BACTERIAL ETIOLOGY OF EMPYEMA THORACIS AND PARAPNEUMONIC PLEURAL EFFUSION IN THAI CHILDREN AGED LESS THAN 16 YEARS

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Abstract. This study aimed to identify the bacterial etiology of empyema thoracis or parapneumonic pleural effusions in Thai children, with a focus on pneumococcus. This hospital-based, descriptive study included children aged ≤16 years, diagnosed with empyema thoracis or parapneumonic pleural effusion, from whom a pleural fluid (PF) sample was taken between January 2008 and November 2009. PF and blood samples were cultured and PF samples were also tested by polymerase chain reaction (PCR) to assess whether evidence of an infection might be identified among culture-negative samples. Serotyping of *Streptococcus pneumoniae*-positive samples was performed by molecular techniques and Quellung reaction. In this study, 29 children with empyema thoracis and 42 children with parapneumonic pleural effusion were enrolled. Potentially pathogenic bacteria were cultured in 13/71 samples at local or central laboratories; the most common bacteria were *Staphylococcus aureus* (8 children) and *S. pneumoniae* (2 children). Molecular techniques detected one or more targeted respiratory pathogens in 18/71 PF samples. *S. pneumoniae* and *Haemophilus influenzae* were identified by PCR in 13 and 6 children, respectively; PCR for *S. aureus* was not performed. The pneumococcal serotypes identified were 1, 3, 5, 6A/B, 9A/V, 14, 15A, 19F and 23A. This study shows that among Thai children with empyema thoracis and parapneumonic pleural effusions, *S. aureus* and *S. pneumoniae* were the most common pathogens identified by culture and PCR, respectively. These findings confirmed that molecular techniques are more sensitive for identification of *S. pneumoniae* and *H. influenzae* and enhance detection of important bacterial causes of empyema.

Keywords: empyema thoracis, uncomplicated parapneumonic pleural effusion, etiology, Thai children

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INTRODUCTION

During the past decade, the incidences of pediatric parapneumonic pleural effusions and empyema thoracis have increased in various parts of the world (Rees *et al*, 1997; Spencer *et al*, 2006; Spencer and Cliff, 2008; Li and Tancredi, 2010). Empyema thoracis is associated with significant morbidity and mortality in children and the consequences of this condition may be severe, including prolonged hospitalization, the need for intensive supportive care and drainage of accumulated fluid, surgical intervention and the use of complex antibiotic regimens (Byington *et al*, 2002; Hsieh *et al*, 2004; Byington *et al*, 2006).

To date, few studies have been conducted regarding the incidence and etiology of parapneumonic pleural effusions and empyema thoracis in the Asia-Pacific region, and there are no data published for Thailand. A previous study conducted in Taiwan between 1997 and 2004 showed that the annual population-based incidence of empyema thoracis reached 10.5 episodes per 100,000 children under 5 years of age and that *Streptococcus pneumoniae* was the most common pathogen identified in this age group (Wu *et al*, 2010). A more recent study conducted in China, Korea, Taiwan, and Vietnam identified *Staphylococcus aureus* followed by *S. pneumoniae* as the most frequently isolated organisms in patients with empyema thoracis (Nyambat *et al*, 2008).

In Thailand, antibiotics can be purchased without prescription and their widespread and incorrect use is at least in part responsible for the high level of antibiotic resistance observed among *S. pneumoniae* isolates here (Reechaipichitkul *et al*, 2006; Srifeungfung *et al*, 2010). Previous antibiotic use by a patient reduces the ability to detect causative pathogens by standard bacterial culture techniques. Molecular techniques may need to be used to identify pathogens and pneumococcal serotypes and they may have a higher sensitivity (Saglani *et al*, 2005; Lahti *et al*, 2006; Le Monnier *et al*, 2006; Tarrago *et al*, 2008; Blaschke *et al*, 2011).

The co-primary objectives of this study were to determine the occurrence and serotypes of *S. pneumoniae* causing empyema thoracis and parapneumonic pleural effusions in Thai children aged less than 16 years, using both molecular and standard microbiological techniques. The secondary objectives were the description of clinical management approaches and outcomes of childhood empyema thoracis and parapneumonic pleural effusion, the identification of other pathogens likely to be responsible for these conditions, and the determination of the antibiotic susceptibilities of *S. pneumoniae* isolates causing both conditions in Thai children.

MATERIALS AND METHODS

Study design

This was a hospital-based, descriptive, etiological study conducted at 9 centers scattered across Thailand between January 2008 and November 2009. The study period at each study center was 12 months to take seasonal variations into account. The study included children younger than 16 years of age admitted to the study hospitals with empyema thoracis or parapneumonic pleural effusions whose clinical condition necessitated a diagnostic thoracocentesis or drainage of pleural fluid (PF), and from whom a PF sample was taken and was available for laboratory testing. The diagnosis of empyema thoracis was based on the macroscopic presence of pus in the PF sample.

Children were not included in the study if they were concurrently participating in another clinical trial, had a known malignancy, collagen vascular disease, or melioidosis thought to be responsible for the pleural effusion, or had been previously enrolled in this study for a previous episode. Before enrolment, informed consent was obtained from the parents/guardians of each study participant. The study was conducted according to Good Clinical Practice, the Declaration of Helsinki, and the local rules and regulations of Thailand. The study protocol and the consent form were reviewed and approved by local ethics committees.

Study procedures

All the children diagnosed with empyema thoracis or parapneumonic pleural effusion at the study hospitals were identified and their basic demographic characteristics were anonymously recorded in a logbook. Reasons for non-enrolment for children who did not meet the inclusion criteria or declined to participate in the study were also collected in the logbook. For each enrolled child, demographic characteristics, medical history, including risk factors and pneumococcal vaccination status, general symptoms and treatment given for the current episode, were collected and a clinical examination was performed at baseline. The potential risk factors recorded in this study included absence of breast feeding, presence of household siblings aged less than five years, prematurity, HIV infection, child care attendance, previous episode of invasive pneumococcal disease, having a congenital chromosomal abnormality, agammaglobulinemia, sickle cell disease, anatomic or functional dysplenia, nephrotic syndrome, chronic renal failure, organ transplantation, diabetes mellitus, congestive cardiac failure, steroid use, cerebrospinal fluid leaks or chronic lung disease. The disease outcome at discharge was recorded for each enrolled child. The disease outcome was recorded as recovered if clinical improvement was observed and if the child was nearly symptom free, had no dyspnea, no tachypnea, and a chest X-ray that was normal or showed only a small pleural effusion. The disease outcome was recorded as recovering if clinical improvement was observed but the child still had some symptoms related to pleural effusion, such as coughing and a chest X-ray that might still have a residual pleural effusion. The biochemical composition of PF sample [pH, lactate dehydrogenase and glucose concentrations, and white blood cells (WBC) counts] and blood samples (WBC counts) were also evaluated and recorded.

At the local hospital laboratories, PF and blood samples were cultured by standard techniques to identify pathogens responsible for empyema thoracis or parapneumonic pleural effusion (Fig 1). A fraction of the PF samples also underwent standard microbiological culture analyses at the Centre for Infectious Diseases and Microbiology (CIDM, Australia). PF samples were also subjected to molecular analyses at the CIDM (Fig 1). PF samples were tested by real-time polymerase chain reaction (PCR) for the presence of genes encoding the pneumococcal autolysin (*lytA*) and species-specific PCR was performed for *Haemophilus influenzae*, *Mycoplasma pneumoniae,* and *Chlamydia pneumoniae*. Samples negative by both culture and species-specific PCR were tested by 16S rDNA PCR to identify other pathogens.

A child was considered *S. pneumoniae* culture-positive if at least one positive culture result for *S. pneumoniae* was obtained at the local laboratory (blood or

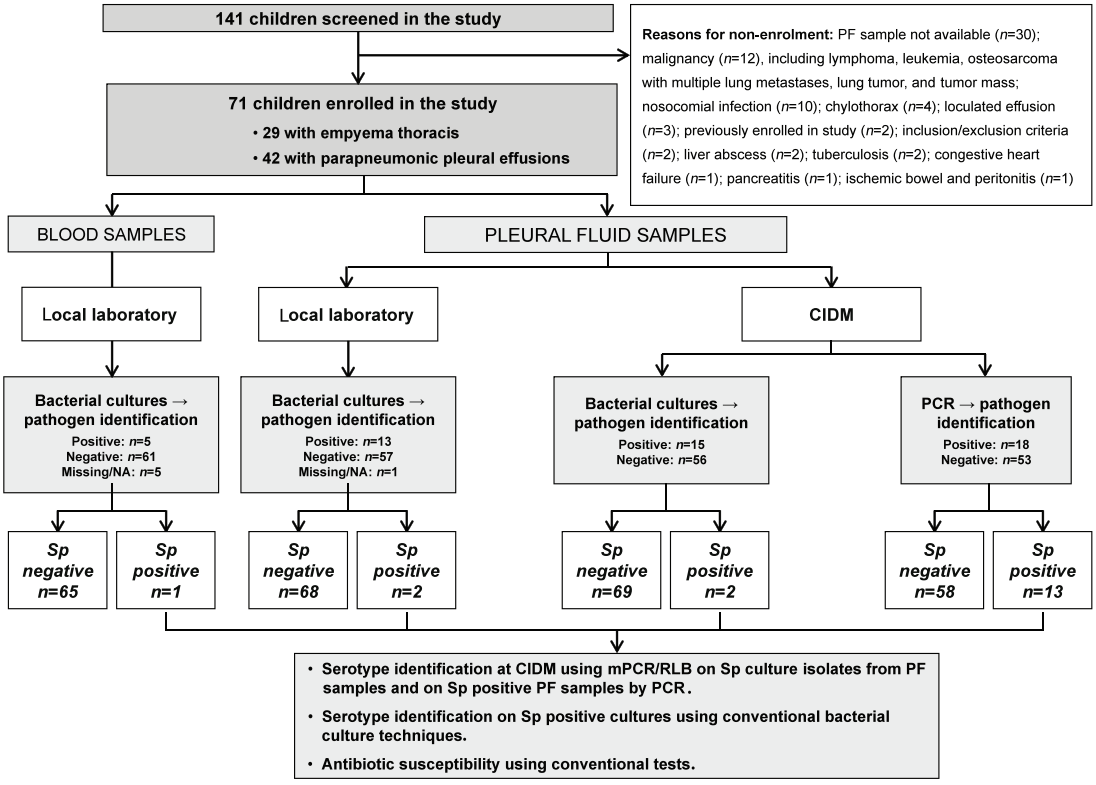

Fig 1–Study procedures. ET, empyema thoracis diagnostic group; UPE, parapneumonic pleural effusion diagnostic group; *n*, number of children in the specified category; CIDM, Centre for Infectious Diseases and Microbiology; PCR, polymerase chain reaction; NA, not applicable; Sp, *Streptococcus pneumoniae*; mPCR/RLB, multiplex polymerase chain reaction-reverse line blot; PF, pleural fluid.

PF sample) or at the CIDM. A child was considered *S. pneumoniae*-positive if *S. pneumoniae* was detected by any method (bacterial culture or PCR) in the blood or PF samples. *S. pneumoniae* culture isolates from PF and blood samples, and *S. pneumoniae* PCR-positive PF samples were further characterized at the CIDM to identify pneumococcal serotypes using multiplex PCR-reverse line blot (mPCR/RLB) assays, in which both *lytA* and pneumococcal pneumolysin (*ply*) were used as positive controls, as previously described (Strachan *et al*, 2011). Serotyping was also performed on all available *S. pneumoniae* culture isolates by conventional microbiological culture techniques using the Quellung reaction.

Antibiotic susceptibilities were tested in *S. pneumoniae* culture-positive samples, using the E-test (penicillin and ceftriaxone) or Kirby-Bauer disc diffusion test (erythromycin and azithromycin). The breakpoints recommended by the Clinical and Laboratory Standard Institute (2009) were used to determine the antibiotic susceptibilities of the pneumococcal isolates.

Statistical analysis

At least 100 children were expected

Table 1
Demographic characteristics and history of pneumococcal vaccination and antibiotic use among Thai children with empyema thoracis or parapneumonic pleural effusions (total cohort).

Variable	*N*=71
Age (years), mean ± SD	5.7 ± 4.75
Age category (years), *n* (%)	
<2	16 (23)
2-5	26 (37)
6-16	29 (41)
Gender, *n* (%)	
Female	26 (37)
Male	45 (63)
At least one dose of pneumococcal vaccine, *n* (%)	
Yes	0 (0)
No	68 (96)
Unknown	3 (4)
Antibiotic pre-treatment, *n* (%)	
Yes	65 (92)
No	6 (8)

N, total number of children; *n* (%), number (percentage) of children in the specified category; SD, standard deviation.

to be enrolled in this study. The screened cohort included all children recorded in the logbook and the total cohort, all children enrolled in the study.

Most study analyses were descriptive. The proportions of *S. pneumoniae*-positive children and of *S. pneumoniae* culture-positive children were calculated with their 95% confidence interval (CI). Comparability between children with empyema thoracis and parapneumonic pleural effusions for descriptive variables was assessed by the Student-*t* or the Wilcoxon-Mann-Whitney tests for continuous variables and by the Fisher exact or chi-square tests for categorical variables. All statistical analyses were performed using SAS®, version 9.2 (SAS Institute, Cary, NC).

RESULTS

Demographic and baseline characteristics of enrolled children

Of the 141 children screened, 29 with empyema thoracis and 42 with parapneumonic pleural effusions were enrolled in the study. The main reasons the others were not enrolled included: non-availability of a PF sample [30/70 insufficient or inadequate PF (*n*=22) and no thoracocentesis (*n*=8)], malignancy (12/70) and nosocomial infection (10/70) (Fig 1). Nearly all the remaining 18 children were excluded because their condition was thought to be due to a non-infectious cause or tuberculosis. More boys than girls were screened (86 boys, 55 girls) and enrolled (45 boys, 26 girls) in the study. The differences in gender distribution between the non-enrolled and enrolled children were not statistically significant (p=0.6). Enrolled children with empyema thoracis were significantly younger than enrolled children with parapneumonic pleural effusions (mean age of 4.5 and 6.6 years, respectively; p = 0.04) (Table 1).

None of the enrolled children had been vaccinated with a pneumococcal conjugate vaccine. Seventy-three percent of the children (52/71) had no risk factor. The risk factors identified in the other 19 children included presence of household siblings aged less than 5 years (*n*=15), absence of breast feeding among children younger than 2 years old (*n*=9), prematurity (*n*=3) and child care attendance (*n*=3). Antibiotic use during the 1 month prior to their enrolment in the study was found in 92% of children (65/71). Antibiotic treatment of at least 5 days before enrolled was found in 74% (48/64).

Table 2
Treatment description of Thai children with empyema thoracis or parapneumonic pleural effusions (total cohort).

Characteristics	Categories	*n* (%)
Diagnostic thoracocentesis performed	Yes	66 (93)
	No	5 (7)
Thoracostomy performed	Yes	38 (54)
	No	33 (46)
Chest drain inserted	Yes	36 (51)
	No	35 (49)
Decortication performed	Yes	6 (16)
	No	32 (84)
	NA	33 (-)
Thoracotomy performed	Yes	11 (29)
	No	27 (71)
	NA	33 (-)
Complications of thoracostomy	Yes	5 (13)
	No	33 (87)
	NA	33 (-)
Analgesia used	Yes	59 (83)
	No	11 (15)
	Unknown	1 (1)

n (%), number (percentage) of children in the specified category; NA, not applicable.

Clinical management

Forty-nine percent of children (35/71) were referred from another hospital. The duration of hospitalization in the study hospital ranged from 4 to 72 days and the mean duration of hospitalization was 23 days. At discharge, 61 children were "recovered or recovering", 3 had "not recovered", 1 had recovered with sequelae and 6 children died during the study period (1 child with empyema thoracis and 5 children with parapneumonic pleural effusion), giving an overall case fatality rate (CFR) of 8% (3% for children with empyema thoracis and 12% for children with parapneumonic pleural effusions). The causes of death were: respiratory failure (*n*=2, the first child had Down's syndrome and morbid obesity as underlying diseases and acute respiratory distress syndrome, acute hepatitis, and obstructive sleep apnea as co-morbidities, and the second child had mucopolysaccharidosis as an underlying disease and acute respiratory distress syndrome, cardiomyopathy, and autoimmune hemolytic anemia as co-morbidities); severe sepsis with disseminated intravascular coagulopathy (*n*=1, the child had systemic lupus erythematosus as underlying disease and rapidly progressive glomerulonephritis, renal insufficiency and severe pneumonia as co-morbidities); lymphoma with lung metastases (*n*=1, the child had superior vena cava obstruction, acute respiratory failure, right pneumothorax, and bacterial pneumonia as co-morbidities); right epidural abscess with subdural empyema

Table 3
Etiology of bacteria identified by culture and by PCR from blood and PF samples among Thai children with empyema thoracis or parapneumonic pleural effusions (total cohort).

Sample origin	Characteristics	Categories	*n* (%)
Blood sample/Bacterial culture/Local laboratory	Culture result	Positive	5 (8)
		Negative	61 (92)
		Missing/NA	5 (-)
	Streptococcus pneumoniae	Positive	1 (2)
	Haemophilus influenzae	Positive	1 (2)
	Staphylococcus aureus	Positive	2 (3)
	Enterobacter spp	Positive	1 (2)
PF sample/Bacterial culture/Local laboratory	Culture result	Positive	13 (19)
		Negative	57 (81)
		Missing/NA	1 (-)
	Streptococcus pneumoniae	Positive	2 (3)
	Haemophilus influenzae	Positive	1 (1)
	Pseudomonas aeruginosa	Positive	1 (1)
	Staphylococcus aureus	Positive	6 (9)
	Streptococcus species[a]	Positive	2 (3)
	Acinetobacter baumannii[b]	Positive	1 (1)
PF sample/Bacterial culture/CIDM	Culture result	Positive	15 (21)
		Negative	56 (79)
	Streptococcus pneumoniae	Positive	2 (3)
	Haemophilus influenzae	Positive	1 (1)
	Staphylococcus aureus	Positive	8 (11)
	Acinetobacter baumanii[b]	Positive	1 (1)
	Streptococcus milleri[a]	Positive	1 (1)
	Skin contaminants[c]	Positive	2 (3)
PF sample/ PCR/CIDM	Any bacteria	Positive	18 (25)
		Negative	53 (75)
	Streptococcus pneumoniae	Positive	13 (18) - 2 mixed[d]
	Haemophilus influenzae	Positive	6 (8) - 2 mixed[d]
	Mycoplasma pneumoniae	Positive	1 (1)

n (%), number (percentage) of children in the specified category; PF, pleural fluid; PCR, polymerase chain reaction; CIDM, Centre for Infectious Diseases and Microbiology; NA, not applicable.
[a]*Streptococcus* species isolated by the local laboratory and *Streptococcus milleri* isolated by CIDM were from the same patient.
[b]*Acinetobacter baumannii* is a recognized opportunistic pathogen and cause of nosocomial pneumonia.
[c]*Staphylococcus epidermis* was in one sample and *Staphylococcus capitis* and *Bacillus* species were in the other sample.
[d]Two children infected with *H. influenzae* were co-infected with *S. pneumoniae.*

(*n*=1); and H1N1 influenza (*n*=1, the child had Aicardi syndrome with epilepsy as underlying disease). Based on the exclusion criteria, the children, who were diagnosed after their enrolment with disseminated intravascular coagulopathy and lymphoma with lung metastasis should have been excluded from the

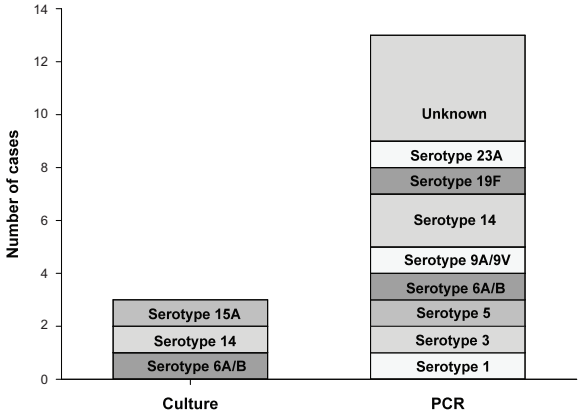

Fig 2–Distribution of pneumococcal serotypes responsible for empyema thoracis and parapneumonic pleural effusions in Thai children assessed by culture at the local laboratory and by PCR at the Centre for Infectious Diseases and Microbiology (total cohort). PCR, polymerase chain reaction; Unknown, below the detection limit.

study, but both children were included in the analysis and may have introduced a bias in the results.

When both current and past conditions were taken into account, respiratory, thoracic and mediastinal underlying conditions were the most common pre-existing conditions, reported in 14% (10/71) of children. On hospital admission, the most frequently reported signs were abnormal auscultatory findings, which were reported in 92% (65/71) of children, followed by tachypnea in 89% (63/71), cough in 83% (59/71), and need for oxygen in 82% (58/71). The therapeutic procedures performed are shown in Table 2. Thoracotomy was performed more often in children with empyema thoracis than in children with a parapneumonic pleural effusions (27/29 versus 11/42; p <0.001); there were no significant differences between the groups in frequency of thoracotomy (9/27 versus 2/11; p= 0.45) or decortication (6/27 versus 0/11; p = 0.15).

Significantly more children with empyema thoracis than parapneumonic pleural effusion had PF samples with a lactate dehydrogenase level >1,000 IU/μl (17/20 versus 15/40; p <0.001), a glucose <60 mg/dl (15/20 versus 5/38; p <0.001), and a white blood cell count >1,000/μl (13/17 versus 18/39; p= 0.045). However, a similar proportion in each group had a PF pH<7.2 (4/14 versus 7/32; p = 0.71).

Identification of pathogens responsible for empyema thoracis and parapneumonic pleural effusion

At the local laboratories, potential pathogens were detected on standard bacterial culture in 3/26 and 2/40 blood samples and in 11/28 and 2/42 PF samples among children with empyema thoracis and parapneumonic pleural effusions, respectively (Table 3). The most frequently cultured pathogen was *S. aureus*, identified in the blood of 1 child with empyema thoracis and 1 child with a parapneumonic pleural effusion and in higher proportions of PF samples (5 versus 2 children; p = 0.031). The other bacteria isolated from the PF samples were *S. pneumoniae*, *H. influenzae*, *Pseudomonas aeruginosa*, *Streptococcus* species, and *Acinetobacter baumannii*.

At the CIDM, pathogens were isolated by standard bacterial culture of PF samples in 13/71 specimens; 10/29 from children with empyema thoracis and 3/42 from children with parapneumonic pleural effusions. These pathogens were the same as those detected at the local laboratories, except for *P. aeruginosa*, which was not isolated at the CIDM. *S. aureus* was detected in a higher proportion of children with empyema thoracis than with parapneumonic pleural effusions, but this was not statistically significant

(6/29 versus 2/42 children; $p = 0.056$). In addition to the 13 pathogens identified by culture, bacteria considered as skin contaminants were cultured from the PF samples of 2 children.

At the CIDM, respiratory pathogens (excluding contaminants) were detected by PCR in 18/71 PF samples; 9/29 from children with empyema thoracis and 9/42 from children with parapneumonic pleural effusions. The most frequently identified pathogen was *S. pneumoniae* (13/71), followed by *H. influenzae* and *Mycoplasma pneumoniae*. Two children infected with *H. influenzae* were co-infected with *S. pneumoniae*. DNA of the non-pathogenic environmental bacteria was detected in 28/39 PF samples tested by 16S rDNA PCR; and were considered to be contaminants. No additional pathogens were identified. This suggests no *S. aureus*-positive samples were missed by culture.

Occurrence and serotype distribution of *S. pneumoniae*

Overall, 13/71 children (18.3%) were *S. pneumoniae*-positive (5 children with empyema thoracis and 8 children with parapneumonic pleural effusions). Of these, 3 were also *S. pneumoniae* culture-positive (2 PF samples and 1 blood sample). Serotypes 6B, 14 and 15A were identified by conventional serotyping methods in these children (Fig 2). The serotypes identified by PCR in the *S. pneumoniae*-positive children were: 14 (n=2), 1, 3, 5, 6B, 9A/V, 19F and 23A (n=1 for each serotype). The serotypes could not be identified by molecular methods in 4 *S. pneumoniae*-positive children. Serotypes identified by bacterial culture were also identified by PCR, except in a child identified as positive for serotype 15A by bacterial culture (from a blood sample), whose PF sample was culture negative and PCR positive, but with an unknown serotype.

Antibiotic susceptibility

Of the 3 *S. pneumoniae* culture-positive samples, all were susceptible to penicillin, ceftriaxone, erythromycin and azithromycin (data not shown).

DISCUSSION

The results are the first published data regarding the etiology of empyema thoracis and parapneumonic pleural effusions in Thailand. A variety of different bacteria were identified by bacterial culture or molecular analyses in PF samples from Thai children diagnosed with both conditions.

For the majority of respiratory pathogens, a higher proportion of PF samples were positive when the PCR analysis was used with the bacterial culture. Using bacterial culture and PCR, 2 and 13 children were *S. pneumoniae*-positive, 1 and 6 were *H. influenzae*-positive and 0 and 1 with *M. pneumoniae*-positive, respectively. *S. aureus* was identified by culture in 8 children. Other opportunistic pathogens, such as *P. aeruginosa*, *Streptococcus* species and *Acinetobacter baumannii* were detected by culture in PF samples; these bacteria can be respiratory contaminants or a significant cause of opportunistic pneumonia, especially in immunocompromised children.

S. aureus was the most frequently identified bacteria by culture at both the local laboratories and the CIDM. This is similar to the results of a previous study, in which *S. aureus* was the most common bacteria identified by culture in Asian children with empyema (Nyambat *et al*, 2008). This is also consistent with an unpublished study from Bangkok during 1993-1997, which found the most common species isolated by culture from children

with parapneumonic effusions were *S. aureus* followed by *S. pneumoniae* and *H. influenzae* (Wiramitchai, 1998). However, these results contrast with those of a recent study conducted in Taiwan, where *S. pneumoniae* was the most common pathogen identified from bacterial cultures in children with empyema thoracis and parapneumonic pleural effusions (Lin *et al*, 2011).

Using molecular techniques, the most frequently identified pathogens in our study were *S. pneumoniae*, followed by *H. influenzae*. This is similar to a study among Taiwanese children, although *S. pneumoniae* was found less frequently in their study than in our study among children with empyema thoracis (17% *vs* 75%) and among children with parapnuemonia pleural effusions (19% *vs* 45%) (Lin *et al*, 2011). The differences between these studies might be due to problems in storage or transport of the samples, leading to degradation of the DNA.

The proportions of *S. pneumoniae*-positive samples did not differ substantially between children with empyema thoracis and parapneumonic pleural effusions, when bacterial culture or molecular techniques were used for pathogen identification. Although all culture-positive samples were also PCR-positive, we observed a greater sensitiviy and specificity with molecular analysis. Three different serotypes were identified from 3 *S. pneumoniae*-positive PF samples by bacterial culture and 8 different serotypes were identified from 13 *S. pneumoniae*-positive PF samples by PCR. Four of the 13 *S. pneumoniae*-positive children had an unknown serotype in the current study. This proportion is similar to other studies using mPCR/RLB for serotype identification (Lin *et al*, 2011; Strachan *et al*, 2011). PCR identifed serotypes 1 and 5, which are included in the 10-valent and 13-valent pneumococcal conjugate vaccines recently approved for use in Thailand (Food and Drug Administration Thailand, 2012a,b). This finding is consistent with studies from other countries (Hausdorff *et al*, 2005; Byington *et al*, 2006; Calbo *et al*, 2006; Fletcher *et al*, 2006; Obando *et al*, 2006). Eight of the 13 serotypes detected by PCR are covered by either the 10- or 13-valent vaccines, similar to the pneumococcal serotype distribution found in other studies from Thailand (Reechaipichitkul *et al*, 2006; Srifeungfung *et al*, 2010). No specific conclusions regarding serotype distribution can be made given the small number of isolates and DNA samples serotyped in this study.

Pneumococcal isolates in this study were susceptible to penicillin, ceftriaxone, erythromycin and azithromycin. However, antibiotic susceptibility analyses were performed in only 3 *S. pneumoniae* culture-positive isolates. Additional studies are needed to evaluate the rates of antibiotic resistance among *S. pneumoniae* in Thai children with empyema thoracis and parapneumonic pleural effusions.

An unexpectedly high number of enrolled children died during this study (6 children: 1 with empyema thoracis and 5 with parapneumonic pleural effusions). Five of the 6 children had major underlying comorbidities: Down's syndrome/morbid obesity, mucopolysaccharidosis, lymphoma, systemic lupus erythematosus and Aicardi syndrome with epilepsy. The investigators found the causes of death were related to serious pre-existing conditions in 3 cases: 2 children had significant viral respiratory infections and 1 child had serious extrapulmonary sepsis. None of the deaths appeared to be due to empyema thoracis or parapneumonic pleural effusions. A higher mortality from

a parapneumonic pleural effusion than empyema thoracis in previously healthy child would be unlikely since empyema thoracis is a more serious complication of pneumonia than a parapneumonic pleural effusion.

An important limitation of this study was the low enrolment rate and the fact that 43% of the eligible participants could not be enrolled because of the non-availability of PF samples, which could be explained by the high proportion of children referred from another hospital. Another limitation was the high number of samples that were culture-negative, which could be explained by the frequent use of antibiotics prior to enrollment. The large number of samples that were negative with molecular analyses, might be due to inadequate handling, long storage or poor transport conditions of the samples. These factors could have introduced bias in pathogen distribution since some bacteria, such as *S. pneumoniae*, are fragile and capable of autolysis from poor handling, while other bacteria, such as *S. aureus,* are more tolerant of handling, transport and long storage periods. The limited panel of pathogens targeted for by PCR in this study may also be a reason for the high proportion of negative samples. Other pathogens might have been present in some samples without being detected.

In conclusion, this study found in Thai children with empyema thoracis and parapneumonic pleural effusions, *S. aureus* was the most frequently detected bacteria with bacterial culture and *S. pneumoniae* was the most frequently detected bacteria with PCR. Molecular analysis of the PF samples improves the sensitivity of detecting bacteria in the PF. Although PCR is more sensitive and specific, there are limitations to this technique. Improvements need to be made, such as the reduction of reducing negative samples and the number of unknown pneumococcal serotypes in children with parapneumonic pleural effusions.

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