=0.005$) (mean±SD; first administration, 0.35±0.48; during intermission, 2.90±3.35; resumption, 0.31±0.54).

of JTT. The frequency of AOM was significantly higher after stopping JTT ($p=0.004$) and decreased again with JTT resumption ($p=0.005$) (Figure 6).

Discussion

JTT is a traditional Japanese herbal medicine, or Kampo, and has a long history of use among Japanese people for the treatment of disturbances in homeostasis and immune disorders. The Japanese Ministry of Health and Welfare has approved 148 Kampo prescription drugs, including JTT, for reimbursement under the National Health Insurance system. JTT has been widely administered to Japanese with very few side effects. Recently, basic and clinical research has demonstrated the effectiveness of Kampo medicines, thereby justifying their wider use [1–9].

Ohya et al. reported the effectiveness of JTT in infants with fistula-in-ano [16]. Fistula-in-ano has a range of clinical features; it is often intractable, has a high relapse rate, and it usually disappears spontaneously before about 15 months of age as immune function matures. They reported that administration of JTT accelerates the recovery of fistula-in-ano patients, and reduces the size of peri-anal abscesses, leading to remission in 90.9% patients.

Otitis media is also a unique disease of infancy. It is thought that the immaturity of immune function, such as lower serum IgG2 levels [13,14] and low concentration of serum anti-P6 IgG [15], is the main reason that a majority of otitis-prone patients are under 3 years of age, and it is also reported that response and development of immune function are poor in otitis-prone children [14,15]. Despite the standard treatment for AOM including oral administration of antibiotics and tympanostomy, some patients are unable to recover from intractable infection, and require hospitalization for intravenous administration of antibiotics or immunoglobulin [17]. Intractable inflammation of the middle ear also occasionally leads to serious conditions, such as mastoiditis, meningitis, cerebritis, subperiosteal abscess of mastoid process, and sigmoid sinus thrombophlebitis. Moreover, although otitis-prone infants might initially recover from otitis media, they are susceptible to relapse as upper airway infections and/or purulent otitis media. Surgical insertion of a tympanostomy tube is one of the subsequent treatment options [18], but this procedure is associated with problems, such as the subsequent necessity of tube removal and the possibility of persistent tympanic perforation.

In the present study, we administered JTT to otitis-prone infants in an effort to improve their immune function. The effects of JTT on otitis-prone individuals had not been investigated before. We showed that the frequency of AOM decreased significantly after JTT administration, as compared with before. Significant decreases in the duration of fever and antibiotics administration, and in number of hospital visits and emergent hospital visits were seen with oral administration of JTT. These results suggest that JTT administration to otitis-prone infants also has benefits with regard to medical economics.

Twenty-one of 24 children (87.5%) had no difficulties in taking JTT. No apparent side effects were recognized. Two of three patients who could not take JTT required surgical ventilation tube insertion for recurrent otitis media. Only 1 of the 21 cases (one of 42 ears) taking JTT needed the surgery after recurrence of otitis media, while the other 20 patients (95.2%) showed remission.

Resumption of JTT administration was considered for children who exhibited frequent relapse of otitis media and/or other infections after the first administration period ended. In fact, 14 of 21 (66.7%) patients started to take JTT again. It was also shown that the frequency of AOM increased significantly during the intermission period and again decreased after resumption of JTT.

JTT is thought to be an effective treatment for otitis-prone cases, and avoidance of the overuse of antibiotics will help prevent the emergence of drug-resistant bacteria. With regard to the onset and progression of infection, increased host immune function may be a viable alternative approach for treatment.

This investigation was an open trial and we observed significant therapeutic effects for JTT. However, a randomized, controlled trial is required to further clarify the efficacy of JTT in otitis-prone children.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Maruyama H, Kawamura H, Takemoto N, Komatsu Y, Aburada M, Hosoya E. Effect of Juzentaihoto on phagocytes. Jpn J Inflamm 1988;8:461–5 (in Japanese).

[2] Kubota A, Okamura S, Shimoda N, Haranaka R, Omori F, Niho Y. A traditional Chinese herbal medicine, Juzen-taiho-to, augments the production of granulocyte/macrophage colony-stimulating factor from human peripheral blood mononuclear cells in vitro. Int J Immunother 1992;8:191–5.

[3] Hamada M, Fujii Y, Yamamoto H, Miyazawa Y, Shui SM, Yung YC, et al. Effect of herbal medicine, Zyuzentaihoto, on the immune reactivity of tumor-bearing mice. J Ethnopharma 1988;24:311–20.

[4] Kiyohara H, Takemoto N, Komatsu Y, Kawamura H, Hosodya E, Yamada H. Characterization of mitogenic peptic polysaccharides from kampo (Japanese herbal) medicine "juzen-taiho-to". Planta Med 1991;57:254–9.

[5] Ohnishi Y, Fujii H, Hayakawa Y, Sakukawa R, Yamaura T, Sakamoto T, et al. Oral administration of a kampo (Japanese herbal) medicine Juzen-taiho-to inhibits liver metastasis of colon 26-L5 carcinoma cells. Jpn J Cancer Res 1998;89:206–13.

[6] Takanashi H, Nakazawa S. Antitumour effect of Juzen-taiho-to, a kampo medicine, for transplanted malignant glioma. Int J Immunother 1995;11:65–9.

[7] Sugiyama K, Ueda H, Ichio Y. Protective effect of Juzen-taiho-to against Carboplatin-induced toxic side effects in mice. Biol Pharm Bull 1995;18:53–8.

[8] Ohnishi Y, Yasuzumi R, Fan H, Liu L, Takao-Liu F, Komatsu Y, et al. Effects of Juzen-taiho-toh (TJ-48), a traditional oriental medicine, on hematopoietic recovery from radiation injury in mice. Exp Hematol 1990;18:18–22.

[9] Abe S, Tansho S, Ishibashi H, Inagaki N, Komatsu Y, Yamaguchi H. Protective effect of oral administration of a traditional medicine, Juzen-Taiho-To, and its components on lethal Candida albicans infection in immunosuppressed mice. Immunopharmacol Immunotoxicol 1998;20:421–31.

[10] Kempen MJ, Vaneechoutte M, Claeys G, Verschraegen GL, Vermeiren J, Dhooge I J. Antibiotic susceptibility of acute otitis media pathogens in otitis-prone Belgian children. Eur J Pediatr 2004;163:524–9.

[11] Sahm DF, Benninger MS, Evangelista AT, Yee YC, Thornsberry C, Brown N P. Antimicrobial resistance trends among sinus isolates of *Streptococcus pneumoniae* in the United States. Otolaryngol Head Neck Surg ;136 2001;2007:385–9.

[12] Jones ME, Karlowsky JA, Draghi DC, Thornsberry C, Sahm DF, Bradley J S. Rates of antimicrobial resistance among common bacterial pathogens causing respiratory, blood, urine, and skin and soft tissue infections in pediatric patients. Eur J Clin Microbiol Infect Dis 2004;23:445–55.

[13] Hayashibara H, Tanimoto K, Nagata I, Harada Y, Shiraki K. Normal levels of IgG subclass in childhood determined by a sensitive ELIZ A. Acta Paediatr Jpn 1993;35:113–7.

[14] Hotomi M, Yamanaka N, Saito T, Shimada J, Suzumoto M, Suetake M, et al. Antibody response to the outer membrane protein P6 of non-typeable *Haemophilus influenzae* and pneumococcal capsular polysaccharides in otitis-prone children. Acta Otolaryngol 1999;119:703–7.

[15] Yamanaka N, Faden H. Antibody response to outer membrane protein of non-typeable Haemophilus influenzae in otitis prone children. J Pediatr 1993;122:212–8.

[16] Ohya T, Usui Y, Okamoto K, Inoue Y, Arii S, Iwai T. Management for fistula-in-ano with Ginseng and Tang-kuei Ten Combination. Pediatr Int 2004;46:72–6.

[17] Ishizaka A, Sakiyama Y, Otsu M, Ozutsumi K, Matsumoto S. Successful intravenous immunoglobulin therapy for recurrent pneumococcal otitis media in young children. Eur J Pediatr 1994;153:174–8.

[18] Rosenfeld R M. Surgical prevention of otitis media. Vaccine 2000;8(Suppl 1):134–9.

資料6

11.5.25

医療技術実用化総合研究（2009−2011年）
H21-臨床研究-一般-007

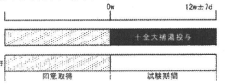
小児反復性中耳炎に対する十全大補湯の有用性に関する、多施設共同二重盲検ランダム化比較試験

統合医療分野の評価技術の開発に関する研究

金沢大学大学院医学系研究科
感覚運動病態学
研究代表者：吉崎 智一

研究の概要 1

- 乳幼児難治性急性中耳炎に対する、十全大補湯の効果を検討し、統合医療分野の評価法を確立する．
- 非盲検



(Randomized, Parallel-group, Open-label, Non-Kampo controlled Trial)

十全大補湯の偽薬開発に技術的な問題が発生．
偽薬完成には相応の時間を要するため、研究期間内に十分な症例数を確保するために、非盲検に変更した．

研究の概要 2

全国7大学、合計25医療施設が参加し、
現在目標症例数の約70%の登録．

急性中耳炎ガイドライン
委員会メンバー 6名
生物統計学専門家 1名

7大学病院
金沢大学
東京医科歯科大学
東北大学
長崎大学
富山大学
和歌山県立医科大学
旭川医科大学

市中病院 6施設
診療所 12施設

研究の背景

反復化・難治化する小児急性中耳炎

急性中耳炎は乳幼児の上気道感染症において最も頻度の高い疾患
生後3歳までに、約50−70%の小児が最低1回は罹患
2歳未満で罹患した場合、約50%が反復化・難治化する

近年、抗菌薬の投与では改善しない、または再発を繰り返す抗菌薬治療の限界を示す難治症例が増加

原因
- 中耳炎起炎菌の薬剤耐性化
- 集団保育の低年齢化

➡ 免疫学的に未熟な2歳未満で大きな問題となる

研究の背景

抗菌薬治療の限界
免疫未熟な乳幼児の
反復性中耳炎の増加

➡ 緊急に抗菌薬を補完する手段が必要！

十全大補湯（漢方補剤）

体力の向上
免疫賦活作用
感染防御効果

細菌を殺す治療から
細菌との共存をめざす
治療から予防へ

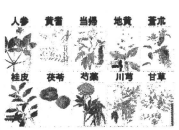
探索的臨床研究の結果

難治性急性中耳炎児症例に対して
十全大補湯エキス　0.14g/kg／日
1日2回投与、3か月間の継続服用

Effects of Japanese Herbal Medicine *Juzen-taiho-to* in otitis-prone children - a preliminary study
Acta Otolaryngol 2009

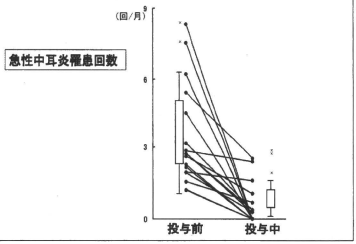
小児反復性中耳炎に対する十全大補湯の効果
耳鼻臨床 2007

小児急性中耳炎診療ガイドライン2009
付記：反復性中耳炎の取り扱い

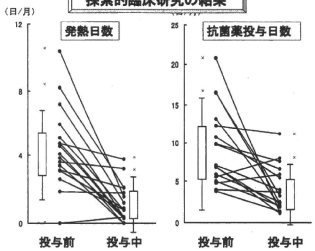


十全大補湯の構成生薬

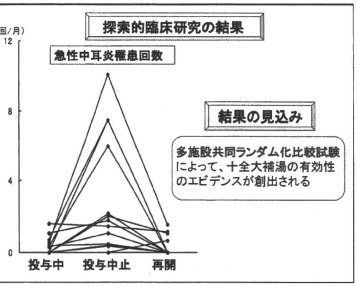
探索的臨床研究の結果



探索的臨床研究の結果



探索的臨床研究の結果



試験の実施計画と進捗状況

	平成21年度	平成22年度	平成23年度
実施計画	プロトコール委員会開催 研究計画の決定 IRB申請・承認 スタートアップミーティング開催 試験開始 患者エントリー	試験実施 結果収集 統計解析計画書を作成	試験実施登録終了 判定委員会（解析用 本審査） 統計解析 解析論文提出

	21年度	22年度
進捗状況	5月：第1回プロトコール委員会 9月：第2回プロトコール委員会 Web登録システムの整備 10月：IRB申請開始，承認 11月：スタートアップミーティング 11月末：臨床試験開始	4月：結果収集開始 5月：第2回班会議 実務上の問題点を改善 11月：統計解析計画書作成 CONSORT2010声明に準拠

症例登録状況

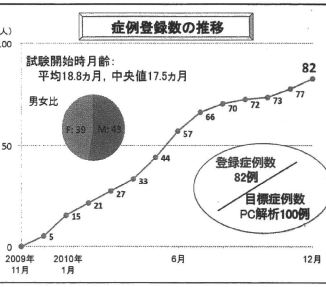
解析対象集団は ① ITT (Intention-to-treat) 解析対象集団
（ランダム割り付けされた症例数）
② PC (Protocol compatible) 解析対象集団
（12週完遂した症例数）

登録症例数の目標

PC 解析対象症例：投与、非投与各50例
合計：100例

2010年12月（**試験開始後1年経過時**）
両群の登録症例数の合計：82例

症例登録数の推移



試験進捗状況

- 2011年1月末の調査票回収状況
  ITT解析対象症例　61 例
  投与群: 28 例, 非投与群: 33 例
  脱落症例数　投与群: 3 例, 非投与群: 3 例
  PC解析症例 55 例
  投与群: 25 例, 非投与群: 30 例
  脱落症例数　投与群: 2 例, 非投与群: 2 例

今後の予定

・目標登録終了後、判定委員会にてデータを評価（本審査）
　評価データについて統計解析、論文作成

研究の期待される結果

・**事業課題との一致**
　さらに症例数を蓄積することによって、
　統合医療分野のエビデンス評価法を確立できる

・**経済効果**
　中耳炎難治化に伴う医療資源・コストの節減

・**社会的効果** ⟹ **少子化対策** ⟸
　頻回な通院による保護者（**特に保育園児の母**）の負担が軽減され、保護者の労働資源の確保

・**危機的状況にある耐性菌の蔓延に対して**
　抗菌薬の濫用を抑制し
　耐性菌の減少に寄与 ⟹ **医療経済的効果は大きい**

統合医療分野の評価技術の開発に関する研究

研究の展望

・反復性中耳炎に対する**我国発の統合医療として、国内外に広く発信**する

　「小児急性中耳炎診療ガイドライン 20XX」に反復性中耳炎のスタンダード治療として記載される

・抗菌薬治療を強力に補完する統合医療として、**他の難治性細菌感染症に対しても応用**可能

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Clonal spread of β-lactamase-producing amoxicillin–clavulanate-resistant (BLPACR) strains of non-typeable *Haemophilus influenzae* among young children attending a day care in Japan

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Pulsed-field gel electrophoresis (PFGE)
Day care

ABSTRACT

Objective: Resistant strains of non-typeable *Haemophilus influenzae* (NTHi) are one of the principal causes of recurrent acute otitis media (otitis prone), rhinosinusitis, and pneumonia in young children. β-Lactamase-nonproducing ampicillin-resistant (BLNAR) strains are particularly common in Japan, and β-lactamase-producing amoxicillin–clavulanate resistant (BLPACR) strains are now emerging. We investigated the nasopharyngeal carriage status of these resistant strains among children attending a same day care center during a 10-year period.

Methods: From 1999 to 2008, we obtained nasopharyngeal swab specimens from young children attending a same day care center and examined the incidence of resistant strains of NTHi. Antimicrobial resistance of NTHi was identified based on PCR analysis of mutation of the penicillin binding protein (PBP) genes. Pulsed-field gel electrophoresis (PFGE) was performed to examine the clonal relationship of each resistant strain.

Results: The prevalence of resistant strains of NTHi among the children attending this day care has significantly increased during the past 10 years and most of this day care children recently have resistant strains with PBP gene mutations in their nasopharynx. Genetically BLPACR (gBLPACR) strains have rapidly increased since 2007 and PFGE analysis demonstrated that all gBLPACR were clonally identical. This is the first report of apparent clonal dissemination of gBLPACR strains of NTHi occurring in a certain environment such as day care.

Conclusions: The rapidly increasing prevalence of resistant strains, in particular gBLPACR, in this day care center may predict a high incidence of these resistant bacteria from clinical isolates in the near future and potential serious medical problems worldwide.

1. Introduction

Non-typeable *Haemophilus influenzae* (NTHi) and *Streptococcus pneumoniae* (*S. pneumoniae*) are the principal causes of acute otitis media (AOM), pneumonia and meningitis in young children. Recently, rapid increases in β-lactamase-nonproducing ampicillin-resistant (BLNAR) strains of NTHi and a penicillin-resistant (Pc^r) strain of *S. pneumoniae* from respiratory tract specimens have been reported in Japan [1,2]. BLNAR strains of NTHi and Pc^r *S. pneumoniae* are the principal causes of recurrent AOM (otitis prone) among young children, especially children attending day care.

Over the past three decades, a rapid increase in Pc^r *S. pneumoniae* has been reported in most areas of the world [3–5]. The high prevalence of Pc^r *S. pneumoniae* has resulted in the limited use of penicillin for the empirical treatment of infectious diseases [6]. These penicillin-resistant bacteria are becoming less susceptible to other commonly prescribed oral antimicrobial drugs, including the extended spectrum cephalosporins [7,8]. A similar

Abbreviations: AOM, acute otitis media; NTHi, non-typeable *Haemophilus influenzae*; gBLPACR, genetically β-lactamase-producing amoxicillin–clavulanate-resistant; gBLNAR, genetically β-lactamase-nonproducing ampicillin-resistant; gBLPAR, genetically β-lactamase-producing ampicillin-resistant; gBLNAS, genetically β-lactamase-nonproducing ampicillin-susceptible; Pc^r, penicillin resistance; *S. pneumoniae* (SP), *Streptococcus pneumoniae*; PCR, polymerase chain reaction; PBP, penicillin binding protein; RTIs, respiratory tract infections; PFGE, pulsed-field gel electrophoresis.

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situation is now emerging among NTHi isolates from patients with respiratory tract infections (RTIs) [9]. In Japan, the BLNAR strains were not identified before 1984 then increased 23.1–37.8% from 1996 to 1999. BLNAR strains are particularly common in Japan and France. BLNAR strains evolved significantly during the past decade in some countries while oral cephalosporin antimicrobial agents were being commonly used for the treatment of RTIs, including AOM and sinusitis. The increase in the percentage of BLNAR strains has led to serious problems in the treatment of infectious disease in Japan. Furthermore, another group of resistant strains with combined mechanisms of altered penicillin binding proteins (PBPs) and TEM β-lactamase classified as β-lactamase-producing amoxicillin–clavulanate-resistant (BLPACR) strains are now emerging [10,11]. BLPACR strains have another resistant mechanism of β-lactamase producing in addition to the BLNAR resistance with PBP gene mutations. BLPACR are highly resistant to amoxicillin/clavulanate and other commonly prescribed oral antimicrobial drugs compared to BLNAR. The high prevalence of BLPACR represents potentially more serious clinical problems than BLNAR.

Children attending day care centers have more frequent episodes of otitis prone than those cared for at home [12–14]. Several lines of studies have shown that the incidence of nasopharyngeal carriage of resistant strains of *S. pneumoniae* and NTHi is high in children attending day care centers, and attendance at a day care center is a risk factor for otitis prone [15,16]. Previously, we described a strong relationship between exposure to other children in day care and nasopharyngeal carriage of Pc^r *S. pneumoniae* among young children in Japan [1]. In addition to *S. pneumoniae*, the prevalence of nasopharyngeal carriage of other respiratory pathogens, such as NTHi and *Moraxella catarrhalis*, in children attending day care is also very high in Japan [1]. In contrast, the carriage rates of these pathogens from children who were healthy and cared for at home were much lower than those from day care children [1].

Recently, Ubukata et al. defined antimicrobial resistances of NTHi genetically based on the mutation status of the PBP genes [10]. BLPACR strains of NTHi were recently isolated and, at least for now, generally continue to be isolated at very low frequencies (0.04–2.5%) worldwide.

The resistance patterns of these RTIs pathogens differ and may change dramatically over time. In the present study, to reveal the bacteriological change in the nasopharyngeal flora of healthy children, the carriage status of antibiotic resistant strains of NTHi were examined at the same day care center over a 10-year period.

2. Materials and methods

From 1999 to 2008, we obtained nasopharyngeal swab specimens once a year from children between 5 months and 3 years of age attending the same day care center during January to March in Kanazawa City, Japan. Each child was sampled only one time in each year. None of the participants in these studies showed any symptoms of RTIs or AOM on the day of the survey. Written informed consent was obtained from the parents of each child. If a parent refused to consent, the child was excluded from the study.

The parents completed a self-reporting questionnaire at the time of consent. The questionnaire included questions about previous hospitalization and the number of episodes of AOM, rhinosinusitis and pneumonia. The number of participants was 34 in 1999, 42 in 2000, 36 in 2005, 35 in 2007 and 31 in 2008. Nasopharyngeal swab specimens for culture were obtained by trained investigators using aluminum shaft ear, nose and throat swabs inserted as far into the nose as possible, parallel to the roof of the mouth. Antimicrobial resistances of NTHi were identified based on polymerase chain reaction (PCR) analysis of mutation of the PBP genes as described by Ubukata et al. [10]. Four sets of primers were obtained from Wakunaga Pharmaceutical Co. (Hiroshima, Japan): P6 primers to amplify the P6 gene which encodes the P6 membrane protein specific for NTHi; TEM-1 primers to amplify a part of the bla_{TEM-1} gene; PBP3-S primers to identify an Asn526 → Lys amino acid substitution in the *ftsI* gene; and PBP3-BLN primers to identify an Asn526 → Lys and Ser385 → Thr amino acid substitution in the *ftsI* gene. On the basis of the PCR-based genotyping, the NTHi strains were classified into four genotypes: genetically β-lactamase-nonproducing ampicillin-susceptible strains (gBLNAS), without amino acid substitutions in the *ftsI* gene and β-lactamase gene; genetically BLNAR strains (gBLNAR), with an amino acid substitution in the *ftsI* gene but without the β-lactamase gene; genetically β-lactamase-producing ampicillin-resistant strains (gBLPAR), with the β-lactamase gene but without an amino acid substitution in the *ftsI* gene; and genetically BLPACR strains (gBLPACR), with β-lactamase gene and an amino acid substitution in the *ftsI* gene. In this study, we have designated PCR-based genotypes as gBLNAS, gBLNAR, gBLPAR and gBLPACR to distinguish them from the phenotypes, which are written without the introductory "g."

Pulsed-field gel electrophoresis (PFGE) was performed with all NTHi isolates obtained in 2008 to examine the clonal relationship of each resistant strain. Association between variables was assayed using the chi-squared test and Fisher Exact Test, using the StatView computer software (Abacus Concepts, Inc., Baltimore, MD, USA).

3. Results

A total of 178 nasopharyngeal swab specimens from young children in the day care center were examined in this study. The mean age of the children was 20.4 months (1999), 25.1 months (2000), 22.9 months (2005), 24.1 months (2007), and 25.1 months (2008). Most of the children entered this day care in April and we obtained nasopharyngeal swab specimens during January to March. These children have been in this day care at least 9 months.

Multiple pathogenic bacteria were cultured from the nasopharynx. The chief bacteria found in children attending day care were *S. pneumoniae* (148 strains, 83.1%), NTHi (155 strains, 87.1%) and MC (138 strains, 77.5%). Nasopharyngeal carriage of these specific respiratory pathogens in each year is listed in Table 1. In 1999, 26 strains (76.5%) of NTHi were isolated from the nasopharynx of 34 children attending the day care center. The carrier rates of NTHi were 39/42 (92.9%) isolates in 2000, 34/35 (97.1%) isolates in 2007 and 29/31 (93.5%) in 2008. Recently, almost all young children attending this day care center were carriers of NTHi. The difference of carriage rates of NTHi was not statistically different in each year.

The percentages of the resistant isolates for NTHi in each year are listed in Table 2. In 1999, gBLNAR were identified in 6/26 (23.1%) NTHi isolates. The other 20/26 (76.9%) strains of NTHi were gBLNAS. None of the children showed AOM or RTIs symptoms on the day of the medical examination. In 2000, antibiotic resistance in NTHi isolates was 11/39 (28.3%) and resistant rate is almost same as 1999. In 2005, the carrier rate of gBLNAR had significantly increased to 21/27 (77.8%) NTHi isolates in the same day care center ($p < 0.05$). In 2007, gBLNAR were identified in 6/34 (17.6%) of isolates. gBLPACR isolates of NTHi were initially identified in 10/34 (29.4%) isolates in 2007. The total carrier rate of antibiotic resistance in NTHi was 16/34 (47.1%) isolates. In 2008, the carrier rate of resistant strains (gBLNAR + gBLPACR) was 28/31 (90.3%) children and the carrier rate of antibiotic resistance in NTHi was 28/29 (96.6%). The incidence of gBLPACR significantly increased to 24/29 (82.8%) isolates ($p < 0.01$) compared with that before 2005. In summary, the prevalence of gBLNAR among the children attending day care significantly increased from 1999–2000 to

Table 1
Nasopharyngeal carriage of specific respiratory pathogens in children attending a day care. Almost all young children attending this day care center were carriers of SP and/or NTHi.

Year	No. of children	No. (%) of SP	No. (%) of NTHi	No. (%) of MC	No. (%) of non-SP, NTHi
1999	34	33 (97.1)	26 (76.5)	17 (50.0)	0 (0)
2000	42	28 (66.7)	39 (92.9)	37 (88.1)	1 (2.4)
2005	36	32 (88.9)	27 (75.0)	36 (100)	3 (8.3)
2007	35	32 (91.4)	34 (97.1)	30 (85.7)	0 (0)
2008	31	23 (74.2)	29 (93.5)	18 (58.1)	1 (3.2)
Total	178	148 (83.1)	155 (87.1)	138 (77.5)	5 (2.8)

Abbreviations: NTHi: non-typeable *Haemophilus influenzae*; SP: *Streptococcus pneumoniae*; MC: *Moraxella catarrhalis*.

2005. Furthermore, gBLPACR strains of NTHi have been rapidly increasing since 2007. As a result, the total carrier rate of all resistant strains of NTHi (gBLNAR and gBLPACR) in the children attending this day care center significantly increased during the past decade. Most of the children in this day care recently have resistant strains of NTHi, which had acquired PBP gene mutations, in their nasopharynx.

3.1. PCR-based genotypes and MICs distributions

The MICs of gBLPACR, gBLNAR and gBLNAS for eight antimicrobial agents against are listed in Table 3. The MIC50s and MIC90s of amoxicillin/clavulanate and other β-lactams for resistant strains of NTHi were higher than those for gBLNAS isolates (Table 3).

3.2. Genetic distribution of NTHi strains by PFGE

PFGE analysis classified the NTHi strains into five PFGE types. Although the PFGE patterns of gBLNAR and gBLNAS (total five strains) were classified into four different patterns, all of the gBLPACR (23 strains) were clonally identical and were different from the other strains of NTHi (Fig. 1). Our PFGE results demonstrate the first evidence that the apparent clonal dissemination of gBLPACR of NTHi occurred in a certain environment such as day care.

4. Discussion

In this study, we focused on resistant strains of NTHi isolated from the nasopharynx of young children attending day care and investigated clonal dissemination of gBLPACR. NTHi and *S. pneumoniae* are recognized as part of the normal nasopharyngeal flora in healthy children but remain a major cause of bacterial infections of the respiratory tract, such as pneumonia, rhinosinusitis, and AOM [1,17]. Hotomi et al. demonstrated that the MIC50s of amoxicillin/clavulanate and cephalosporin for gBLNAR isolates were 4–64 times higher than those for gBLNAS isolates [18]. PBP gene mutations of NTHi, identified by PCR, were related to antibiotic resistance. Intractable infections due to resistant strains of these pathogens have become a significant clinical problem worldwide. Single or occasional episodes of AOM are relatively easy to treat, but recurrent episodes of AOM (otitis prone) would represent an onerous burden for both child and caregiver. The high prevalence of BLNAR and Pc^r *S. pneumoniae* in Japan is a serious problem and is thought to be the principal cause of otitis prone seen among young children. BLNAR isolates of NTHi were first reported in 1980 [19] and generally continue to be isolated at low frequencies in western Europe and the USA [20–23]. However, recent surveillance studies have reported higher proportions of BLNAR isolates [10,11,17,24,25]. There have been reports from many countries such as Spain, France, Poland, India, Korea and Japan, in which 9.3%, 18.6%, 12.8%, 14.4%, 29.3% and 13.9–65.1%, respectively, of NTHi isolates were BLNAR strains [2,11,24–29]. In Japan, this strain appeared in 1997 and increased rapidly among isolates from patients with RTIs, AOM and rhinosinusitis. The mechanism of resistance in the BLNAR strains involves decreased affinities of PBPs for β-lactam antibiotics [29]. In the present study, we have reported a high prevalence of resistant strains of NTHi among young children attending a single day care in Japan. The carrier rates of gBLNAR in the NTHi isolates increased from 23.1–28.2% in 1999–2000 to 77.8% in 2005. The carrier rate of resistant strains (gBLNAR and gBLPACR) in the NTHi isolates was quite high (96.6%) in 2008. Furthermore, NTHi were isolated from about 95% of children attending this day care center recently and these carrier rates were much higher than those from clinical specimens [25,28–31]. Thus the carrier rate of resistant strains (gBLNAR and gBLPACR) of NTHi in children attending this day care center has been rapidly increasing during the past decade. Because day care attendance is a risk factor for upper RTIs and otitis prone, both the high prevalence of resistant strains in the isolates and the very high isolate rates of NTHi from healthy young children potentially pose significant clinical problems.

The prevalence of resistance strains of NTHi varies in different countries but gBLPACR strains of NTHi are isolated at very low frequencies around the world [11,31], except in Korea where its frequency is relatively high (8.3%) [27]. In Japan, β-lactamase-producing strains were identified in 2.5% of isolates and β-lactamase-producing strains with mutations in ftsI (gBLPACR) were identified in 0.3% of isolates [25]. The most important finding in the present study was that gBLPACR of NTHi were initially

Table 2
Nasopharyngeal carriage of NTHi in children attending a day care. Changes in resistance among NTHi strains, by year, as identified by PCR. The carrier rates of gBLPACR were dramatically increased after 2007.

No. (%) of NTHi isolates	1999	2000	2005	2007	2008
gBLPACR	0	0	0	10 (29.4)	24 (82.8)
gBLNAR	6 (23.1)	11 (28.2)	21 (77.8)	6 (17.6)	3 (10.3)
gBLNAS	20 (76.9)	28 (71.8)	6 (22.2)	18 (52.9)	2 (6.9)
Total NTHi	26 (100)	39 (100)	27 (100)	34 (100)	29 (100)

Abbreviations: NTHi: non-typeable *Haemophilus influenzae*; gBLPACR: genetically β-lactamase-producing amoxicillin–clavulanate-resistant; gBLNAR: genetically β-lactamase-nonproducing ampicillin-resistant; gBLNAS: genetically β-lactamase-nonproducing ampicillin-susceptible.

Table 3
MIC distributions for eight antimicrobial agents against 24 isolates of gBLPACR (upper), 3 isolates of gBLNAR (middle) and 2 isolates of gBLNAS (lower) in 2008.

	PCR-based genotype	No. of isolates with MIC (μg/ml)											MIC (μg/ml)		
		≤0.008	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	Range	50%	90%
GRNX	gBLPACR	21	3										≤0.008–0.015	≤0.008	0.015
	gBLNAR	3											≤0.008	≤0.008	≤0.008
	gBLNAS	2											≤0.008	≤0.008	≤0.008
LVFX	gBLPACR		24										0.015	0.015	0.015
	gBLNAR		3										0.015	0.015	0.015
	gBLNAS	1	1										≤0.008–0.015	≤0.008	0.015
GFLX	gBLPACR		23	1									0.015–0.03	0.015	0.015
	gBLNAR	3											≤0.008	≤0.008	≤0.008
	gBLNAS	2											≤0.008	≤0.008	≤0.008
MFLX	gBLPACR		24										0.015	0.015	0.015
	gBLNAR		3										0.015	0.015	0.015
	gBLNAS		2										0.015	0.015	0.015
CDTR-PI	gBLPACR				24								0.06	0.06	0.06
	gBLNAR			2			1						0.03–0.25	0.03	0.25
	gBLNAS	1	1										≤0.008–0.015	≤0.008	0.015
AMPC/CVA	gBLPACR										1	23	4–8	8	8
	gBLNAR							1	1			1	0.5–8	1	8
	gBLNAS						1	1					0.25–0.5	0.25	0.5
AZM	gBLPACR					1		6	17				0.125–1	1	1
	gBLNAR							2		1			0.5–>2	0.5	2
	gBLNAS						1	1					0.25–0.5	0.25	0.5
ABPC	gBLPACR									24(>2)			>2	>2	>2
	gBLNAR							2		1 (>2)			0.5–>2	0.5	>2
	gBLNAS							2					0.5	0.5	0.5

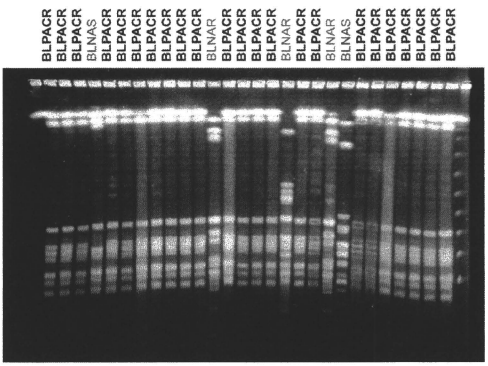

Fig. 1. PFGE analysis of NTHi isolates in 2008. All of the gBLPACR were clonally identical and were different from the other strains of NTHi.

identified in 29.4% of subjects in 2007 and then this rate markedly increased to 82.8% in 2008. The present study is the first report to show that gBLPACR strains of NTHi are capable of spreading in a certain environment such as a day care center. Because gBLPACR of NTHi are resistant to amoxicillin/clavulanate and other commonly prescribed oral antimicrobial drugs, the high prevalence of gBLPACR will cause significant problems in the clinical setting of treating community-acquired infections, including pneumonia, AOM, and rhinosinusitis.

Another important finding of the present study is that all gBLPACR isolates were clonally identical by PFGE analysis and were different from the other strains of NTHi. Our PFGE analysis revealed clonal spread of only one gBLPACR isolate with altered PBPs in this day care center. This represents the first evidence of the apparent clonal epidemic of gBLPACR of NTHi occurred. The contributions of clonality to the isolation of gBLPACR were not determined in previous studies. The present study demonstrated that gBLPACR of NTHi are capable of broad dissemination worldwide, similar to the gBLNAR. The clinical significance of gBLPACR of NTHi is as yet unknown. However, the MICs of amoxicillin–clavulanate for all gBLPACR in the present study were high (8 μg/ml). Furthermore, gBLPACR isolates are generally highly ampicillin-resistant and show elevated levels of resistance to expanded and broad-spectrum cefalosporins compared to non-gBLPACR. The high prevalence of gBLPACR from the clinical isolates may cause more serious problems than gBLNAR for the treatment of patients with otitis prone, intractable RTIs and rhinosinusitis. The rapidly increasing prevalence of resistant strains of NTHi in this day care center may predict a high incidence of these resistant bacteria being isolated from clinical samples in the near future and possible serious medical problems worldwide.

5. Conclusions

Most of the children attending this day care center recently had resistant strains of NTHi with PBP gene mutations in their nasopharynx. gBLPACR strains have rapidly increased since 2007. PFGE analysis demonstrated that all gBLPACR strains in 2008 were clonally identical. This represents the first report in the would about the apparent clonal dissemination of gBLPACR of NTHi occurred like an epidemic. Because BLPACR strains are generally multi-drug resistant, BLPACR strains are now emerging and clonal spread of BLPACR observed in this study might be an alarm of increasing more serious bacterial infectious diseases with NTHi in the future.

Conflict of interest

We have no conflict of interest with any other people or organizations.

Acknowledgements

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References

[1] M. Ito, K. Ito, T. Yoshizaki, T. Nishimura, T. Miwa, M. Furukawa, Nasopharyngeal penicillin-resistant *Streptococcus pneumoniae* strains among young children in Japan, Otol. Neurotol. 23 (2002) 349–352.
[2] K. Hasegawa, N. Chiba, R. Kobayashi, S.Y. Murayama, S. Iwata, K. Sunakawa, et al., Rapidly increasing prevalence of beta-lactamase-nonproducing, ampicillin-resistant *Haemophilus influenzae* type b in patients with meningitis, Antimicrob. Agents Chemother. 48 (2004) 1509–1514.
[3] N. Chiba, R. Kobayashi, K. Hasegawa, M. Morozumi, E. Nakayama, T. Tajima, et al., Antibiotic susceptibility according to genotype of penicillin-binding protein and macrolide resistance genes, and serotype of *Streptococcus pneumoniae* isolates from community-acquired pneumonia in children, J. Antimicrob. Chemother. 56 (2005) 756–760.
[4] S.S. Richter, K.P. Heilmann, S.L. Coffman, H.K. Huynh, A.B. Brueggemann, M.A. Pfaller, et al., The molecular epidemiology of penicillin-resistant *Streptococcus pneumoniae* in the United States, 1994–2000, Clin. Infect. Dis. 34 (2002) 330–339.
[5] C.Y. Lu, P.I. Lee, P.R. Hsueh, S.C. Chang, T.F. Chiu, H.C. Lin, et al., Penicillin-nonsusceptible *Streptococcus pneumoniae* infections in children, J. Microbiol. Immunol. Infect. 32 (1999) 179–186.
[6] K. Nagai, Y. Shibasaki, K. Hasegawa, T.A. Davies, M.R. Jacobs, K. Ubukata, et al., Evaluation of PCR primers to screen for *Streptococcus pneumoniae* isolates and beta-lactam resistance, and to detect common macrolide resistance determinants, J. Antimicrob. Chemother. 48 (2001) 915–918.

[7] K. Nagai, T.A. Davies, M.R. Jacobs, P.C. Appelbaum, Effects of amino acid alterations in penicillin-binding proteins (PBPs) 1a, 2b, and 2x on PBP affinities of penicillin, ampicillin, amoxicillin, cefditoren, cefuroxime, cefprozil, and cefaclor in 18 clinical isolates of penicillin-susceptible, -intermediate, and -resistant pneumococci, Antimicrob. Agents Chemother. 46 (2002) 1273–1280.
[8] M.R. Jacobs, D. Felmingham, P.C. Appelbaum, R.N. Gruneberg, The Alexander Project 1998–2000: susceptibility of pathogens isolated from community-acquired respiratory tract infection to commonly used antimicrobial agents, J. Antimicrob. Chemother. 52 (2003) 229–246.
[9] K. Suzuki, T. Nishimura, S. Baba, Current status of bacterial resistance in the otolaryngology field: results from the Second Nationwide Survey in Japan, J. Infect. Chemother. 9 (2003) 46–52.
[10] K. Ubukata, Y. Shibasaki, K. Yamamoto, N. Chiba, K. Hasegawa, Y. Takeuchi, et al., Association of amino acid substitutions in penicillin-binding protein 3 with beta-lactam resistance in beta-lactamase-negative ampicillin-resistant *Haemophilus influenzae*, Antimicrob. Agents Chemother. 45 (2001) 1693–1699.
[11] H. Dabernat, C. Delmas, M. Seguy, R. Pelissier, G. Faucon, S. Bennamani, et al., Diversity of beta-lactam resistance-conferring amino acid substitutions in penicillin-binding protein 3 of *Haemophilus influenzae*, Antimicrob. Agents Chemother. 46 (2002) 2208–2218.
[12] E.R. Wald, N. Guerra, C. Byers, Frequency and severity of infections in day care: three-year follow-up, J. Pediatr. 118 (1991) 509–514.
[13] E.R. Wald, B. Dashefsky, C. Byers, N. Guerra, F. Taylor, Frequency and severity of infections in day care, J. Pediatr. 112 (1988) 540–546.
[14] J.P. Collet, P. Burtin, M.S. Kramer, D. Floret, N. Bossard, T. Ducruet, Type of day-care setting and risk of repeated infections, Pediatrics 94 (1994) 997–999.
[15] K.E. Arnold, R.J. Leggiadro, R.F. Breiman, H.B. Lipman, B. Schwartz, M.A. Appleton, et al., Risk factors for carriage of drug-resistant *Streptococcus pneumoniae* among children in Memphis, Tennessee, J. Pediatr. 128 (1996) 757–764.
[16] J.L. Pons, M.N. Mandement, E. Martin, C. Lemort, M. Nouvellon, E. Mallet, et al., Clonal and temporal patterns of nasopharyngeal penicillin-susceptible and penicillin-resistant *Streptococcus pneumoniae* strains in children attending a day care center, J. Clin. Microbiol. 34 (1996) 3218–3222.
[17] K. Hasegawa, R. Kobayashi, E. Takada, A. Ono, N. Chiba, M. Morozumi, et al., High prevalence of type b beta-lactamase-non-producing ampicillin-resistant *Haemophilus influenzae* in meningitis: the situation in Japan where Hib vaccine has not been introduced, J. Antimicrob. Chemother. 57 (2006) 1077–1082.
[18] M. Hotomi, K. Fujihara, D.S. Billal, K. Suzuki, T. Nishimura, S. Baba, et al., Genetic characteristics and clonal dissemination of beta-lactamase-negative ampicillin-resistant *Haemophilus influenzae* strains isolated from the upper respiratory tract of patients in Japan, Antimicrob. Agents Chemother. 51 (2007) 3969–3976.
[19] S.M. Markowitz, Isolation of an ampicillin-resistant, non-beta-lactamase-producing strain of *Haemophilus influenzae*, Antimicrob. Agents Chemother. 17 (1980) 80–83.
[20] G.V. Doern, A.B. Brueggemann, G. Pierce, H.P. Holley Jr., A. Rauch, Antibiotic resistance among clinical isolates of *Haemophilus influenzae* in the United States in 1994 and 1995 and detection of beta-lactamase-positive strains resistant to amoxicillin–clavulanate: results of a national multicenter surveillance study, Antimicrob. Agents Chemother. 41 (1997) 292–297.
[21] J.A. Karlowsky, I.A. Critchley, R.S. Blosser-Middleton, E.A. Karginova, M.E. Jones, C. Thornsberry, et al., Antimicrobial surveillance of *Haemophilus influenzae* in the United States during 2000-2001 leads to detection of clonal dissemination of a beta-lactamase-negative and ampicillin-resistant strain, J. Clin. Microbiol. 40 (2002) 1063–1066.
[22] D. Felmingham, J. Washington, Trends in the antimicrobial susceptibility of bacterial respiratory tract pathogens—findings of the Alexander Project 1992–1996, J. Chemother. 11 (Suppl. 1) (1999) 5–21.
[23] G.V. Doern, Antimicrobial resistance among lower respiratory tract isolates of *Haemophilus influenzae*: results of a 1992–93 western Europe and USA collaborative surveillance study. The Alexander Project Collaborative Group, J. Antimicrob. Chemother. 38 (Suppl. A) (1996) 59–69.
[24] F. Marco, J. Garcia-de-Lomas, C. Garcia-Rey, E. Bouza, L. Aguilar, C. Fernandez-Mazarrasa, Antimicrobial susceptibilities of 1,730 *Haemophilus influenzae* respiratory tract isolates in Spain in 1998–1999, Antimicrob. Agents Chemother. 45 (2001) 3226–3228.
[25] M. Hotomi, K.F. Sakai, D.S. Billal, J. Shimada, M. Suzumoto, N. Yamanaka, Antimicrobial resistance in *Haemophilus influenzae* isolated from the nasopharynx among Japanese children with acute otitis media, Acta Otolaryngol. 126 (2006) 130–137.
[26] A. Skoczynska, M. Kadlubowski, I. Wasko, J. Fiett, W. Hryniewicz, Resistance patterns of selected respiratory tract pathogens in Poland, Clin. Microbiol. Infect. 13 (2007) 377–383.
[27] I.S. Kim, C.S. Ki, S. Kim, W.S. Oh, K.R. Peck, J.H. Song, et al., Diversity of ampicillin resistance genes and antimicrobial susceptibility patterns in *Haemophilus influenzae* strains isolated in Korea, Antimicrob. Agents Chemother. 51 (2007) 453–460.
[28] T. Kubota, F. Higa, N. Kusano, I. Nakasone, S. Haranage, M. Tateyama, et al., Genetic analyses of beta-lactamase negative ampicillin-resistant strains of *Haemophilus influenzae* isolated in Okinawa, Japan, Jpn. J. Infect. Dis. 59 (2006) 36–41.
[29] A. Sakai, M. Hotomi, D.S. Billal, K. Yamauchi, J. Shimada, S. Tamura, et al., Evaluation of mutations in penicillin binding protein-3 gene of non-typeable *Haemophilus influenzae* isolated from the nasopharynx of children with acute otitis media, Acta Otolaryngol. 125 (2005) 180–183.
[30] N. Yamanaka, M. Hotomi, D.S. Billal, Clinical bacteriology and immunology in acute otitis media in children, J. Infect. Chemother. 14 (2008) 180–187.
[31] K. Hasegawa, K. Yamamoto, N. Chiba, R. Kobayashi, K. Nagai, M.R. Jacobs, et al., Diversity of ampicillin-resistance genes in *Haemophilus influenzae* in Japan and the United States, Microb. Drug Resist. 9 (2003) 39–46.

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A case of chronic otitis media caused by *Mycobacterium abscessus*☆

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Abstract

Although it appears very uncommon in adult COM, *Mycobacterium abscessus* should be considered as a possible cause of a chronically draining ear. Multi-antibiotic chemotherapy including high-dose clarithromycin can effectively treat adult COM cased by *M. abscessus*. The first case report of adult chronic otitis media (COM) caused by *M. abscessus* is described here. A 61-year-old woman presented persistent otorrhea for 2 months, despite treatment with standard antimicrobial drugs. Physical examination revealed a small perforation of the tympanic membrane and edematous middle ear mucosa. Mycobacterial cultures and PCR yielded non-tuberculous mycobacteria (NTM); *M. abscessus*. Intravenous panipenem/betamipron and amikacin and oral clarithromycin were administered for 36 days. Computed tomography of the temporal bone showed improved aeration in the tympanic cavity, but soft tissue shadow remained unchanged in the mastoid 31 days after starting medication. She therefore underwent tympano-mastoidectomy at 36 days. At surgery, inflammation remained in the middle ear, and edematous pale mucosal tissue was noted around the stapes and ossicular chain. Histopathologic examination showed inflammation and granulation tissue, but no caseating necrosis or acid-fast bacilli. After surgery the symptoms resolved and remained well without evidence of infection recurrence 12 months after the operation.

Keywords: Non-tuberculous mycobacteria; *Mycobacterium abscessus*; Chronic otitis media; Multi-antibiotic chemotherapy; Surgery

1. Introduction

Most cases of infectious diseases caused by non-tuberculous mycobacteria (NTM) are resistant to antibiotic therapy. Among NTM, *Mycobacterium abscessus* (*M. abscessus*) is the most pathogenic and chemotherapy-resistant, and the most rapidly growing [12]. It is therefore a problematic infection requiring specific diagnosis and treatment. Ear, nose, and throat (ENT) infections caused by *M. abscessus* are very infrequent now, and most are cervico-facial lymphadenitis [14–16]. Only 10 cases of chronic otitis media (COM) caused by *M. abscessus* have been reported in the literature and all of them were intractable cases in children [5–8,10]. To the best of our knowledge, we present here the first case report of adult COM caused by *M. abscessus*. Multi-antibiotic chemotherapy including high-dose clarithromycin is potentially effective for adult *M. abscessus* otitis media.

2. Case report

A 61-year-old woman presented with recurrent right-sided otorrhea for seven years, and perforation of the tympanic membrane was diagnosed. In the episode in which she presented she had experienced right ear fullness and persistent otorrhea for 2 months, despite treatment with standard antimicrobial drugs. She did not have undue

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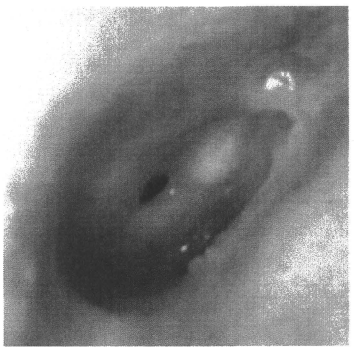

Fig. 1. The tympanic membrane showed a small perforation with copious, serous otorrhea.

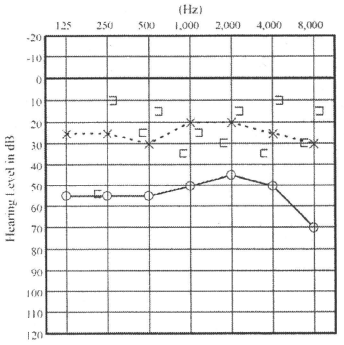

Fig. 3. Audiometry on March 3, 2008 revealed right conductive healing loss.

susceptibility to infections other than otitis media. Otoscopy revealed a small perforation of tympanic membrane with copious, serous otorrhea. The tympanic membrane and mucosa of the tympanic cavity showed edematous swelling (Fig. 1). Initial cultures did not yield any bacteria. Computed tomography (CT) showed a soft tissue shadow occupying the middle ear and the mastoid cavity but no bone destruction (Fig. 2). Laboratory studies revealed a normal white blood cell count and CRP. We suspected middle ear tuberculosis, and acid-fast bacillus stains revealed mycobacteria (Gaffky 3). However, polymerase chain reaction (PCR) for *M. tuberculosis* was negative. Eventually mycobacterial cultures and PCR yielded *M. abscessus*, a type of NTM. Chest CT revealed no evidence of lung mycobacterial infection. Audiometry revealed right conductive hearing loss (Fig. 3).

She received treatment with multiple antibiotics (600 mg/day oral clarithromycin, 200 mg/day intravenous amikacin, and 0.5 g/day intravenous panipenem/betamipron) for 36 days. The otorrhea stopped after 25 days of chemotherapy. CT of the temporal bone showed improved aeration in the tympanic cavity, but the soft tissue shadow remained unchanged in the attic and mastoid cavity 31 days after starting medication. Hence tympano-mastoidectomy was performed 36 days after starting medication. At operation, although serous otorrhea had already stopped, inflamed tissue remained in the middle ear cavity, and edematous pale mucosal tissue was noted around the stapes and ossicular chain. Histopathologic examination showed inflammation and granulation tissue, but no caseating necrosis or acid-fast bacilli.

After surgery the symptoms resolved and the right ear remained dry. She remained well without evidence of infection recurrence 12 months after the operation. Follow-

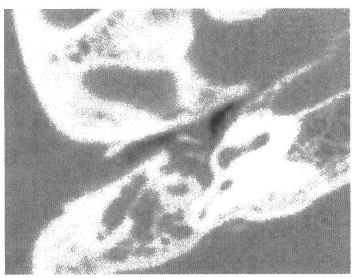

Fig. 2. Computed tomography on April 17, 2008 showed a soft tissue shadow occupying the middle ear and mastoid cavity.

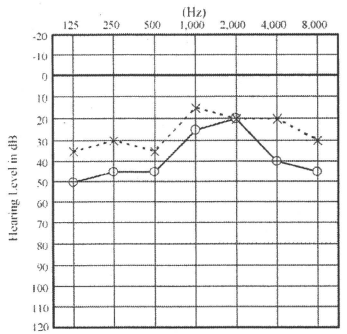

Fig. 4. Audiometry on July 11, 2008 showed improvement of the conductive healing loss.

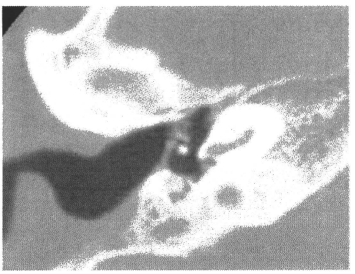

Fig. 5. Computed tomography on August 29, 2008 demonstrated good aeration in the tympanic cavity.

up audiometry revealed improvement of the conductive hearing loss (Fig. 4). Follow-up CT demonstrated good aeration in the tympanic cavity (Fig. 5).

3. Discussion

Runyon first classified NTM into 4 groups based on growth rate and pigment production [2] and *M. abscessus* belongs to the rapidly growing group (visible colony formation within 7 days of subculture) [11]. To become infected with NTM, it is necessary to aspirate the organism or become inoculated with it from a natural reservoir; so far there is no evidence of transmission from one human to another [12]. *M. abscessus* was first recognized by Moore and Frerichs in 1953 [3]. It is a ubiquitous organism that is commonly found in soil and water. *M. abscessus* can cause disseminated disease in immunocompromized individuals but is not as serious in immunologically normal hosts. Previously reported patterns of *M. abscessus* infection include pulmonary disease, lymphadenitis, and ulcerative skin lesions. *M. abscessus* infections typically follow surgery, or penetrating trauma, particularly that causing retained foreign bodies.

ENT infections caused by NTM, particularly *M. abscessus*, are very infrequent. The most common ENT manifestation of NTM infections is cervico-facial lymphadenitis [14–16]. We found only 10 cases of COM caused by *M. abscessus* in the literature. Most of these patients were systemically well children who presented with painless chronic otorrhea that failed to settle with aural toilet and antimicrobial chemotherapy. To the best of our knowledge, we herein report the first case of *M. abscessus* COM in an adult (Table 1).

NTM and *M. tuberculosis* infections of the ear have similarities in their clinical presentation, such as edematous tissue in the middle ear and chronic otorrhea that fails to settle with antimicrobial chemotherapy. On the other hand, *M. Tuberculosis* causes necrosis of the tympanic membrane and skin of the external auditory canal and can result in facial paralysis and necrosis of the nasopharynx, while NTM is less pathogenic and does not cause such destructive changes. CT of the temporal bone cannot differentiate NTM from other forms of otitis media or granulomatous disease but is generally performed to rule out bone erosion and intracranial complications [13,17,18]. Differentiation from other granulomatous diseases is performed by biopsy of the granulations and by culture looking specifically for NTM [9,18]. The diagnosis of *M. abscessus* infection is generally straightfor-

Table 1
M. abscessus patient information.

Authors	Age (year)/sex	Antibiotic treatment	Surgical treatment	Surgical revision	Outcome (hearing level)
Nylen et al. [10]	6/M	Clarithromycin	Radical mastoidectomy	Mastoidectomy	Residual 40-dB conductive hearing loss
Franklin et al. [5]	1/F	Erythromycin	Tympano-mastoidectomy	None	Residual mild conductive hearing loss
Franklin et al. [5]	2/M	Erythromycin	Examination, debridement, biopsy	Tympanoplasty	Not noted
Franklin et al. [5]	3/M	Erythromycin	Tympano-mastoidectomy	None	Normal hearing
Franklin et al. [5]	6/M	Erythromycin	Tympano-mastoidectomy	None	Residual mild mixed conductive and sensorineural hearing loss
Franklin et al. [5]	4/F	Erythromycin	Tympano-mastoidectomy	Tympano-mastoidectomy	Residual 40-dB conductive hearing loss
Franklin et al. [5]	1/M	Clarithromycin	None	None	Not noted
Van Aerem et al. [7]	2/M	Clarithromycin/ ciprofloxacin/ ethionamide	Mastoidectomy	Mastoidectomy (twice)	Residual 34-dB conductive hearing loss
Ferguson et al. [8]	5/M	Clarithromycin	None	None	Not noted
Limmans et al. [6]	4/M	Clarithromycin	Combined-approach tympanoplasty	Second look	Normal hearing
Our case	61/F	Clarithromycin/ panipenem/ betamipron	Tympano-mastoidectomy	None	Residual 30-dB conductive hearing loss

ward, i.e., the organism is an acid-fast, Gram-positive rod that resembles diphtheroid group on a Gram-stained smear. *M. abscessus* grows well on routine bacterial culture media. The cultures must be maintained for more than 4 days to allow sufficient time for growth of the organism [1,5]. In recurrent or persistent COM with otorrhea, mycobacterial cultures should be obtained to diagnose NTM and tuberculosis.

Until recently the treatment of *M. abscessus* was considered difficult. Rapidly growing mycobacteria are routinely resistant to standard anti-tuberculous drugs, and *M. abscessus* is particularly drug resistant. Spontaneous recovery is accordingly rare in these infections. Therapy consists of surgical debridement, removal of all foreign bodies, and long-term multi-antibiotic chemotherapy [4]. In the literature, surgical excision of the infected tissue is recommended for *M. abscessus* COM and long-term antimycobacterial chemotherapy with clarithromycin and amikacin is also recommended for *M. abscessus*. Clarithromycin should be given at high doses (600–800 mg/day) and the chief disadvantage of multi-antibiotic chemotherapy is gastrointestinal symptoms due to high-dose clarithromycin. When performing surgical debridement, as much infected tissue must be removed as possible to avoid multiple surgical interventions, because one study showed that almost 50% of all cases needed multiple surgical debridements before the infection resolved [5,7,9,10].

In the present adult case, the infection was successfully eradicated with multi-antibiotic treatment and tympanomastoidectomy. Multi-antibiotic chemotherapy including high-dose clarithromycin was very effective, and no bacteria including NTM or residual suppuration were observed in the middle ear on the day of surgery. On the basis of our experience with this case, we recommend multi-antibiotic chemotherapy including high-dose clarithromycin for the initial treatment for adult cases of COM caused by *M. abscessus*.

M. abscessus otitis media is usually found in well children presenting with painless chronic otorrhea. Although it appears very uncommon in adult otitis media, *M. abscessus* should be considered as a possible cause of a chronically draining ear in an adult. It is important to obtain mycobacterial cultures to diagnose *M. abscessus* infection.

Acknowledgements

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References

[1] Wallace Jr RJ, Swenson JM, Silcox VA. Spectrum of disease due to rapidly growing mycobacteria. Rev Infect Dis 1983;5:657–79.
[2] Runyon EH. Anonymous mycobacteria in pulmonary disease. Med Clin North Am 1959;43:273–90.
[3] Moor M, Frerichs JB. An unusual acid-fast infection of the knee with subcutaneous, abscess-like lesions of the gluteal region. J Invest Dermatol 1953;20:133–69.
[4] Lowry PW, Jarvis WR, Oberle AD. Chelonae causing otitis media in an ear-nose-throat practice. N Engl J Med 1988;319:978–82.
[5] Franklin DJ, Starke JR, Brady MT, Brown BA, Wallace Jr RJ. Chronic otitis media after tympanostomy tube placement caused by *Mycobacterium abscessus*. Am J Otol 1994;15(3):313–20.
[6] Limmans JJ, Stokroos RJ, Linssen CFM. *Mycobacterium abscessus*, an uncommon cause of chronic otitis media. Arch Otolaryngol Head Neck Surg 2008;134(9):1004–6.
[7] van Aerem A, Muytjens HL, Smits MM, Cremers CWRJ. Recurrent therapy resistant mastoiditis by *Mycobacterium cheilonae abscessus*, a nontuberculous mycobacterium. Int J Pediatr Otorhinolaringol 1998;43(1):61–72.
[8] Ferguson PJ, Saulscury FT. Successful treatment of chronic *Mycobacterium abscessus* otitis media with clarithromycin. Pediatr Infect Dis J 1996;15(4):384–5.
[9] Flint D, Mahadevan M, Gunn R, Brown S. Nontuberculous mycobacterial otomastoidaitis in children. Int J Pediatr Otorhinolaryngol 1999;51(2):121–7.
[10] Nylen O, Alestig K, Fath A. Infections of the ear with nontuberculous mycobacteria in three children. Pediat Infect Dis J 1994;13(7): 653–6.
[11] Prtrini B. *Mycobacterium abscessus*: an emerging rapid-growing potential pathogen. APMIS 2006;114(5):319–28.
[12] de Zins LOR, Tironi A, Nassif N, Ghizzardi D. Temporal bone infection caused by atypical mycobacterium: case report and review of the literature. Otol Neurol 2003;24:843–9.
[13] Cho Y-S. A clinical and radiologic analysis of 52 patients. Laryngoscope 2006;116(6):921–7.
[14] Cleary KR, Batsakis JG. Mycobacterial disease of the head and neck: current perspective. Ann Otol Rhinol Laryngol 1995;104: 830–3.
[15] Starke JR. Nontuberculous mycobacterial infection in children. Adv Pediatr Infect Dis 1992;7:123–59.
[16] Williams RG, Douglas-Jones T. Mycobacterium marches back. J Laryngol Otol 1995;109:5–13.
[17] Dalovisio JR, Pankey GA, Wallace RJ. Clinical usefulness of amikacin and doxycycline in the treatment of infection due to *Mycobacterium fortuium* and *Mycobacterium chelonei*. Rev Infect Dis 1981;3:1068–74.
[18] Moerman M, Dierick J, Mestdagh J. Mastoiditis caused by atypical *Mycobacteria*. Int J pediatr Otorhinolaryngol 1993;28:69–76.

Clonal Spread of β-lactamase-producing, amoxicillin-clavulanate-resistant (BLPACR) Strains of Non-typeable *Haemophilus influenzae* among Young Children Attending a Day Care in Japan

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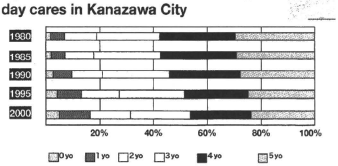
Is Day Care a risk factor for Otitis Prone?

Children attending day care centers have more frequent episodes of AOM than those cared for at home. Collet JP, Pediatrics 1994

Day Care is a risk factor for AOM and Otitis Prone !

Age distribution of children attending day cares in Kanazawa City



Need to know "Colonization of the nasopharynx in day care attendance"

Antimicrobial resistances of Non-typable *H. influenzae* (NTHi) were identified based on PCR analysis of mutation of the *pbp* genes.

gBLPACR: β-lactamase producing AMPC/CVA resistant with β-*lactamase* gene and mutation in the *ftsI* gene

gBLNAR: β-lactamase-nonproducing ampicillin-resistant with an amino acid substitution in the *ftsI* gene

gBLPAR: β-lactamase-producing ampicillin-resistant

gBLNAS: without mutation in the *ftsI* gene and β-*lactamase* gene

MATERIALS AND METHODS 1

We obtained nasopharyngeal swabs from healthy children in 1999 – 2000.

- Children (6m to 3 yo) attending a day care center (n=107)
- Children (6m to 3 yo) with acute otitis media (AOM) (n=144)
- Children (1.5 yo) at a public health examination (n=72)

Nasopharyngeal carriage of specific respiratory pathogens in each groups

(1999-2000)

groups	No.of swabs	No.(%)of Streptococcus pneumoniae	No.(%)of Heamophilus influenzae	No.(%)of Moraxella catarrhalis	No.(%)of Noncarriers
Day care center					
Children	107	88 (82)	90 (86)	78 (73)	6 (6)
Adults (Staffs)	15	0 (0)	7 (47)	2 (13)	6 (40)
Health examination					
Total	72	33 (46)	31 (43)	37 (51)	16 (22)
Day care attendance	10	10 (100)	7 (70)	10 (100)	0 (0)
Non-day care	62	23 (37)	24 (39)	27 (44)	16 (26)
Acute otitis media					
Total	144	118 (82)	70 (49)	55 (38)	8 (6)
≦3 years old	56	52 (93)	28 (50)	46 (56)	0 (0)