Original Article

Characteristics and etiology of hospitalized pediatric community-acquired pneumonia in Taiwan

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Background/purpose: The purpose of this study was to determine the pathogens and to estimate the incidence of pediatric community-acquired pneumonia (CAP) in Taiwan.

Methods: This prospective study was conducted at eight medical centers from November 2010 to September 2013. Children aged 6 weeks to 18 years who met the radiologic criteria for pneumonia were enrolled. To detect classical and atypical bacteria and viruses, blood and pleural fluids were cultured, and respiratory specimens were examined by multiple conventional and molecular methods.

Results: At least one potential pathogen was identified in 705 (68.3%) cases of 1032 children enrolled, including bacteria in 420 (40.7%) cases, virus in 180 (17.4%) cases, and mixed viral-bacterial infection in 105 (10.2%) cases. *Streptococcus pneumoniae* (31.6%) was the most common pathogen, followed by *Mycoplasma pneumoniae* (22.6%). Adenovirus (5.9%) was the most common virus. RSV was significantly associated with children aged under 2 years, *S. pneumoniae* in children aged between 2 and 5 years, and *M. pneumoniae* in children aged >5 years. The annual incidence rate of hospitalization for CAP was highest in children aged 2—5 years (229.7 per 100,000). From 2011 to 2012, significant reduction in hospitalization rates pertained in children under 5 years of age, in pneumonia caused by pneumococcus, adenovirus or co-infections and complicated pneumonia.

Conclusion: CAP related pathogens have changed after increased conjugated pneumococcal vaccination rates. This study described the latest incidences and trends of CAP pathogens, which are crucial for prompt delivery of appropriate therapy.

Introduction

Childhood community-acquired pneumonia (CAP) is a common and serious health care problem, responsible for one fifth of children's deaths around the world.[1] Even as recently as in 2015, pneumonia accounted for 15% of all deaths of children under 5 years old, killing an estimated 922,000 children in this age range.[2] Despite this large disease burden, critical gaps in our knowledge about pediatric pneumonia persist.[3] CAP is usually caused by bacterial infection, and *Streptococcus pneumoniae* is the most common pathogen in the pediatric population.[4,5] Nevertheless, pneumonia of viral origin, including influenza virus, adenovirus, human metapneumovirus, parainfluenza virus, and respiratory syncytial virus (RSV), are often endowed with similar radiographic findings.[6,7] In recent years, improvement in the sensitivity and specificity of molecular methods has provided new opportunities to delineate the causative CAP pathogens. It is becoming evident viruses are also important pathogens of CAP in children.[7,8] This has also helped to uncover the interplay among the different pathogens during acute respiratory infections.[8,9]

The incidence estimates of pediatric CAP hospitalizations based on prospective data collection are limited.[10] We conducted a prospective, multicenter study in Taiwan and aimed to perform a comprehensive analysis on the incidence rates and the pathogens of pediatric CAP in Taiwan. At the time of the study, pneumococcus conjugated vaccine (PCV) was only available in the private market (approximately 16.2% in 2008, 22.3% in 2009, 30.2% in 2010, and 33.6% in 2011[11] and 40% in 2012 of children less than 5 years of age received one or more doses of the PCV7 or PCV13 vaccine, manufacturer estimates; Wyeth Pharmaceuticals Inc., a subsidiary of Pfizer,

Inc., Taiwan). We also compared and analyzed the demographic, clinical, and laboratory features of children with different etiologies.

Materials and methods

Study design

From November 2010 to September 2013, children aged 6 weeks to 18 years who met the World Health Organization's radiologic criteria for pneumonia[12] were prospectively enrolled at eight participating medical centers, including Chang-Gung Memorial Hospital Linkou branch, National Taiwan University Hospital, Mackay Memorial Hospital, China Medical University Hospital, Buddhist Tzu Chi General Hospital, National Taiwan University Hospital Yun-Lin Branch, National Cheng-Kung University Hospital, and Chang-Gung Memorial Hospital, Kaohsiung branch. These eight medical centers belong to the Taiwan Pediatric Infectious Disease Alliance (TPIDA), a study group funded by the National Health Research Institutes, Taiwan. Children were excluded if they had chronic renal failure, dialysis, indwelling devices, thalassemia major, chronic cardiovascular diseases, chronic lung disease of prematurity, nephrotic syndrome, liver cirrhosis, diabetes mellitus, congenital immunodeficiency, HIV infection, asplenia or malignancy, or were receiving immunosuppressant agents.

The study was approved by the Institute of Review Board of each participating hospital, and a written informed consent was obtained from a parent/guardian of each subject. Upon inclusion, all medical records, including demographics, medical history, clinical signs and symptoms,

diagnoses, and treatments of enrolled inpatients, were collected and kept in an electronic database.

Definition of alveolar pneumonia

Chest radiographs, obtained within 24 h of admission, were interpreted prospectively and independently by two pediatricians masked to patients' clinical conditions. Pneumonia on a plain film was defined as a dense opacity with a fluffy consolidation of any size within a lobe, or the entire lung, with or without visible air bronchogram and pleural effusion. Two pediatricians, one of which a pediatric infectious disease specialist, interpreted the chest X rays independently and the diagnosis of CAP was confirmed if their interpretations agree.

Patients were grouped based on the radiological severity of alveolar pneumonia: 1) sub-lobar, 2) lobar, 3) with pleural effusion, 4) complicated pneumonia. Pleural effusion was defined as blunting of the costophrenic angle.[13] Complicated pneumonia was defined as the presence of empyema or/and necrotizing pneumonia.[14]

Sample collection and processing

Blood samples, acute-phase serum specimens, and pleural fluids (if present; obtained by thoracentesis or thoracostomy) were collected after enrollment. Nasopharyngeal specimen was sampled using sterile swabs (Eswab; Copan Diagnostics Inc., Murrieta, Calif., USA). The swabs were introduced through a nostril and advanced until resistance was met. Following specimen collection, each swab was suspended in 1 ml of liquid Amies transport medium. A throat swab sample was obtained using a sterile nylon swab (Regular Flocked swab, Cat. No.520CS01, Copan Diagnostics Inc., Murrieta, Calif., USA) and placed in virus transport medium upon collection. Specimens were stored and transported at 4 °C to National Taiwan University Hospital for testing.

Bacterial study

Blood samples and pleural-fluid specimens were submitted for bacterial culture at each study site and processed according to standard techniques. Urinary S. pneumoniae antigens were detected using immunochromatographic tests (Binax NOW, Portland, Oregon, USA). A positive urine pneumococcal antigen test (Binax NOW) in children with CAP was considered a probable case of pneumococcal pneumonia.[10] Real-time polymerase-chain-reaction (PCR) assays targeting the S. pneumoniae lytA gene were performed on pleural fluid.[13] Detection of Chlamydophila pneumoniae and Mycoplasma pneumoniae were performed by PCR on nasopharyngeal swabs as previously described.[15,16] Serum samples were tested for the presence of M. pneumoniae antibodies by using the IgM-specific Mycoplasma Immuno-Card, an enzyme immunoassay purchased from Meridian Bioscience (Cincinnati, OH), and the Mycoplasma pneumoniae IgG/IgM Antibody Test System (FTI-SERODIA-myco II test; Fujirebio Inc., Taipei, Taiwan) per manufacturers' instructions. M. pneumoniae infection was confirmed if any following was present: (1) seropositivity of mycoplasma IgM in acute stage, (2) positive detection of M. pneumoniae in nasopharyngeal swab by PCR, or (3) four-fold or greater increase in the mycoplasma IgG titer in the acute stage and convalescent stage.

Detection of respiratory viruses

All nasopharyngeal swabs were subjected for viral isolation, including the followings (performed at each study site): fluorescent immunoassay for the detection of influenza A/B and RSV; and viral culture, with the cell lines usually including MK2, MRC-5, and MDCK cells. Real-time PCR (RT-PCR) was performed at the central laboratory to detect viruses of interest as follow: 200 uL of virus transport medium was placed in a MagNA Pure Compact instrument for automated nucleic acid extraction using MagNA Pure Compact Nucleic Acid Isolation Kit I (Roche Applied Science). The resulting RNA was reversely transcribed into cDNA using Transcriptor Reverse Transcriptase (Roche Applied Science) at National Taiwan University Hospital, and the cDNA sample was then sent to the Centers for Diseases Control of Taiwan (central laboratory) where multiplex real-time PCR was performed for detection of viruses, including adenovirus, coronavirus (229E, HKU1, OC43 and NL63), enterovirus, human metapneumovirus, influenza virus (types A and B), parainfluenza virus (types 1−3, 4A, and 4B), RSV, rhinovirus, bocavirus, polyomavirus, parechovirus, parvovirus, and human herpes virus 7.[17,18]

Statistical analyses

The National Health Insurance (NHI) program, which was initiated in 1995 by the government, covers 99.6% of Taiwan's population and 93% of the country's hospitals and clinics are NHI-contracted. The eight participating medical centers are established, long standing hospitals in the northern, middle and southern part of Taiwan and together serviced nearly 20% of the population covered by NHI.[19] The annual incidence rates of hospitalization for CAP were calculated from January 2011 to December 2011, and from January 2012 to December 2012. The study intended to enroll at least 60% of children with CAP at each site. The actual number of participants therefore were adjusted according to the numbers of eligible subjected at each site. The annual population was adjusted according to the proportion of served population at each study site for the corresponding year. Age-specific population was provided by the Department of Household Registration Affairs of the Interior Ministry.

Statistical comparisons of incidence rate were performed using Poisson distribution with 95% confidence intervals; 95% confidence intervals for which the upper and lower bounds did not include 0 were interpreted as statistically significant. The x2 test or Fisher's exact test was used to assess group differences in categorical variables. For continuous variables, Student's t test or One-way Analysis of Variance (ANOVA) was used. A P value less than 0.05 was considered statistically significant. All probabilities were 2-tailed. All statistical analyses were performed using SPSS software, version 15.0 (SPSS Inc., Chicago, IL).

Results

A total of 1032 children with CAP were enrolled during the study period. A total of 494 (47.9%) were male. Their age ranged from 6 weeks to 17.9 years with a median of 4.7 years. Of these, 94 (9.1%) were under 2 years of age, 611 (59.2%) were between 2 and 5 years, and 327 (31.7%) were above 5 years. The annual incidence rates of hospitalization for CAP in children under 18 years in Taiwan was 69.5 cases per 100,000 population. The annual incidence rates by age was highest in children aged 2–5 years (229.7 cases per 100,000 population), followed by children aged < 2 years (69.8/100,000) and was lowest in children aged > 5 years (30.5/100,000).

Among the participants, 69 (6.7%) children had an underlying condition, including chromosome anomaly, metabolic disease, neurologic disorder, congenital heart disease, and hematologic disorder. A potential pathogen was identified in 705 (68.3%) of the 1032 children (Table 1). Isolated bacterial infection was detected in 420 (40.7%) children, isolated viral infection in 180 (17.4%), and viral-bacterial co-infection in 105 (10.2%). Bacterial infection (43.4%) was the most common cause of CAP in children aged 2–5 years, and viral infections (31.9%) in children under 2 years old, whereas the majority (42.8%) of CAP in children older than 5 years old had no identifiable pathogens (Table 1).

The mean duration of hospitalization was 8.4 days (range: 1–84 days). A total of 377 (36.5%) children received supplemental oxygen therapy, and 246 (23.8%) children required intensive care. Children with CAP pathogens identified were younger (median, 4.5 years old vs. 5.3 years old, $P < 0.001$), and had longer hospital stay (mean, 9.3 days vs 6.5 days, $P < 0.001$), higher serum C-reactive protein (CRP) level (mean, 15.3 mg/dL vs. 11.4 mg/dL, $P < 0.001$), higher ICU admission rate (28.6% vs 13.7%, $P < 0.001$), and higher rate of O2 supplementation (41.2% vs 26.5%, $P < 0.001$) (Table 2). Pathogens were also more likely to be isolated in children with complicated pneumonia than those with lobar pneumonia (88.9% vs 64.6%, $P < 0.001$) (Table 2). Patients required intensive care were younger (median, 5.0 years old

vs. 5.8 years old, $P = 0.001$), and had higher serum CRP level (mean, 22.2 mg/dL vs. 11.5 mg/dL, $P < 0.001$), longer hospital stays (mean, 17.2 days vs 5.7 days, $P < 0.001$), and higher rates of O2 supplementation (82.9% vs 22%, $P < 0.001$). ICU admission rates were also higher among cases with pleural effusion, complicated pneumonia, bacterial infection, and mixed viral-bacterial infection (Table 2).

The most identified pathogen was 326 *S. pneumoniae* (31.6%). 56 patients had culture or PCR-confirmed pneumococcal pneumonia and the remaining 276 patients were probable pneumococcal pneumonia. Among the confirmed patients, serotype 19A were identified in 35 patients, serotype 3 in 5 patients, serotype 6A in 3 patients, serotype 19F, 6B, 14 in 2 patients; serotype 23F in 1 patient and unknown in 6 patients. Other pathogens identified were 233 *M. pneumoniae* (22.6%), 61 adenovirus (5.9%), 51 influenza virus (4.9%), 51 RSV (4.9%), 50 rhinovirus (4.8%), and 30 para-influenza virus (2.9%) (Fig. 1A).

The pathogens exhibited age-specific patterns. RSV was significantly associated with children aged under 2 years ($P < 0.001$), *S. pneumoniae* in children aged between 2 and 5 years ($P < 0.001$), and *M. pneumoniae* in children aged >5 years ($P < 0.001$) (Fig. 1B). Pneumonia caused by *S. pneumoniae* was significantly associated with leukocytosis, high C-reactive protein and complicated pneumonia compared to those caused by *M. pneumoniae* and virus alone ($P < 0.001$) (Table 3).

Pneumonia was reported in all months of the year. *S. pneumoniae* CAP occurred year-round, but incidence was lower from May to July (Fig. 2A). *M. pneumoniae* was also detected year-round, but the incidence was lower from February to April (Fig. 2A). Influenza related CAP peaked in the winter season whereas adenovirus related CAP was more frequently seen in June and July (Fig. 2A). RSV was detected year-round but had bimodal peaks in February to March and August to September (Fig. 2B). Rhinovirus peaked in April (Fig. 2C).

Adenovirus, influenza virus, rhinovirus, parainfluenza virus, coronavirus, enterovirus, polyomavirus, parechovirus, *S. pneumoniae*, and *M. pneumoniae* were more likely to be

Table 1 Etiologic identification of case with community-acquired pneumonia in each age group.

Etiology	Total, N (%) (n = 1032)	<2 yr (n = 94)	2–5 yr (n = 611)	>5 yr (n = 327)	P value
Negative	327 (31.7%)	29 (30.9%)	158 (25.9%)	140 (42.8%)	<0.001
Bacteria alone	420 (40.7%)	26 (27.7%)	265 (43.4%)	129 (39.4%)	0.01
S. pneumoniae	326 (31.6%)	28 (29.8%)	257 (42.1%)	41 (12.5%)	<0.001
Urine antigen only	270 (26.2%)	22 (23.4%)	210 (34.4%)	38 (11.6%)	
Culture/PCR	56 (5.4%)	6 (6.4%)	47 (7.7%)	3 (0.9%)	
Blood	28 (2.7%)	4 (4.2%)	22 (3.6%)	2 (0.6%)	
Pleural fluid	25 (2.4%)	1 (1.1%)	23 (3.8%)	1 (0.3%)	
Blood and Pleural fluid	3 (0.3%)	1 (1.1%)	2 (0.3%)	0 (0%)	
M. pneumoniae	233 (22.6%)	6 (6.4%)	115 (18.8%)	112 (34.3%)	<0.001
Throat PCR	93 (9%)	6 (6.4%)	33 (5.4%)	54 (16.5%)	
Serology	108 (10.5%)	0 (0%)	69 (11.3%)	39 (11.9%)	
PCR and serology	32 (3.1%)	0 (0%)	13 (2.1%)	19 (5.9%)	
Virus alone	180 (17.4%)	30 (31.9%)	115 (18.8%)	35 (10.7%)	<0.001
Mixed virus-bacteria	105 (10.2%)	9 (9.6%)	73 (11.9%)	23 (7%)	0.06

Table 2 Comparisons of 1032 cases with community-acquired pneumonia based on etiology identification and ICU admission.

Characteristic	Positive* (n = 705)	Negative* (n = 327)	P value	ICU (n = 246)	No ICU (n = 786)	P value
Age (years), median (range)	4.5 (0–17.9)	5.3 (0–17.9)	<0.001	5.0 (0.1–17.7)	5.8 (0–17.9)	0.001
Male	344 (48.9)	150 (45.7)	0.3	125 (50.8)	369 (46.9)	0.3
Prematurity	80 (11.9)	31 (9.5)	0.2	25 (10.2)	90 (11.5)	0.6
Underlying diseases	36 (5.1)	33 (10.1)	0.003	22 (8.9)	47 (6.0)	0.1
Mean CRP, mg/dL	15.3 ± 12.7	11.4 ± 10.8	<0.001	22.2 ± 12.8	11.5 ± 10.9	<0.001
CRP> 4	538 (76.4)	216 (65.9)	<0.001	219 (89.0)	535 (68.1)	<0.001
CRP>10	401 (57.0)	142 (43.3)	<0.001	195 (79.3)	348 (44.3)	<0.001
Length of Stay, days	9.3 ± 9.3	6.5 ± 6.4	<0.001	17.2 ± 12.7	5.7 ± 3.9	<0.001
ICU admission	201 (28.6)	45 (13.7)	<0.001	—	—	—
O2 supply	290 (41.2)	87 (26.5)	<0.001	204 (82.9)	173 (22.0)	<0.001
Chest radiograph						
Sublobar	177 (25.1)	94 (28.7)	0.2	27 (11.0)	244 (31.0)	<0.001
lobar	336 (47.7)	184 (56.1)	0.01	57 (23.2)	463 (58.9)	<0.001
With pleural effusion	92 (13.1)	37 (11.3)	0.4	69 (28.0)	60 (7.6)	<0.001
Complicated pneumonia	96 (13.6)	12 (3.7)	<0.001	93 (37.8)	15 (91.9)	<0.001
Etiology						
Virus alone	—	—	—	47 (19.1)	151 (19.2)	1.0
Bacteria alone	—	—	—	123 (50.0)	316 (40.2)	0.008
Mixed virus and bacteria	—	—	—	34 (13.8)	53 (6.7)	0.001

*Positive: cases with identified any potential pathogens, Negative: cases with no identified potential pathogen.

present with other pathogens (Table 4). *M. pneumoniae*, rhinovirus, and influenza virus were frequently isolated with *S. pneumoniae* and adenovirus frequently with *M. pneumoniae* (Table 4).

Table 5 showed the Annual incidence of hospitalized childhood CAP between 2011 and 2012. From 2011 to 2012, a significant reduction in CAP hospitalization rates pertained to children aged under 5 years of age. Pneumococcal pneumonia related hospitalization rated decreased by 37% (95% CI: −52% to −18%) in 2012. Pneumonia secondary to mixed virus-bacterial infections (−43%; 95% CI: −63% to −12%) also had decreased. Pneumonia needs ICU admission (−35%; 95% CI: −52% to −12%), sublobar pneumonia (−18%; 95% CI: −33%−1%), pneumonia with pleural effusion (−37%; 95% CI: −58% to −5%) and complicated pneumonia (−63%; 95% CI: −77% to −40%) had also significantly decreased. Hospitalization rates for adenovirus pneumonia and viral pneumonia were high in 2011 but had decreased by 83% (95% CI: −91% to −65%) and 56% (95% CI: −70% to −37%) in 2012, respectively.

Discussion

The hospitalization rate for CAP under 18 years of age in this study was 69.5 cases per 100,000 population, which was 30%–40% lower than that of the United States (157–225 cases per 100,000 population).[8,20] The incidence rates in this study was lower because we intended to recruit only cases with radiologically-confirmed pneumonia including sub-lobar, lobar, with pleural effusion, or complicated pneumonia. The incidences for complicated pneumonia including empyema, necrotizing pneumonia, were otherwise similar in both countries (4.4–10.7 cases vs. 5.4 to 9.6 cases per 100,000 population).[20]

In accordance with our expectations, *S. pneumoniae* was the leading cause of CAP in children under 5-year-old. Most of the pneumococcal infections were diagnosed via urinary antigen tests, but the specificity of the urinary Binax NOW assay in the diagnosis of pneumococcal pneumonia was variable.[21] Literatures had correlated positive urinary antigen results to higher nasopharyngeal carriage rate of pneumococci.[22,23] However, only 14.1% of Taiwanese children aged 2–60 months were colonized by pneumococci in the nasopharynx, a frequency that was much lower than other studies.[24]

In the present study, pneumococcus was documented to contribute to 31.6% of the CAP, similar to the incidence rates of 37–46% reported previously in Taiwan[11] and other countries.[25,26] The leading disease burden of invasive pneumococcal disease (IPD) in Taiwan was bacteremic pneumonia/empyema in children aged between 2 and 5 years.[27] Surveillance data from the CDC-Taiwan revealed that the incidence rate of IPD in children aged between 2 and 5 years had increased from 16.8 cases per 100,000 person-years in 2008–2010 to 22.8 cases per 100,000 person-years in 2011–2012.[28] During this time of suboptimal vaccination coverage of PCV, there were many children aged 2–4 years who suffered from severe pneumococcal pneumonia with empyema.[29,30] The increase was attributed to the emergence and surge of serotype 19A during the studied time period.[28–30] The emergence of serotype 19A was presumably why *S. pneumoniae* still stood out as the predominant pathogen in children with CAP in our study. The 13-valent PCV (PCV13) has been available in the private market in Taiwan since 2011. Within one year of optional PCV13 immunization, the annual CAP hospitalization rates had lowered in children under 5 years of age. The annual incidence rates of pneumococcal pneumonia and complicated pneumonia had also decreased by 30%–60% in 2012. Reduction in incidence rates of hospitalization was also observed in cases with mixed viral-bacterial CAP, which agreed with previous observations by Madhi et al.[31] Our results provided valid evidence and demonstrated the
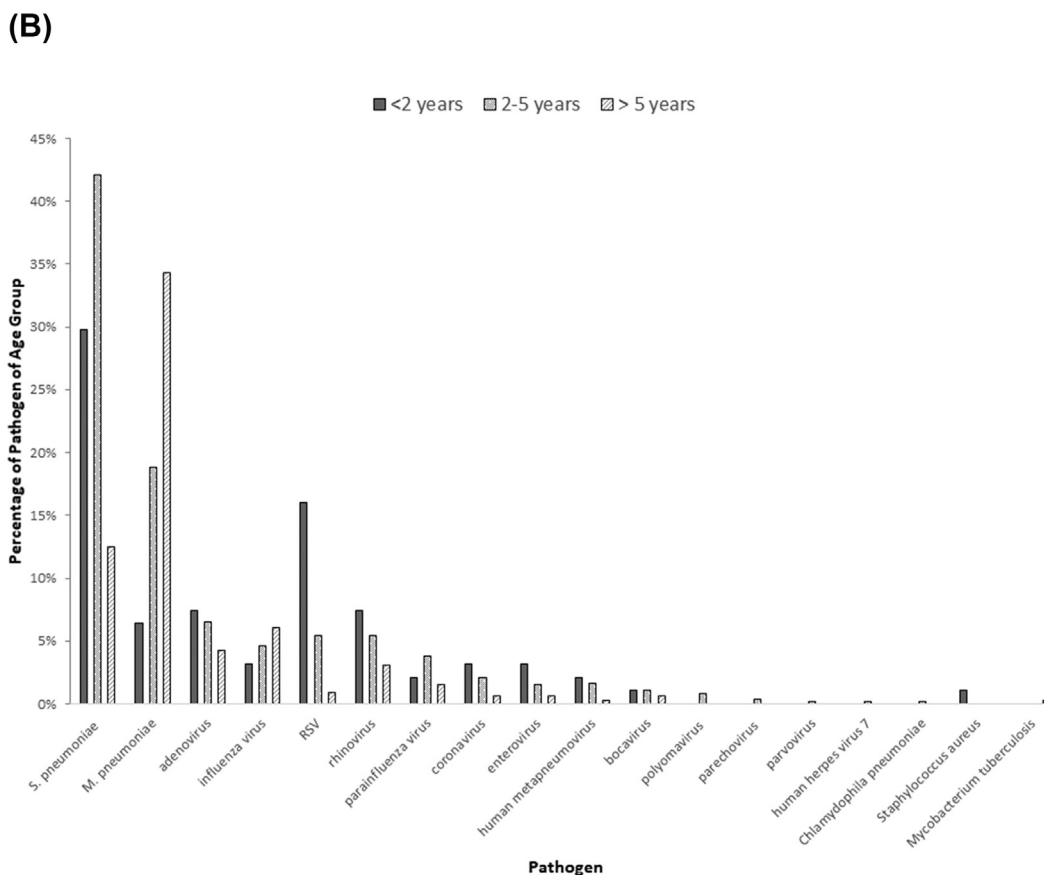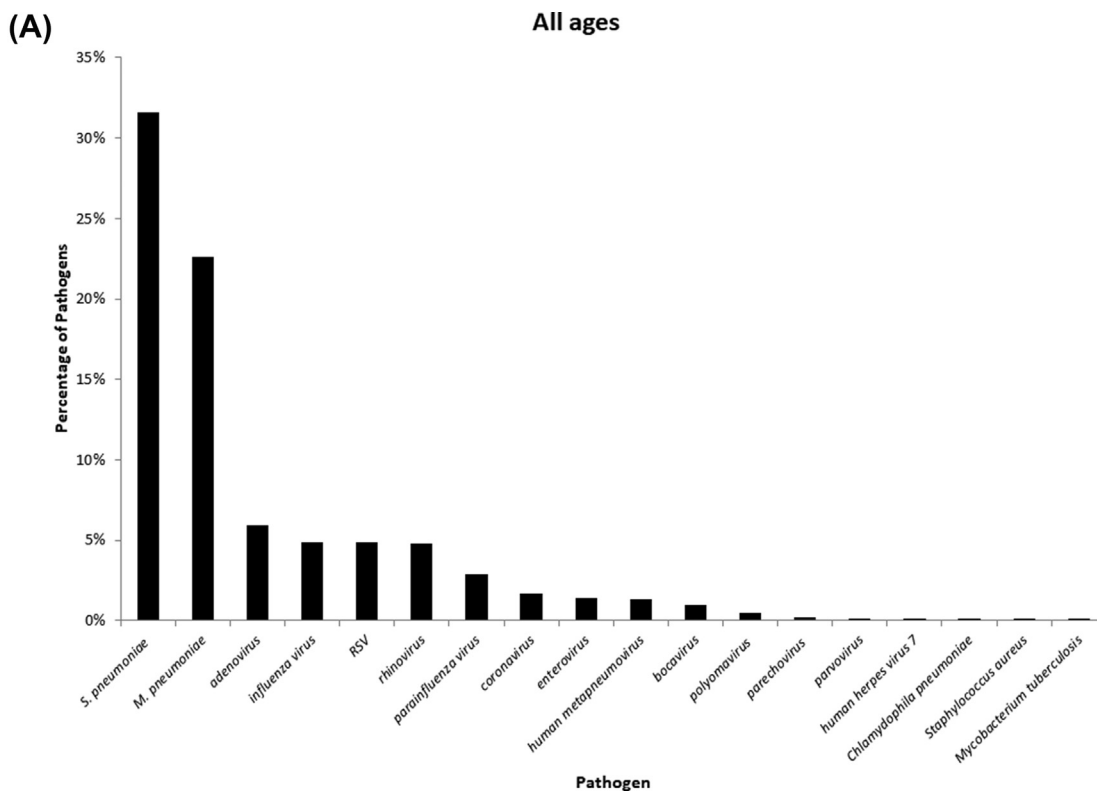
(A)

(B)

Figure 1   (A) Pathogens identified in our study. (B) Percentages of each pathogen in different age groups. RSV: respiratory syncytial virus.

Table 3 Comparisons of cases with community-acquired pneumonia based on different etiology.

Characteristic	S. pneumoniae (n = 326)	M. pneumoniae (n = 233)	Virus alone (n = 180)	P value
Age (years), median (range)	4.2 (0–17.7)	6.8 (0.5–17.9)	3.9 (0.2–17.6)	<0.001
Male	172 (52.89)	91 (46.2)	81 (45)	0.2
Fever	282 (86.5)	156 (79.2)	149 (82.8)	0.09
Cough	315 (96.6)	193 (98)	171 (95)	0.3
Abdominal pain	217 (66.6)	141 (71.6)	117 (65)	0.3
Mean white blood cell count (x10^3/uL)	15.1 ± 5.9	8.9 ± 3.9	11 ± 5.6	<0.001
Hemoglobin level, g/dL	10.5 ± 4.6	11.9 ± 1.3	11.4 ± 1.5	<0.001
Mean CRP, mg/dL	22 2 ± 12.4	7.9 ± 7.9	11 ± 11	<0.001
CRP> 4	299 (91.7)	115 (58.4)	57 (68.3)	<0.001
CRP>10	267 (81.9)	55 (27.9)	78 (43.3)	<0.001
Chest radiograph				
Sublobar	49 (15)	142 (27.9)	73 (40.6)	<0.001
lobar	134 (41.1)	123 (62.4)	79 (43.9)	<0.001
With pleural effusion	51 (15.6)	17 (8.6)	23 (12.8)	0.07
Complicated pneumonia	89 (27.3)	2 (1.0)	5 (2.8)	<0.001
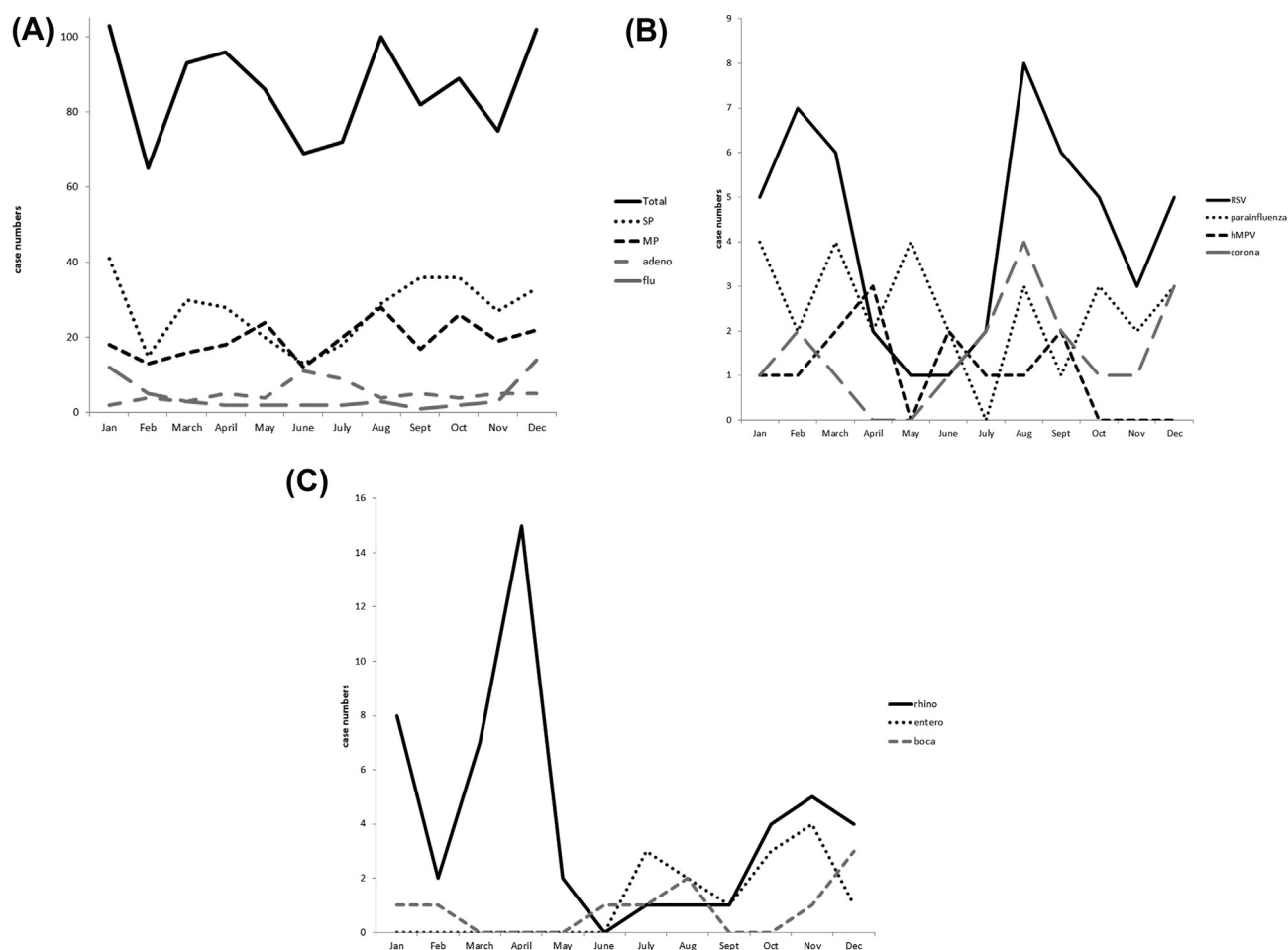
Figure 2 Case numbers of pathogen detection in each month during November 2010 to September 2013. (A) Total pathogens, SP, MP, adeno, and flu; (B) RSV, parainfluenza, hMPV, corona; (C) rhino, entero, and boca. SP: *S. pneumoniae*, MP: *M. pneumoniae*, adeno: adenovirus, and flu: influenza virus, RSV: respiratory syncytial virus, parainfluenza: parainfluenza virus hMPV: human metapneumovirus, corona: coronavirus, rhino: rhinovirus, entero: enterovirus, and boca: bocavirus.

Table 4 Distribution of various pathogens in 705 patients with community-acquired pneumonia.

Pathogen (No.)	Single infection (n = 550) No. (%)	Mixed infection (n = 155) No. (%)	P value	Mixed with		
				Other viruses (No.)	S. pneumoniae (No.)	M. pneumoniae (No.)
Adenovirus (61)	35 (6.4)	26 (16.8)	<0.001	11	7	13
Influenza (51)	27 (4.9)	24 (15.5)	<0.001	3	17	7
Respiratory syncytial virus (51)	40 (7.3)	11 (7.1)	1.0	4	7	2
Rhinovirus (50)	22 (4.0)	28 (18.1)	<0.001	6	18	4
Parainfluenza (30)	15 (2.7)	15 (9.7)	0.002	7	8	4
Coronavirus (18)	5 (0.9)	13 (8.4)	<0.001	5	4	5
Enterovirus (14)	3 (0.5)	11 (7.1)	<0.001	4	7	3
Human metapneumovirus (13)	9 (1.6)	4 (2.6)	0.5	3	1	0
Bocavirus (10)	6 (1.0)	4 (2.6)	0.2	1	3	0
Polyomavirus (5)	1 (0.2)	4 (2.6)	0.009	0	4	0
Parechovirus (2)	0 (0.0)	2 (1.3)	0.05	0	2	0
Parvovirus (1)	0 (0.0)	1 (0.6)	0.2	0	0	1
Human herpes virus 7 (1)	0 (0.0)	1 (0.6)	0.2	0	0	1
Chlamydophila pneumoniae (1)	0 (0.0)	1 (0.6)	0.2	0	1	0
Streptococcus pneumoniae (326)	221 (40.3)	105 (67.7)	<0.001	73	—	36
Mycoplasma pneumoniae (233)	164 (29.7)	69 (44.5)	0.002	37	36	—
Staphylococcus aureus (1)	1 (0.2)	0 (0.0)	—	0	0	0
Mycobacterium tuberculosis (1)	1 (0.2)	0 (0.0)	—	0	0	0

Table 5 Annual incidence of hospitalized childhood community-acquired pneumonia between 2011 and 2012.

Characteristics	Incidence (no. of cases per 100,000 children per year)			Relative Changes in Incidence (2011 vs 2012)	
	Overall	2011	2012	Percentage (95% CI)	P value
Total	69.5	78.5	60.3	−30 (−40 to −20)	<0.001
Age group (year-old)					
<2	69.8	93.5	49.6	−47 (−68 to −12)	0.01
2-5	229.7	270.3	187.1	−31 (−43 to −16)	<0.001
>5	30.5	30.0	31.1	3.6 (−20 to 33.6)	0.8
Pathogen					
S. pneumoniae	21.4	25.2	17.5	−37 (−52 to −18)	0.001
M. pneumoniae	15.6	16.6	14.6	−20 (−41 to 8)	0.2
Adenovirus	5.4	8.9	1.7	−83 (−91 to −65)	<0.001
Influenza	4.2	3.7	4.8	15.3 (−36 to 107)	0.6
Respiratory syncytial virus	3.0	3.4	2.7	−28 (−64 to 44)	0.4
Parainfluenza	2.3	2.8	1.7	−45 (−76 to 27)	0.16
Human metapneumovirus	0.9	1.3	0.6	−60 (−90 to 53)	0.2
Virus-bacteria coinfection	8.4	10.3	6.5	−43 (−63 to −12)	0.01
Requiring ICU admission	18.1	25.4	10.6	−67 (−72 to −48)	<0.001
Sublobar pneumonia	16.4	19.0	13.7	−35 (−52 to −12)	0.005
Lobar pneumonia	36.3	38.0	34.6	−18 (−33 to 1)	0.06
Pneumonia with pleural effusion	9.0	10.6	7.4	−37 (−58 to −5)	0.02
Complicated pneumonia	7.5	10.7	4.4	−63 (−77 to −40)	<0.001

critical role of *S. pneumoniae* in the development of virus-associated pneumonia. A national vaccination catch-up program providing 1 dose of PCV13 to children aged 2–5 years was launched in 2013, followed by a program providing 2 doses in children aged 1–2 years old in 2014 and a 2 + 1 national infant immunization program in 2015.[32] Based on the surveillance data from the CDC-Taiwan, the incidence rate of IPD in children aged 2–5 years had decreased to 11.9/100,0000 person-years in 2013.[28] The

incidence rate of pneumococcal pneumonia is expected to continue falling under the current PCV13 immunization policy.

The current diagnosis of *M. pneumoniae* infections relies on serology or molecular diagnosis by using real-time PCR to detect bacterial DNA from respiratory samples. Studies have shown that *M. pneumoniae* was responsible for 8%–35% of CAP, confirmed either by serology and/or PCR.[8,9,25] In the present study, diagnoses of mycoplasma infection

were demonstrated from RT-PCR, serology, or both in 12.1%, 13.6%, and 3.1% of our cases (respectively). Spuesens et al.[33] reported that *M. pneumoniae* is present in the upper respiratory tract in asymptomatic children and concluded the current diagnostic modalities are unable to differentiate symptomatic infection from asymptomatic carriage of *M. pneumoniae*. Furthermore, serological data do not correlate well with PCR results.[33] Thus, it remains challenging to establish a definite diagnosis of mycoplasma infection.

Case-controlled studies exploring the causal relationship between pathogens and pneumonia, had associated RSV, influenza, hMPV, parainfluenza, adenovirus, and coronavirus with CAP, especially the first three.[8,34,35] Self et al.[35] demonstrated an age-dependent relationship between rhinovirus, adenovirus and CAP. Rhinovirus was associated with CAP in adults, but not in children. Adenovirus associated CAP occurred only in children aged under 2 years old. Geographical, seasonal, and epidemiological factors could have contributed to the apparent differences. In the present study, adenovirus was the most frequently detected virus in children with CAP. In 2011, Taiwan experienced a community outbreak of co-circulating adenovirus type 2, 3 and 7.[36] Among the 203 cases of adenovirus infection identified at a tertiary center, 39% had consolidation on radiograph, 15.3% required intensive care, and 3.4% died.[36] The outbreak was within the period of this study, which explains why adenovirus was the most common virus identified in this study.

It is worth mentioning that most of the studies have tested nasopharyngeal specimens using quantitative real-time PCR in CAP patients. Although highly sensitive, quantitative real-time PCR should be utilized cautiously. Viruses can often be isolated from the respiratory tracts. Given, rhinovirus, enterovirus, and bocavirus have been detected frequently in asymptomatic children, the clinical significance of PCR results should be interpreted carefully.[8,37] A previous study from Taiwan reported that the viral detection rates among asymptomatic children in Taiwan were 9.7% (vs. 4.8% in the present study) for rhinovirus, 4.4% (vs. 1.4% in the present study) for enterovirus, and 0.9% (vs. 1.7% in the present study) for coronavirus, respectively.[38] Influenza and hMPV were not detected in asymptomatic children in that study.[38] Furthermore, the nasopharynx is distant from the lung; nasopharyngeal sampling is not representative of bronchopulmonary specimens.

The major drawbacks of the present study were (1) the lack of controls without pneumonia to assess the strength of association between a positive test and pneumonia etiology, (2) the use of positive urinary antigen test as the diagnosis of pneumococcal infection, which might be subjected to substantial over-diagnosis, and (3) the dearth of diagnostic tools with sensitivities sufficient to permit identification of bacterial sources of infection. The latter issue was presumably the reason for the relatively low detection rate of pathogens in our study. We might need an integrated analytic approach on studies of pneumonia etiology with different perspectives in the future. However, the strength of the present study is that this work was a prospective, multicenter study, that had spanned for 3 years, providing thorough inspections of CAP pathogens in different seasons.

In this study, we observed improved conjugated pneumococcal vaccination coverage was associated with reduction in the incidence rates of CAP secondary to *S. pneumoniae*, Adenovirus, and viral-bacterial coinfections. The implementation of PCV13 will render *M. pneumoniae*, adenovirus, influenza, RSV, parainfluenza and hMPV significant CAP agents. This study described the latest incidences and trends of CAP pathogens, which are crucial for prompt delivery of appropriate therapy.

Declaration of Competing Interest

The authors have no conflicts of interest relevant to this article.

Acknowledgments

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