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ORIGINAL ARTICLE

Acute otitis media caused by *Streptococcus pneumoniae* serotype 19A ST320 clone: epidemiological and clinical characteristics

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Abstract *Background: Streptococcus pneumoniae* serotype 19A ST320, a highly multiresistant and virulent clone, has emerged as a common pathogen causing acute otitis media (AOM) in children.
Methods: Patients aged 0–18 years with AOM who presented at Mackay Memorial Hospital, Taipei, Taiwan were prospectively enrolled between December 1, 2009, and November 30, 2012. For each patient, a specimen of middle-ear fluid was obtained and cultured. *S. pneumoniae* isolates were tested by serotyping, antibiotic-resistance profiling, and multilocus sequence typing. Demographic characteristics and clinical history of patients with pneumococcal AOM were recorded.
Results: Pneumococcal AOM was observed in 108 (24.8%) of 436 episodes. One hundred and four isolates of *S. pneumoniae* were available for study. The most common serotypes were 19A (67 isolates, 64.4%), followed by 19F (16 isolates, 15.4%), and 3 (7 isolates, 6.7%). Among the 85 sequence-typed isolates, Serotype 19A ST320 (50, 58.8%) was the most frequent.

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Children with AOM caused by Serotype 19A ST320 were younger (33.9 ± 21.4 months vs. 46.7 ± 35.9 months, $p = 0.04$) and had a higher rate of spontaneous rupture of the tympanic membrane (64.0% vs. 40%, $p = 0.05$) than those caused by isolates of other sequence types. Serotype 19A ST320 caused 90% of AOM episodes in children aged ≤ 12 months and had had higher resistance rates to penicillin according to meningeal breakpoints ($p = 0.011$), amoxicillin ($p < 0.001$) and trimethoprim/sulfamethoxazol ($p < 0.001$).
Conclusions: It is better to use pneumococcal conjugate vaccine effective against Serotype 19A in early infancy to prevent the first and subsequent episodes of AOM in children in Taiwan.

Introduction

Streptococcus pneumoniae serotype 19A ST320 has emerged in many countries as a cause of invasive disease, severe pneumonia, acute otitis media (AOM), and hemolytic uremic syndrome, despite the implementation of a 7-valent pneumococcal conjugate vaccine (PCV7).[1–6] AOM is an important infectious disease of the respiratory tract among children and is a leading cause of physician visits and antibiotic prescriptions.[7–9] It has been reported that Serotype 19A ST320 is the predominant cause of pneumococcal mastoiditis, a suppurative complication of AOM,[10] and that this serotype causes persistent symptoms even when treated with high doses of β-lactam antibiotics.[11]

In Taiwan, Serotype 19A ST320 clone was highly prevalent under a low PCV7 vaccination rate.[12] About 50% of cases of invasive pneumococcal disease among children aged < 5 years are caused by Serotype 19A ST320. Clonal expansion of Serotype 19A ST320 resulted in an increase in overall invasive pneumococcal disease among children in Taiwan. In view of Serotype 19A ST320 being a highly virulent and antibiotic-resistant clone,[11] the aim of this study was to investigate the epidemiological and clinical features of AOM caused by the Serotype 19A ST320 clone. Antimicrobial treatment of AOM caused by Serotype 19A ST320 also was characterized.

Materials and methods

Setting, patients, and study design

The Mackay Memorial Hospital Institutional Review Board (Taipei, Taiwan) approved the study. We prospectively collected the *S. pneumoniae* isolates obtained from children with a diagnosis of AOM who were attended at Mackay Memorial Hospital from December 2009 to November 2012. Middle ear fluid (MEF) for culture was collected from children by tympanocentesis or following spontaneous rupture of tympanic membrane. Isolates for recruitment were either from children with a new episode of AOM who had not yet received antibiotics for the episode, or from children who had a diagnosis of AOM and had received antibiotic therapy within the past 48—72 hours but remained symptomatic at the time of study entry. Where indicated, the tympanocentesis was performed by an otorhinolaryngologist; alternatively, for patients with spontaneous tympanic membrane perforation, a swab was obtained within 24 hours of the perforation. Previous antibiotic use was defined as exposure to antibiotics in the 3 months previous to the recruitment episode. The immunization status of children was obtained by review of medical records from the child's primary care provider. The study year was divided into three 12-month periods: 2010 (December 2009 to November 2010), 2011 (December 2010 to November 2011), and 2012 (December 2011 to November 2012).

Bacterial isolates, antimicrobial susceptibility testing, and serotyping

Each MEF sample was immediately applied to a sterile swab and preserved in transport medium (Amies agar gel with charcoal; COPAN Italia Inc., Brescia, Italy) and was submitted for culturing at the clinical microbiology laboratory. Isolates were identified in our laboratory by standard microbiological methods that included Gram strain morphology, optochin sensitivity, bile solubility, antigenic testing, and biochemical methods. Polysaccharide capsule types were determined on the basis of the Quellung test with factor-specific sera (Pneumotest-latex kit; Statens Serum Institute, Copenhagen, Denmark). Antimicrobial susceptibility tests were performed according to the reference guidelines of the Mackay Memorial Hospital laboratory. Susceptibility of *S. pneumoniae* to antibiotic agents was determined by the minimum inhibitory concentration (MIC) method using Vitek 2 (bioMerieux Inc.; Hazelwood, MO, USA). Definitions of antimicrobial susceptibility were based on the Clinical and Laboratory Antimicrobial Standards Institute 2009 criteria.[13] Isolates with intermediate or full resistance were defined as nonsusceptible. For our analyses, β-lactam antibiotics included penicillin, amoxicillin, cefotaxime, ceftriaxone, and imipenem.[14] Resistance to more than one β-lactam antibiotic was considered β-lactam antibiotic resistance. Resistance to ≥ 3 antibiotic classes was considered multidrug resistance. For analysis, we recorded the patients treated with high- dose amoxicillin—clavulanate (Augmentin) as Group 1 and the patients treated with cefixime, ceftriaxone/cefotaxime, vancomycin, or levofloxacin as Group 2.

Multi-locus sequencing type

The nucleotide sequences of 450-bp internal regions from the *aroE*, *gdh*, *gki*, *recP*, *spi*, *xpt*, and *ddl* genes were amplified by polymerase chain reaction, using the previously described primers and reaction conditions.[15] The sequences then were compared (using the BioEdit Sequence Alignment Editor, Ibis Biosciences, Carlsbad, CA) with those of all of the recognized alleles of each gene listed in the pneumococcal multilocus sequence typing (MLST) website database (http://spneumoniae.mlst.net). The Web database (www.mlst.net) was used for assigning allele numbers for particular loci; the sequence types (STs) of isolates were designated on the basis of the resulting allelic profiles.

Statistical analysis

We used χ^2 analysis or Fisher's exact test for categorical variables and Student's *t* test or Mann–Whitney *U* test for continuous variables to test for significant differences between groups. All variables with p values < 0.05 were included in the multivariate model. A p value < 0.05 was considered statistically significant. All probabilities were two-tailed. All statistical analyses were performed using SPSS version 15.0 (SPSS Inc., Chicago, IL, USA).

Results

During the 3-year period of the study, 436 MEF samples of AOM patients were collected for culture. Among them, 108 isolates (24.8%) were *S. pneumoniae*, 24 isolates (5.5%) were *Haemophilus influenzae*, three isolates (0.7%) were *Moraxella catarrhalis*, and three isolates (0.7%) were *Streptococcus pyogenes*. One hundred and four isolates of *S. pneumoniae* were available for further study. Forty-eight episodes (46.2%) occurred in men and 56 episodes (53.8%) occurred in women. Age ranged from 3.9 months to 187 months. The mean ± standard deviation was 40.6 ± 27.7 months (median, 39.9 months). Culture specimens from 49 episodes (47.1%) were obtained by tympanocentesis; culture specimens from 55 episodes (52.9%) were obtained by collection of pus draining from the ear. The distribution of episodes in children was as follows: 43 episodes (42.3%) occurred in children aged 25–60 months, 25 episodes (23.1%) in children aged 13–24 months, 24 episodes (20.2%) in children aged $>$ 60 months, and 12 episodes (14.4%) in children aged $\leq$ 12 months.

Serotype distribution and MLST analysis

A total of eight serotypes were identified (Table 1). The most common serotype was 19A (67 isolates, 64.4%), followed by 19F (16 isolates, 15.4%), 3 (7 isolates, 6.7%), 23F (5 isolates, 4.8%), 14 (3 isolates, 2.9%), 6B (3 isolates, 2.9%), 15A (2 isolates, 1.9%), and 35B (1 isolate, 1%). Serotype 19A was the most common type in each age group, and Serotype 19A significantly caused AOM episodes in children aged $\leq$ 12 months (91.7%, $p = 0.04$) (Table 1). In contrast, Serotype 3 caused a significantly larger fraction of AOM episodes among children aged $>$ 60 months (20.8%, $p = 0.007$).

Table 1 Serotype distribution in each age group.

Serotype	Age group (mo), *n* (%)				*p*
	$\leq$ 12 ($n = 12$)	13–24 ($n = 25$)	25–60 ($n = 43$)	$>$ 60 ($n = 24$)	
19A	11 (91.7)	15 (60)	30 (69.8)	11 (45.8)	0.04
19F	0 (0)	3 (12)	7 (16.3)	6 (25)	0.3
3	1 (8.3)	1 (4.0)	0 (0)	5 (20.8)	0.007
23F	0 (0)	2 (8.0)	2 (4.7)	1 (4.2)	0.9
14	0 (0)	2 (8.0)	1 (2.3)	0 (0)	0.6
15A	0 (0)	2 (8.0)	0 (0)	0 (0)	0.2
35B	0 (0)	0 (0)	1 (2.3)	0 (0)	1.0
6B	0 (0)	0 (0)	2 (4.7)	1 (4.2)	0.7

Percentage of Serotype 19A increased from 36.8% (2009–2010) to 64.3% (2010–2011) and 74.1% (2011–2012) (p for trend $= 0.007$) (Fig. 1).

Among the 85 typed isolates, 23 clones were identified and the most frequent clone was Serotype 19A ST320, followed by Serotype 3 ST180 (5, 5.9%) and Serotype 19F ST236 (4, 4.7%). For Serotype 19F ST271, Serotype 19F ST320, Serotype 19F ST1464, Serotype 19F ST7123, and Serotype 23F ST83, each clone accounted for two isolates. For Serotype 19A ST873, Serotype 19A ST2652, Serotype 19A ST3111, Serotype 19A ST3164, Serotype 19F ST1465, Serotype 19F ST8525, Serotype 14 ST13, Serotype 14 ST320, Serotype 14 ST2108, Serotype 23F ST81, Serotype 23F ST242, Serotype 6B ST76, Serotype 6B ST90, Serotype 15A ST63, and Serotype 35B ST558, each clone accounted for one isolate.

AOM caused by Serotype 19A ST320

The percentage of Serotype 19A ST320 dramatically increased from 29.4% in 2009–2010, to 59.5% in 2010–2011, and to 76.9% in 2011–2012 (p for trend $= 0.003$; Table 2). The median age of patients with AOM due to Serotype 19A ST320 (median age, 32.1 months) was younger than the median age of patients with AOM due to non-Serotype 19A ST320 (median age, 44 months) ($p = 0.04$). There was no significant difference in total fever days and rates of PCV7 vaccination, antibiotic use within 3 months, mastoiditis, and pneumonia between AOM due to Serotype 19A ST320 and non-Serotype 19A ST320. AOM due to Serotype 19A ST320 had a borderline higher rate of spontaneous rupture of tympanic membrane compared with that due to non-Serotype 19A ST320 (64.0% vs. 40.0%, $p = 0.05$).

Antimicrobial susceptibility

Antimicrobial susceptibility of pneumococcal strains causing AOM is shown in Table 3. All 104 strains were susceptible to levofloxacin, moxifloxacin, and vancomycin. The percentage of resistance to erythromycin and trimethoprim–sulfamethoxazole (TMP/SMX) was 99.0% and 84.6%, respectively. The percentage of resistant strains according to meningeal (oral penicillin) and nonmeningeal (parenteral) breakpoints was 87.5% and 20.2%, respectively. According to the nonmeningeal criteria, the resistance rates to amoxicillin and cefotaxime were 66.3% and 20.2%, respectively. The nonsusceptibility rates to

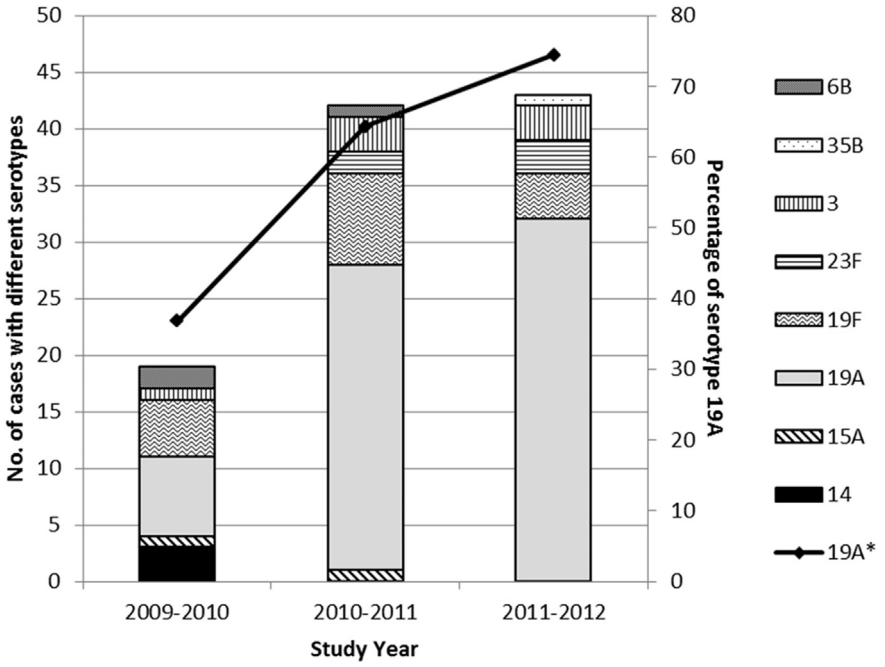

Figure 1. Annual number of cases (bars) by serotype and percentage of cases (dots) caused by *Streptococcus pneumoniae* serotype 19A from December 2009 to November 2012. A "study year" is defined as the 12-month interval: 2009–2010 (December 2009 to November 2010), 2010–2011 (December 2010 to November 2011), and 2011–2012 (December 2011 to November 2012).

Table 2 Demographic and clinical characteristics of children with acute otitis media.

	19A ST320	Non-9A ST320	*p*
No. of isolates	50	35	
Gender, male	25 (50.0)	18 (51.4)	0.93
Age (mo), mean ± standard deviation	33.9±21.4	46.7±35.9	0.04
Age distribution (mo)			
≤ 12	9 (18.0)	1 (2.9)	0.03
13–24	8 (16.0)	11 (31.4)	0.16
25–60	24 (48.0)	13 (37.1)	0.44
> 60	9 (18.0)	10 (28.6)	0.38
Study year			
2009–2010	5 (10.0)	12 (34.3)	0.013
2010–2011	25 (50.0)	17 (48.6)	0.93
2011–2012	20 (40.0)	6 (17.1)	0.044
PCV7 vaccination	10 (20.0)	6 (22.9)	0.75
Antibiotic use within 3 mo	11 (22.0)	8 (22.9)	1.0
Treatment with amoxicillin–clavulanate	30 (60.0)	20 (57.1)	0.97
Total fever duration (d)	6.2 ± 4.0	6.3 ± 4.5	0.97
Spontaneous rupture of tympanic membrane	32 (64.0)	14 (40.0)	0.05
Mastoiditis	3 (6.0)	3 (8.6)	0.29
Pneumonia	8 (16)	6 (17.1)	0.88

Data are presented as *n* (%) of children, unless otherwise indicated.
PCV = pneumococcal conjugated vaccine.

amoxicillin and penicillin were 77.9% and 70.2%, respectively. Seventy-four isolates (71.2%) were resistant to at least one of the β-lactam antibiotics and 96 (92.3%) were resistant to multiple drugs. Among isolates resistant to at least one of the β-lactam antibiotics, 95.9% of the isolates also were resistant to TMP/SMX, and all the isolates were resistant to erythromycin. Among the 50 strains of *S. pneumoniae* serotype 19A ST320, all isolates were resistant to erythromycin and susceptible to chloramphenicol (Table 3). Compared to non-19A ST320, 19A ST320 isolates had higher nonsusceptibility rates to penicillin according to either meningeal breakpoints ($p < 0.001$) or nonmeningeal breakpoints ($p = 0.003$) and higher resistance rates to penicillin according to meningeal breakpoints ($p = 0.011$), amoxicillin ($p < 0.001$) and TMP/SMX ($p < 0.001$). The *S. pneumoniae* serotype 19A ST320 had a higher rate of β-lactam antibiotic resistance ($p < 0.001$) and multidrug resistance ($p = 0.004$) than non-19A ST320 had.

Treatment of AOM due to Serotype 19A ST320

Among 50 patients with AOM due to Serotype 19A ST320, 30 were treated with amoxicillin–clavulanate (Group 1) and 20 with further antibiotics (Group 2). We compared the clinical course and laboratory data of Serotype 19A ST320 AOM cases between Groups 1 and 2 (Table 4). Total fever duration was shorter in Group 1 than Group 2 (4.7 ± 4.0 days vs. 7.8 ± 3.4 days, $p = 0.008$). Similarly, the fever duration after antibiotics treatment was shorter in Group 1 than Group 2 (2.3 ± 2.7 days vs. 3.7 ± 2.5 days, $p = 0.041$). We further analyzed the rate of spontaneous rupture in Serotype 19A AOM cases that had not received antibiotic treatment before the culture was done. Specifically, out of 35 AOM cases infected with Serotype 19A ST320 and who

Table 3 Antimicrobial susceptibility of *Streptococcus pneumoniae* isolates causing acute otitis media.

MIC (mg/L)	All (n = 104) No. of strains (%)	Typed (n = 85) No. of strains (%)	19AST320 (n = 50) No. of strains (%)	Non-19AST320 (n = 35) No. of strains (%)	p
Penicillin (meningeal and oral breakpoints)					
$<$ 0.12 μg/mL (S)	7 (6.7)	5 (5.9)	0 (0.0)	5 (14.3)	0.01
0.12–1 μg/mL (I)	6 (5.8)	3 (3.5)	2 (4.0)	1 (2.9)	0.43
$\geq$ 2 μg/mL (R)	91 (87.5)	77 (90.6)	48 (96.0)	29 (82.9)	0.04
Penicillin (nonmeningeal and parental breakpoints)					
$\leq$ 2 μg/mL (S)	31 (29.8)	26 (30.6)	9 (18.0)	17 (48.6)	0.003
4 μg/mL (I)	52 (50.0)	41 (48.2)	28 (56.0)	13 (37.1)	0.087
$\geq$ 4 μg/mL (NS)	73 (70.2)	59 (69.4)	41 (82.0)	18 (51.4)	0.003
$\geq$ 8 μg/mL (R)	21 (20.2)	18 (21.2)	13 (26.0)	5 (14.3)	0.19
Amoxicillin (nonmeningeal breakpoints)					
$\leq$ 2 μg/mL (S)	23 (22.1)	16 (18.8)	2 (4.0)	14 (40.0)	$<$ 0.001
4 μg/mL (I)	12 (11.5)	11 (12.9)	5 (10.0)	6 (17.1)	0.33
$\geq$ 4 μg/mL (NS)	81 (77.9)	69 (81.2)	48 (96.0)	21 (60.0)	$<$ 0.001
$\geq$ 8 μg/mL (R)	69 (66.3)	58 (68.2)	43 (86.0)	17 (42.9)	$<$ 0.001
Cefotaxime (nonmeningeal breakpoints)					
$\leq$ 1 μg/mL (S)	59 (56.7)	47 (55.3)	24 (48.0)	23 (65.7)	0.11
2 μg/mL (I)	24 (23.1)	17 (20.0)	12 (24.0)	5 (14.3)	0.27
$\geq$ 2 μg/mL (NS)	45 (43.3)	38 (44.7)	26 (52.0)	12 (34.3)	0.11
$\geq$ 4 μg/mL (R)	21 (20.2)	21 (24.7)	14 (28.0)	7 (20.0)	0.40
Ceftriaxone (nonmeningeal breakpoints)					
$\leq$ 1μg/mL (S)	62 (59.6)	51 (60.0)	28 (56.0)	23 (65.7)	0.37
2 μg/mL (I)	22 (21.2)	15 (27.6)	9 (18.0)	6 (17.1)	0.92
$\geq$ 2 μg/mL (NS)	42 (40.4)	34 (40.0)	22 (44.0)	12 (34.3)	0.37
$\geq$ 4 μg/mL (R)	20 (19.2)	19 (22.4)	13 (26.0)	6 (17.1)	0.33
Erythromycin					
$\leq$ 0.25μg/mL (S)	1 (1.0)	1 (1.2)	0 (0.0)	1 (2.9)	0.41
0.5 μg/mL (I)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	-
$\geq$ 1 μg/mL (R)	103 (99.0)	84 (98.8)	50 (100.0)	34 (97.1)	0.41
TMP/SMX					
$\leq$ 0.5 μg/mL (S)	13 (12.5)	9 (10.6)	1 (2.0)	8 (22.9)	0.003
1–2 μg/mL (I)	4 (3.8)	4 (4.7)	2 (4.0)	2 (5.8)	0.36
$\geq$ 4μg/mL (R)	87 (83.7)	72 (85.7)	47 (94.0)	25 (71.3)	0.005
Chloramphenicol					
$\leq$ 4 μg/mL (S)	95 (91.3)	78 (91.8)	50 (100.0)	28 (80.0)	0.001
$\geq$ 8 μg/mL (R)	9 (8.7)	7 (8.2)	0 (0.0)	7 (20.0)	0.001
Tetracycline					
$\leq$ 1 μg/mL (S)	3 (2.9)	3 (3.5)	1 (2.0)	2 (5.7)	0.30
2 μg/mL (I)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	–
$\geq$ 4 μg/mL (R)	101 (97.1)	82 (96.5)	49 (98.0)	33 (94.3)	0.30
BLR	74 (71.2)	62 (72.9)	45 (90.0)	17 (48.6)	$<$0.001
MDR	96 (92.3)	79 (92.9)	50 (100.0)	29 (82.9)	0.004

The classifications of the susceptible (S), intermediate (I), or resistant (R) were according to the Clinical Laboratory Standards Institute 2009 criteria for MICs of *Streptococcus pneumoniae* [13]. Isolates with intermediate (I) or full resistance (R) were defined as non-susceptible (NS). BLR = β-lactam antibiotics resistance; MDR = multidrug resistance ($\geq$ 3 antibiotic classes; TMP/SMX = trimethoprim/sulfamethoxazole.

had not previously been treated with antibiotics, 21 (60%) presented with spontaneous rupture of the tympanic membrane.

Discussion

In this prospective study of 436 episodes of AOM in children during a 3-year study period, we found that *S. pneumoniae* was the most common bacterial pathogen in children with bacterial AOM. Clonal expansion of highly resistant Serotype 19A ST320 accounted for 58.8% of children with pneumococcal AOM. Serotype 19A ST320 is prevalent in many countries,[1–3] and has been observed among children with AOM.[5] The virulence characteristics of Serotype 19A ST320 clone are adaptations that increase the persistence of the strain within a host during colonization.[12] Childhood AOM caused by Serotype 19A ST320 occurs at a significantly younger age and is associated with a higher rate of spontaneous rupture of the tympanic membrane.

Table 4 Clinical and laboratory characteristics of Serotype 19A ST320 cases of acute otitis media treated by different group of antibiotics.

	Group 1 (n = 30)[a]	Group 2 (n = 20)[b]	p
Age (mo), mean ± SD	32.7 ± 23.9	35.8 ± 17.0	0.40
Gender, male, no (%)	14 (46.7)	11 (55)	0.77
WBC, mean cells × 10^3/mm^3 ± SD	14.0 ± 4.5	14.1 ± 5.2	0.90
Band, mean cells × 10^3/mm^3 ± SD	3.1 ± 4.5	2.4 ± 3.9	0.60
CRP, mean, mg/dL ± SD	9.9 ± 11.9	8.3 ± 6.3	0.97
Total fever duration (d), mean ± SD	4.7 ± 4.0	7.8 ± 3.4	0.008
Fever duration post antibiotics use (d), mean ± SD	2.3 ± 2.7	3.7 ± 2.5	0.041
Spontaneous rupture of tympanic membrane, no (%)	21 (70)	10 (50)	0.26
Mastoiditis, no (%)	0 (0)	3 (15)	0.06

[a] High-dose amoxicillin–clavulanate.
[b] Cefixime, ceftriaxone/cefotaxime, vancomycin or levofloxacin.
CRP = C-reactive protein; SD = standard deviation; WBC = white blood cell count.

Among the four bacterial pathogens [*S. pneumoniae*; non-typeable *H. influenzae*; *Moraxella catarrhalis*; *S. pyogenes* (Group A streptococcus, GAS)] typically implicated in AOM, pneumococcal AOM is the most commonly encountered, and frequently causes high temperature, severe otalgia, tympanic membrane redness and bulging, and accumulation of middle ear fluid.[16–18] *H. influenzae* AOM is associated with conjunctivitis and recurrent disease,[16] and *M. catarrhalis* is characterized by a higher rate of mixed infection,[19] whereas GAS AOM is accompanied by higher rates of tympanic perforation and mastoiditis.[20] Segal et al[20] demonstrated that spontaneous perforation of tympanic membrane occurred in 66.7% of GAS AOM, 20.8% of pneumococcal AOM, 17.5% of *H. influenzae* AOM, and 14.1% of *M. catarrhalis* AOM. A rapid and virulent progression to tympanic perforation is a major characteristic of GAS AOM, compared with AOM caused by any of the other three pathogens. In the present study, spontaneous perforation occurred in 64.0% of AOM caused by Serotype 19A ST320, and 40.0% of AOM caused by non-Serotype 19A ST320. A high-dose amoxicillin/clavulanate formulation (90/6.4 mg/kg/d) has been developed to sustain amoxicillin/clavulanate concentrations in MEF at levels that would predict eradication of *S. pneumoniae* with amoxicillin/clavulanate MICs $\leq$ 4 μg/mL.[21] However, 86% of our Serotype 19A ST320 strains had amoxicillin/clavulanate MICs $>$ 4 μg/mL. In our study, amoxicillin–clavulanate was the most frequently prescribed empirical antibiotic used in children with AOM. The higher rate of tympanic perforation in cases with AOM caused by Serotype 19A ST320 may reflect failure of amoxicillin–clavulanate treatment.[22] However, we noted that the tympanic perforation rate among cases with AOM caused by Serotype 19A ST320 was 70% in the amoxicillin–clavulanate group, while a 60% tympanic perforation rate was observed in children with AOM caused by Serotype 19A ST320 in the absence of prior antibiotic treatment. Thus, the high tympanic perforation rate in AOM with Serotype 19A ST320 infection cannot be totally attributed to amoxicillin–clavulanate treatment failure. Serotype 19A ST320 might be locally aggressive, as is GAS. After tympanic perforation, amoxicillin–clavulanate was continued clinically in most cases because fever and otalgia improved. Tympanocentesis is recommended in order to accurately identify the causative pathogen and determine antibiotic susceptibility in selected cases of failed antibiotic treatment or complicated AOM. Given the higher rates of antibiotic resistance and spontaneous rupture rate in cases with AOM caused by Serotype 19A ST320, tympanocentesis might be the most prudent treatment strategy for AOM, especially for infections caused by Serotype 19A ST320.

The previous study showed that children aged 25–60 months exhibited the highest incidence of invasive pneumococcal disease and pneumonia in Taiwan.[23] In our study, we also found that highest frequency of AOM occurred in the same age group. However, this profile differs from that in other countries, where children aged $\leq$ 24 months exhibited the highest rate of AOM.[5,24] Differing rates and ages of attendance at child care centers, host genetic factors, and differences in the prevalent pneumococcal clones among regions and cultures may explain the disparity in the epidemiology of AOM in Taiwan compared to other countries.

It has been reported that 61–93% of children will develop AOM at least once before 3 years of age, and 20% will develop recurrent AOM or chronic otitis media with effusion.[25–27] The first episode of AOM causes inflammation and subsequent damage to the middle-ear mucosa and Eustachian tube, therefore, recurrent AOM usually ensues after the first episode. Once recurrent otitis media has been established, no reduction in the number of otitis media cases has been seen, even after implementation of PCV.[28] Therefore, the efficacy of PCVs depends upon immunization in early infancy to prevent initial AOM. The present study demonstrated that Serotype 19A was the most common serotype in each age group of AOM. Importantly, Serotype 19A was associated with AOM in 91.7% of children aged $\leq$ 12 months.

There were limitations to this study. Our study was unable to estimate the true rates, as our patients were enrolled only if MEF cultures were obtained. Only patients with pus or persistent symptoms were included, so the serotypes of the milder cases might have been ignored. Given the high proportion of highly antimicrobial-resistant pneumococcal isolates recovered, it is likely that antibiotic-susceptible strains were under-represented in our survey.

In conclusion, due to the rapid expansion of highly resistant Serotype 19A ST320 of AOM, our work supports

prevention of AOM among children in Taiwan by use of Serotype 19A-containing PCV. The surveillance of pneumococcal serotypes and clones after the introduction of 13-valent vaccine in order to adjust prophylaxis and treatment strategies is needed.

Conflicts of interest

All authors declare no conflict of interest.

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