



ORIGINAL ARTICLE

Bacterial etiology of acute otitis media in the era prior to universal pneumococcal vaccination in Taiwanese children



Yen-Hsin Kung^a, Nan-Chang Chiu^{a,b}, Kuo-Sheng Lee^c,
Lung Chang^a, Daniel Tsung-Ning Huang^a, Fu-Yuan Huang^a,
Yu-Chia Hsieh^d, Li-Min Huang^{e,f}, Hsin Chi^{a,b,e,*}

^a Department of Pediatrics, Mackay Memorial Hospital, Taipei, Taiwan

^b Mackay Medicine, Nursing and Management College, Taipei, Taiwan

^c Department of Otorhinolaryngology, Mackay Memorial Hospital, Taipei, Taiwan

^d Department of Pediatrics, Chang Gung Children's Hospital, Chang Gung Memorial Hospital, Chang Gung University, College of Medicine, Taoyuan, Taiwan

^e Graduate Institute of Clinical Medicine, National Taiwan University College of Medicine, Taipei, Taiwan

^f Department of Pediatrics, National Taiwan University Hospital, Taipei, Taiwan

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Background: Acute otitis media (AOM) is one of the most frequent bacterial infections in children. *Streptococcus pneumoniae* and nontypeable *Haemophilus influenzae* (NTHi) are the two major bacterial pathogens. Pneumococcal conjugate vaccine was introduced into Taiwan in 2005 and only some children were vaccinated. This retrospective study assessed the bacterial etiology of AOM and its antimicrobial susceptibility in the era prior to universal pneumococcal vaccination in Taiwan.

Methods: From December 2009 to November 2011, children presenting with AOM and having a middle ear effusion sample collected by tympanocentesis were enrolled. The study period was divided into two parts. Demographic data of patients and antibiotic susceptibility of the pathogens were collected and analyzed. Serotypes of *S. pneumoniae* were identified.

Results: Among the 151 episodes, 46% of samples found bacterial pathogens. *S. pneumoniae* and NTHi were the leading causes of AOM, detected in 55.7% and 22.9% of bacterial AOM episodes, respectively. The prevalent serotypes of *S. pneumoniae* were 19 A and 19 F.

* Corresponding author. Department of Pediatrics, Mackay Memorial Hospital, Number 92, Section 2, Chung-Shan North Road, Taipei, 10449, Taiwan.

E-mail address: chi.4531@ms1.mmh.org.tw (H. Chi).

Significantly more pneumococcal and serotype 19 A AOM were found in the later study period (18.4% vs. 33.3%, $p = 0.0036$; 10.5% vs. 24.0%, $p = 0.028$). Among the 39 *S. pneumoniae* isolates, 11 strains (28.2%) were penicillin-susceptible. Of the 16 NTHi, 10 (62.5%) were susceptible to amoxicillin/clavulanate and all were susceptible to cefotaxime.

Conclusion: *S. pneumoniae* and NTHi were the leading causes of AOM in Taiwanese children in the study period. An increase in patient numbers and proportion of pneumococcal and serotype 19 A AOM occurred. Antimicrobial nonsusceptibility was common in the predominant pathogens.

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Introduction

Acute otitis media (AOM) is one of the most frequently encountered bacterial infections in children worldwide. The peak incidence of AOM is in the first 2 years of life, often between 6 months and 18 months of age.¹ Up to 80% of children experienced at least one episode of otitis media prior to age 3 years.² AOM is also the major cause for physician visits and the most common reason for antibiotic prescriptions in children.^{2–5} *Streptococcus pneumoniae* and nontypeable *Haemophilus influenzae* (NTHi) are the two major bacterial pathogens of AOM worldwide.^{6,7} Similar common causes of AOM were found in a previous study in Taiwan.⁸

The seven-valent pneumococcal conjugate vaccine (PCV7; Prevenar, Pfizer/Wyeth, New York) has been available as self-paid vaccine in Taiwan since October 2005. The 10-valent pneumococcal *H. influenzae* protein D conjugate vaccine (PHiD-CV; Synflorix, GlaxoSmithKline Biologicals, Belgium) was marketed in Taiwan in April 2010. The 13-valent pneumococcal conjugate vaccine (PCV13; Prevenar 13, Pfizer/Wyeth, New York) was marketed in Taiwan in April 2011. The publicly funded pneumococcal vaccine is only for high-risk groups from July 2009. The high-risk groups included children younger than 5 years with impaired splenic function, congenital or acquired immune deficiency, implantation or replacement of cochlear prosthetic device, chronic disease (chronic kidney, heart, or pulmonary disease and diabetes mellitus), cerebrospinal fluid leakage, malignancy and post-transplantation. The pneumococcal vaccinations for children aged 2 years to 5 years have been included in Taiwan's national immunization program since March 2013.

Information on the leading pathogens of AOM and their antibiotic susceptibility may influence empirical antibiotic choice and treatment outcome. Concerning the coverage rate of pneumococcal vaccines, the distribution of pneumococcal serotypes is also important. Therefore, we conducted this study to investigate bacterial etiology including antimicrobial susceptibility and serotypes of *S. pneumoniae* that cause AOM in the era prior to universal pneumococcal vaccination in Taiwan.

Materials and methods

This retrospective study was approved by the institutional review board of Mackay Memorial Hospital. We collected

middle ear fluid (MEF) samples that were cultured at Mackay Memorial Hospital (MMH) from December 2009 to November 2011. The samples were obtained from children in whom AOM was diagnosed and a tympanocentesis procedure was performed. AOM was diagnosed by a pediatrician or otolaryngologist after otoscopic examination of the ear drum, and they decided whether patients needed tympanocentesis. The procedure was performed by an otolaryngologist. The MEF sample obtained by tympanocentesis was immediately applied onto a sterile swab preserved in transport medium (Amies Agar Gel-With Charcoal, COPAN Italia Inc., Brescia, Italy) and was sent for culture at a clinical microbiology laboratory. The samples were cultured in sheep blood agar and chocolate agar that was incubated aerobically at 35 °C for 48 hours. Isolation of bacterial pathogens and determination of antimicrobial susceptibility profiles were done. The antimicrobial susceptibility test was performed according to the reference guidelines in the laboratory in MMH. Susceptibility testing of antibiotic agents for *S. pneumoniae* was determined by the minimum inhibitory concentration (MIC) using Vitek 2 (bioMérieux Inc., Hazelwood, MO, USA). Definitions of antimicrobial susceptibility were based on the Clinical and Laboratory Antimicrobial Standards Institute (CLSI) 2009 criteria.⁹ If *S. pneumoniae* was identified, its serotypes were also analyzed. Pneumococcal isolates were typed by the latex agglutination using serotype-specific antisera (Statens Serum Institut, Copenhagen, Denmark). In each patient within one episode, only the result of the first sample was included. If a patient had more than one episode of AOM with tympanocentesis during this period, each episode was analyzed separately.

We reviewed patients' medical charts and recorded demographic data, culture results and antibiotic susceptibility test reports, pneumococcal vaccine status, and history of antibiotic administration in the past 3 months prior to MEF culture.

Our study period was 24 months, divided into two periods: from December 2009 to November 2010 and December 2010 to November 2011. The positive bacterial culture rates, pneumococcal isolation rates, pneumococcal serotype distribution, and NTHi-positive rates of the two periods were compared.

Fisher exact test was performed for categorical variables. We performed the Mann-Whitney U test for continuous variables. A $p < 0.05$ was considered statistically significant. All statistical analyses were performed using SPSS version 12.0 software (SPSS, Inc., Chicago, IL, USA).

Results

We enrolled 151 episodes from 146 patients in whom AOM was diagnosed and performed tympanocentesis during the study period. The age of patients was 43.3 ± 30.3 months (mean \pm standard deviation), and ranged from 4 months to 213 months. The demographic data is presented in Table 1. Seventy samples (46.4%) yielded bacteria and two (1.3%) yielded fungi. Normal flora of the skin accounted for 30 samples (19.9%) and 49 samples (32.5%) yielded no bacterial or fungi.

Among 70 samples yielding bacteria, the most common isolated pathogen was *S. pneumoniae*, which was found in 55.7% of episodes (39 of 70). NTHi was the second common pathogen, and it accounted for 22.9% of episodes (16 of 70; Fig. 1). Five serotypes of *S. pneumoniae* were identified. The most common serotypes among 39 *S. pneumoniae* strains were 19 A (26 strains, 66.7%) and 19 F (7 strains, 17.9%). Two episodes (5.1%) were identified from serotypes 3, 6B, and 14, respectively.

The age distribution of culture-positive episodes was shown in Fig. 2. *S. pneumoniae* was the most common isolated bacteria in each age group. There was no NTHi isolated in children older than 5 years.

The enrolled episodes of the earlier and later study periods were 76 and 75, respectively. *S. pneumoniae* accounted for 18.4% (14 of 76) and 33.3% (25 of 75) of AOM episodes in the two periods, and it had a significantly higher percentage in the later period ($p = 0.036$). The AOM episodes caused by pneumococcal serotype 19 A were also significantly increased in the later episode ($p = 0.028$; Fig. 3). However, there was no significant difference in serotypes 3, 6B, 14, and 19 F between the two periods. No significant difference of NTHi-positive rates was recorded between the two periods (9.2% vs. 12%, $p = 0.578$).

Among 39 children with 39 episodes of pneumococcal AOM, only seven (17.9%) had received at least one dose of

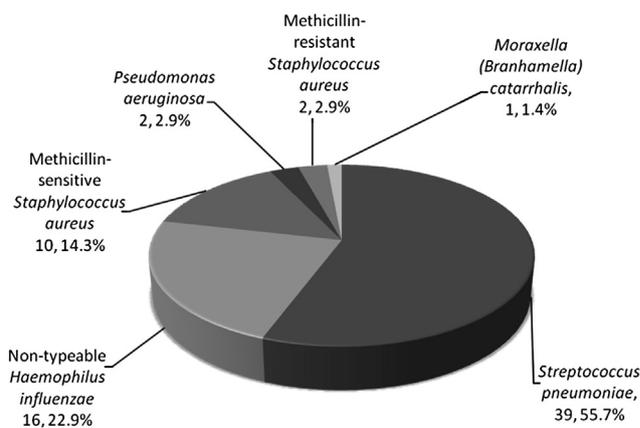


Figure 1. Episode numbers and percentages of bacteria isolated from middle ear effusion received via tympanocentesis in children with acute otitis media.

PCV7 prior to their AOM episodes. None of the seven patients had received PHI-D-CV or PCV 13. Five pneumococcal strains were serotype 19 A and two pneumococcal strains were serotype 19 F in these seven children.

Eighty-two AOM episodes (54.3%) received antibiotic treatment within 3 months prior to MEF cultures (Fig. 4). In this group, 40.2% of episodes yielded bacteria and 40.2% episodes had no pathogen. In those without previous antibiotic use, 53.6% of episodes yielded bacteria and 23.2% yielded no pathogen. The culture-negative rate of patients with previous antibiotic therapy was significantly higher than that of those without previous antibiotic therapy ($p = 0.036$). The bacterial culture-positive rate of patients with or without antibiotic treatment within 3 months did not reach statistical significance ($p = 0.106$).

Among the 39 isolated *S. pneumoniae* strains, 28.2% (11 of 39) were found to be susceptible to penicillin, 53.8% (21 of 39) were susceptible to third-generation cephalosporins (cefotaxime and ceftriaxone) and 97.4% (38/39) to levofloxacin. The isolated *S. pneumoniae* strains were all resistant to erythromycin (Table 2). Among serotype 19 A strains, 28.2% (5 of 26) were susceptible to penicillin, 50.0% (13 of 26) were susceptible to cefotaxime and ceftriaxone, and 100% to levofloxacin. All 16 NTHi strains were susceptible to cefotaxime and ceftriaxone but only 75.0% (12 of

	No. of episodes	%
Sex		
Male	78	51.7
Female	73	48.3
Age (mo)		
4–11	12	7.9
12–23	35	23.2
24–35	27	17.9
36–47	18	11.9
48–60	23	15.2
>60	36	23.8
Pneumococcal vaccination		
Vaccinated (PCV7)	16	10.6
Vaccinated (PCV10)	1	0.7
Unvaccinated	135	89.4
Previous antibiotic use within 3 mo	82	54.3

PCV7 = 7-valent pneumococcal conjugate vaccine; PCV10 = 10-valent pneumococcal *Haemophilus influenzae* protein D conjugate vaccine.

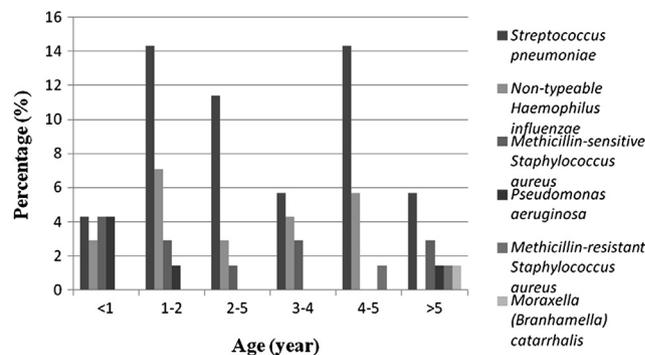


Figure 2. The age distribution of culture-positive episodes from children with acute otitis media.

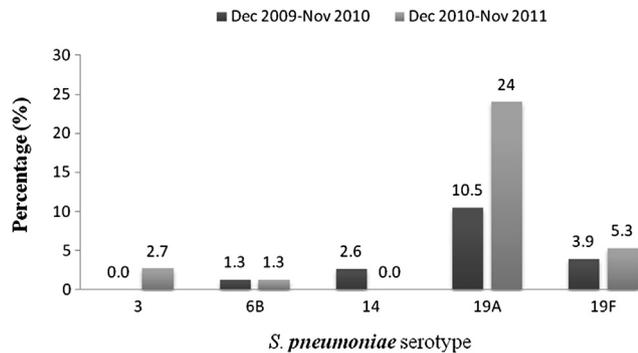


Figure 3. Comparison of the serotypes of *S. pneumoniae* identified from children with acute otitis media.

16) were susceptible to cefuroxime. In addition, 62.5% of NTHi strains (10 of 16) were susceptible to amoxicillin/clavulanate and 93.8% (15 of 16) were susceptible to moxifloxacin.

Discussion

In this study we found that *S. pneumoniae* and NTHi were still the two leading pathogens of bacterial AOM in the era prior to universal pneumococcal vaccination in Taiwan, the same as our previous epidemiological study prior to when the PCV7 were marketed.⁸ The other studies conducted in the United States and Europe also revealed *S. pneumoniae* as the most common causative bacteria of AOM in children prior to pneumococcal vaccination.^{7,10,11} Block et al¹⁰ reported that serotypes 19 F, 3, 23 F, and 6B were the predominant serotypes in children with pneumococcal AOM in the United States during the prevaccination era. Hausdorff et al¹¹ found serotypes 19 F, 23 F, 14, and 6B as major serotypes isolated in their datasets from Finland, France, Greece, Israel, several East European countries, the United States, and Argentina. However, serotype 19 A was only counted at 3.2% and 6.6% of pneumococcal AOM in these two studies prior to when pneumococcal vaccines were available.

The pneumococcal conjugate vaccine showed efficacy in the prevention of AOM caused by the serotypes included in

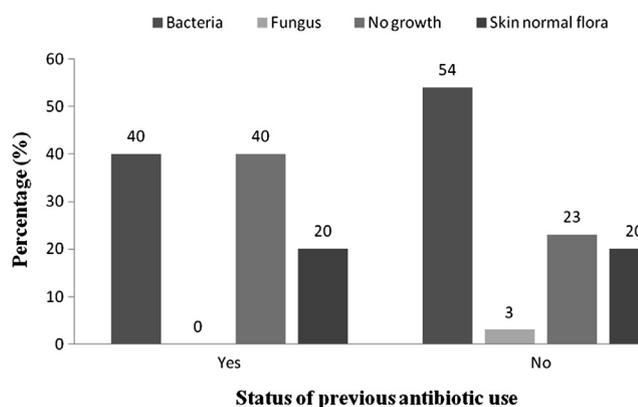


Figure 4. The culture-positive rate of patients with or without antibiotic treatment in the previous 3 months.

Table 2 Susceptibility of *Streptococcus pneumoniae* and *Haemophilus influenzae* isolates from middle ear effusion of children with acute otitis media

	<i>S. pneumoniae</i> (%)	NTHi (%)
Amoxicillin/clavulanate	N/A	62.5
Cefotaxime	53.8	100
Ceftriaxone	53.8	100
Cefuroxime	N/A	75
Chloramphenicol	94.9	6.8
Erythromycin	0	N/A
Imipenem	15.4	N/A
Levofloxacin	97.4	N/A
Moxifloxacin	100	93.8
Penicillin	28.2	N/A
SXT	12.8	43.8
Vancomycin	100	N/A

NTHi = non-typeable *H. influenzae*; N/A = not available; SXT = trimethoprim/sulfamethoxazole.

the vaccine.¹² Changes of the AOM pathogens was found after the PCV7 was introduced. In some studies, reduction in pneumococcal AOM and increase in NTHi were noted,^{13–16} including our previous data.¹⁷ NTHi even once became the predominance pathogen in the United States.¹³ A later study in Mexico showed the incidence of bacterial AOM caused by *S. pneumoniae* elevated again and was almost equal to NTHi.¹⁸ It was suggested that this should be the result from the increasing of non-PCV7 serotypes. Our study revealed a different situation; the *S. pneumoniae* culture-positive rate was much higher than NTHi. The nasopharyngeal carriage rate of *H. influenzae* type B was low in Taiwan both prior to and after pneumococcal vaccination.^{19,20} AOM caused by NTHi was less in Taiwan compared with other countries.⁸ Even vaccine-covered serotypes decreased, but pneumococcal AOM were still more common than NTHi AOM.

Among pneumococcal AOM, the most frequent isolated serotypes were 19 A and 19 F in our study. We compared the former and the later study periods and found significantly increased pneumococcal AOM, especially serotype 19 A. Although the prevalent pneumococcal serotypes varied in different geographical areas, pneumococcal vaccination could influence serotype distribution.²¹ Pichichero and Casey²² reported changes in otopathogens that the non-PVC7 serotypes *S. pneumoniae* increased in isolation from children with AOM. The non-PVC7 serotypes causing invasive pneumococcal disease (IPD) also increased worldwide.^{23–26} Seven patients in this study received at least one dose of PCV7 prior to their MEF cultures, and five of them yielded PCV7 noncontained serotype 19 A. Hsieh et al²⁷ conducted a study after PCV7 vaccination in Taiwan issued the importance of serotype 19 A in IPD and found the peak age of disease among children was 2–4 years. In January 2011, a Taiwan epidemiology bulletin revealed the increased number of IPD and serotype 19 A epidemic.²⁸ Our results suggested that an increase in serotype 19 A not only occurs in IPD, but also in less invasive diseases, just like AOM.

Two children in our study who received PCV7 previously got 19 F pneumococcal AOM even though serotype 19 F was included in PCV7. The immunogenicity of pneumococcal serotypes is variable after vaccination. In general, the higher antibody concentration provided better protection from disease. However, the studies have shown that this association for serotype 19 F is weak.²⁹ Even high antibody levels against 19 F do not seem to protect against AOM caused by 19 F.³⁰ This finding might explain why two patients experienced 19 F pneumococcal AOM after acceptance of PCV7.

In our previous study, only 4.2% of AOM *S. pneumoniae* strains were penicillin-susceptible in the 1990s.⁸ The penicillin-susceptible *S. pneumoniae* increased to 28.2% in this study. However, the CLSI published new *S. pneumoniae* breakpoints for penicillin in 2008. Under the former criteria, susceptible MIC breakpoint for penicillin was ≤ 0.06 $\mu\text{g}/\text{mL}$. The new susceptible MIC breakpoint for non-meningitis patients who are treated with intravenous penicillin is ≤ 2 $\mu\text{g}/\text{mL}$. It might not be reasonable to compare these two penicillin-susceptible rates of *S. pneumoniae*. However, Tsai et al.³¹ reported a transient decrease in penicillin nonsusceptibility, especially by nonmeningitis criteria in Taiwan. More than 80% of *S. pneumoniae* strains were susceptible to third-generation cephalosporins (cefotaxime and ceftriaxone) in the 1990s.³² We found susceptibility of third-generation cephalosporins decreased and only half of the strains were susceptible in the study. About 80% of NTHi isolated in our hospital in the 2000s were susceptible to amoxicillin/clavulanate,¹⁷ but this percentage decreased to 62.5% in this study. We need to monitor the resistance condition to adjust empirical antibiotic treatment in the future.

Tympanocentesis is thought to be a reliable diagnostic method to isolate and confirm the pathogenic bacteria.³³ The culture of otorrhea fluid draining from the spontaneous perforated eardrum may be contaminated by bacteria of the external ear canal or skin unless they are cleaned prior to swabbing otorrhea fluid. *Staphylococcus aureus* may be a contamination of the ear canal skin and was excluded in previous studies.^{17,34,35} In this study, our specimens were all obtained directly from tympanocentesis; the isolated *S. aureus* could be regarded as a true pathogen and was enrolled. However, the coagulase-negative staphylococci (CoNS) were thought to be normal flora of the skin and not true pathogens in this study. In our previous study, the cultures of MEF were obtained mainly by myringotomy, which is easily contaminated, and CoNS occupied 33.1% specimens.⁸ Because specimens were only collected by tympanocentesis in this study, the proportion of normal flora of the skin is lower (19.9%). However, tympanocentesis is not commonly done in children with AOM because parents usually do not agree to the procedure. It is often accepted by parents when the patients have persistent symptoms of AOM after empirical antibiotic treatment, or impending rupture of bulging ear drums. Although the isolated bacterial strains by tympanocentesis would be reliable, they might exclude the mild to moderate AOM strains. Our results indicate data collected from children with moderate to severe AOM.

In conclusion, *S. pneumoniae* and NTHi were the two most common pathogens of bacterial AOM in the era prior to universal pneumococcal vaccination in Taiwan. Non-

PCV7 pneumococcal serotypes, especially 19 A, became predominant. Continuous monitoring of the causative pathogens is needed. More effective and broad-spectrum vaccines would be indicated.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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