

INVASIVE PNEUMOCOCCAL DISEASE AMONG HOSPITALIZED CHILDREN AGED 28 DAYS TO 60 MONTHS IN JARKARTA

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Abstract. *Streptococcus pneumoniae* is a leading cause of morbidity and mortality among children worldwide. Prevention of invasive pneumococcal disease (IPD) with a pneumococcal conjugate vaccine (PCV) is an effective approach to reduce the burden of pneumococcal disease. Nationwide epidemiological data is required prior to considering universal pneumococcal immunization for Indonesia. This preliminary study aimed to quantify the burden of IPD among hospitalized children at Cipto Mangunkusumo Hospital and Fatmawati Hospital, Jakarta. We studied 205 subjects aged 28 days to 60 months who were admitted with the diagnosis of pneumonia, meningitis, sepsis, and suspected occult bacteremia. *Streptococcus pneumoniae* was isolated from 1 of 205 blood specimens, giving an IPD proportion of 0.5%. The IPD case in this study was a 3-month-old baby with meningitis and bilateral lobar pneumonia. The Quellung test demonstrated serotype 7F. The isolate was susceptible to amoxicillin and Cotrimoxazole. Incidence of IPD could not be calculated due to low number of cases; this underscores the importance of surveillance of pneumococcal disease in Indonesia.

Keywords: invasive pneumococcal disease, bacteremic pneumococcal pneumonia, pneumococcal meningitis, sepsis, occult bacteremia, pneumococcal conjugate vaccine

INTRODUCTION

Pneumococcal disease is a significant cause of childhood morbidity and mortality worldwide (WHO, 2007). Clinical manifestations of pneumococcal disease vary depending on the site of infection. The collective term invasive pneumococ-

cal disease (IPD) refers to infection of a normally sterile site with *Streptococcus pneumoniae*, which commonly presents as bacteremia pneumonia, meningitis, bacteremia or sepsis (Dagan *et al*, 2009).

The World Health Organization estimated one million children under five years die every year from IPD (WHO, 2007). The incidence of IPD varies by country, it has been reported to be 130-597/100,000 in developing countries (O'Dempsey *et al*, 1996; Karstaedt *et al*, 2000; Brent *et al*, 2006; Roca *et al*, 2006).

Increasing numbers of antibiotic-resistant invasive pneumococci have led

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to higher risk of treatment failure. In the USA, the resistance rate of pneumococci to penicillin has increased significantly, from 1.3% in 1992 to 24% in 1998 (Whitney, 2000). High levels of penicillin resistance have also been found in Singapore (44%, 1997-2004) (Chong *et al*, 2008) and Thailand (41.3%, 2003) (Sangthawan *et al*, 2003). Data regarding pneumococcal penicillin resistance in Indonesia from sputum, throat swabs, urine, and sinus specimens showed 100% susceptibility to penicillin in 2003. The emergence of resistant strains underscores the need for preventing pneumococcal disease rather than using curative modalities.

The remarkable success of the pneumococcal conjugate vaccine, as seen in the surveillance of IPD after routine infant immunizations in North America, the United Kingdom and Australia, challenges policy makers throughout the world to quantify the burden of IPD in their regions and to estimate the potential benefits of vaccine use. There is a paucity of data regarding the incidence of IPD in Indonesia.

This study aimed to estimate IPD burden among hospitalized children at two referral hospitals in Jakarta. We also determined the serotype, clinical presentation and antibiotic susceptibility patterns.

MATERIALS AND METHODS

Subjects

This was a prospective, hospital-based study conducted at two academic referral hospitals in Jakarta: Cipto Mangunkusumo Hospital and Fatmawati Hospital. Both are government hospitals that serve mainly the middle- and low-middle income populations. We intended to find the proportion of IPD, not incidence, since the denominator (population

served by our study hospitals) could not be determined.

All studies regarding IPD incidence are surveillance studies and use a time period instead of a sample size calculation. We could not carry out surveillance due to limited resources. Sample size was calculated using a formula for a single proportion. The only previous data regarding IPD is the proportion of *S. pneumoniae* positivity in cerebrospinal fluid cultures among children aged 2 days to 12 years, that is 6% (Ruspondji *et al*, 1981). We estimated a proportion of 10%, since our subjects are younger and therefore presumed to have a higher proportion of IPD. The sample size calculation using an α of 0.05, a z_{α} of 1.96 and a proportion of 10% showed we needed a minimal sample size of 150 subjects. Subjects were recruited consecutively.

Subjects were children aged 28 days to 60 months admitted to hospital with pneumonia, sepsis or meningitis. Children aged 28 days to 36 months with suspected occult bacteremia were also included in this study. Exclusion criterion was previous administration of intravenous antibiotics. Those who required more than 48 hours for specimen delivery time to the laboratory were also excluded presuming the viability of the microorganism would dramatically decrease after 48 hours in transport media.

Case definitions

A case of IPD was defined as isolation of *S. pneumoniae* from a normally sterile body fluid (blood or CSF) (Pilishvili *et al*, 2009). Pneumococcal meningitis was diagnosed when pneumococcus was cultured from the CSF regardless of the results of blood culture or isolation of pneumococcus from the blood of a patient with a CSF leukocyte count >10 leukocytes/mm³ and

CSF glucose <40 mg/dl or CSF protein >100 mg/dl.

Laboratory procedures

Blood specimens (2-3 ml) were collected aseptically from all subjects for culture and susceptibility testing. Specimens of CSF (1-2 ml) were obtained from all subjects suspected of having meningitis, for analysis and culture. Specimens were injected into a Pediatric Bactec bottle (Becton Dickinson®, Franklin Lakes, NJ) and sent at room temperature to the Laboratory of Microbiology, Faculty of Medicine, University of Indonesia within 48 hours. Positive cultures were Gram stained and subcultured on sheep blood agar, McConkey agar, and chocolate agar. Identification was done according to standard microbiology protocols. Pneumococcal isolates were serogrouped and typed using the Quellung reaction with antisera from Statens Serum Institut (Copenhagen, Denmark). Antimicrobial susceptibility testing was done by the disc diffusion method.

Data management

Data were entered into and analyzed using SPSS version 17.0. The number of IPD cases was reported as a proportion (percentage of IPD cases per all study subjects).

Ethics

This study was approved by the Ethics Committee, Faculty of Medicine, University of Indonesia.

RESULTS

During the study period (June 2008 through December 2009), 292 children fulfilled inclusion criteria, 165 were treated at Cipto Mangunkusumo Hospital and 127 were treated at Fatmawati Hospital.

Of the 292 patients, 55 were excluded due to previous intravenous antibiotic administration and 32 were excluded due to a long holiday which caused the specimens to not be delivered within 48 hours of collection. The excluded patients had pneumonia (38), sepsis (21), a complex febrile seizure (10), pneumonia and sepsis (7), febrile neutropenia (5), viral infection with hyperpyrexia (3), fever without source (2) and meningitis (1).

A total of 205 children met eligibility criteria and were enrolled in this study, 118 from Cipto Mangunkusumo Hospital (CMH) and 87 from Fatmawati Hospital. Characteristics of the subjects are shown in Table 1. The majority of children (95%) were aged 28 days to 36 months and pneumonia was the most common diagnosis (72.6%).

Of the 205 subjects, 1 (0.5%) had blood culture positive for *S. pneumoniae*. Cultures of CSF were performed in 5 subjects but none yielded *S. pneumoniae*. The pneumococcal serotype was 7F, as determined by the Quellung test. The isolate was susceptible to amoxicillin and Cotrimoxazole, intermediately sensitive to chloramphenicol and resistant to tetracycline.

Positive blood cultures were found in 35 subjects (17.1%) (Fig 1). *Staphylococcus epidermidis* was the most frequent isolate and considered as a contaminant, giving a contamination rate of 11.7%. Of the 5 CSF cultures, 1 yielded *Candida albicans* and the remainder were negative for growth.

The positive IPD case was a 3-month-old baby diagnosed with meningitis, bilateral lobar pneumonia and failure to thrive. He was the third child of three siblings, living in a crowded house and had no history of immunizations. Clinical manifestations included fever, cough,

Table 1
Characteristics of subjects.

Variable	N	%
Age (months)		
28 days to 36 months	195	95.0
37-60 months	10	5.0
Sex		
Male	123	60.0
Female	82	40.0
Diagnosis ^a		
Pneumonia	149	72.6
Suspected occult bacteremia		
Complex febrile seizures	20	9.6
Viral infection with hyperpyrexia	2	1.0
Febrile neutropenia	4	2.0
Fever without source	3	1.5
Sepsis	10	4.9
Sepsis and pneumonia	9	4.4
Meningitis	2	1.0
Meningitis and pneumonia	4	2.0
Meningitis and sepsis	2	1.0
Prior oral antibiotic		
Yes	43	21.0
Unknown	31	15.0
No	131	64.0
Immunization		
PCV7 immunization (complete or incomplete)	0	0
Hib immunization (complete or incomplete)	3	1.5
Outcome		
Survived	177	86.3
Died	28	13.7

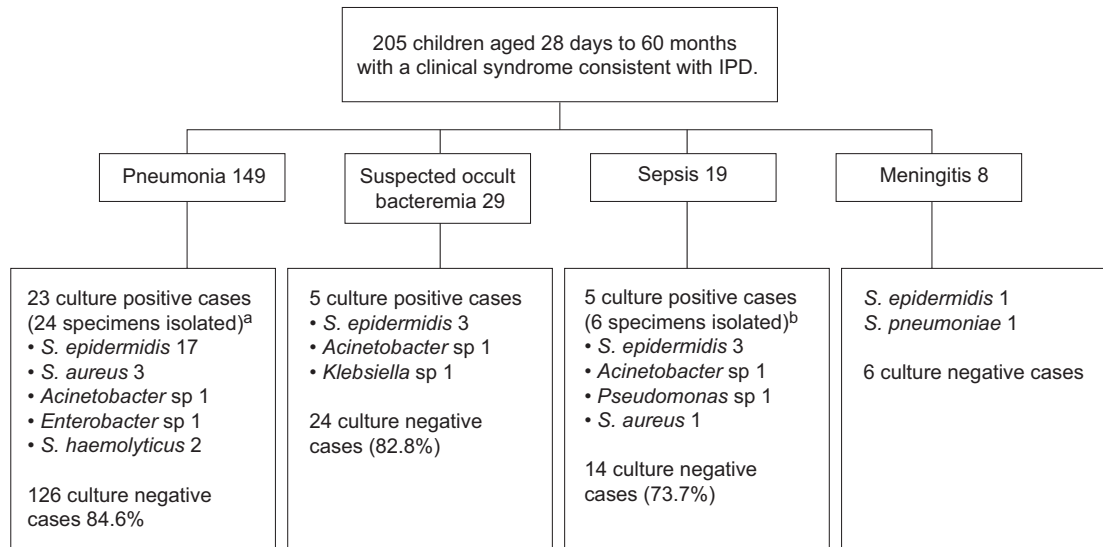
^aDiagnosis at recruitment, final diagnosis on discharge might be different

respiratory distress, a seizure and decreased consciousness. Upon arrival at the Emergency Unit, the patient was found to be somnolent with a bulging anterior fontanelle and spastic extremities. He had lower chest indrawing and rales in both lungs.

A complete blood count showed anemia (Hb 6.3 g/dl), leukocytosis (25,100/ μ l), a neutrophil count of 75% and thrombocytosis (783,000/ μ l). Blood gas

analysis showed a PaO₂ of 163.7 mmHg with oxygen by mask at 5 liter/minute. A qualitative CRP examination was positive. Analysis of CSF revealed a cell count of 1,700/mm³ (75% polymorphonuclear), a glucose of 20 mg/dl, and a protein of 65 mg/dl. Culture of the CSF could not be performed due to lack of specimen. The chest X-ray demonstrated bilateral consolidation.

The IPD patient was hospitalized



The most severe diagnosis listed for subjects with more than one diagnosis

^aTwo microorganisms were isolated from one subject (*S. aureus* and *S. haemolyticus*)

^bTwo microorganisms were isolated from one subject (*S. epidermidis* and *Acinetobacter* sp)

Fig 1—Blood culture results by clinical presentation.

in the pediatric intensive care unit and treated with cefotaxime and gentamicin. Unfortunately, he died on the fifth day of hospitalization due to respiratory failure.

DISCUSSION

This is the first study to evaluate the epidemiology of IPD in Jakarta. Similar studies including outpatients and inpatients were conducted in Surabaya, Bali, and Bandung (PneumoNet Study). The results were presented at the 5th ASEAN Society of Pediatric Infectious Disease (ACPID) Meeting, September 2010 in Taipei, Taiwan.

Instead of incidence, we studied the proportion of IPD. The incidence of IPD could not be calculated due to difficulty in defining the catchment area. Variations

in IPD incidences have been reported elsewhere; several differences in characteristics of the study settings and population may have greatly contributed to this variability (Roca *et al*, 2006; Arifeen *et al*, 2009). These differences included: 1) differences in eligibility criteria; 2) methods used for the study, as demonstrated by a study in Belgium which reported passive surveillance caused under-reporting (Vergison *et al*, 2006); 3) differences in laboratory capabilities in isolating pneumococcus; 4) differences in case definitions; 5) different blood culture procedures; and 6) variations by geographical area and period of study. There are also likely to be true differences in disease incidences (Roca *et al*, 2006; Arifeen *et al*, 2009).

The proportion of IPD found in this study was 0.5%, comparable to studies from Bangladesh (0.3%) and Nepal (0.9%)

(Saha *et al*, 2009; Shah *et al*, 2009). Similar studies from other cities in Indonesia also showed comparable results (Bandung, 2 positive IPD cases out of 466 subjects, giving a proportion of 0.4%; Surabaya, 5 positive IPD cases out of 1,040 subjects, giving proportion of 0.5%) (Kartasasmita *et al*, 2010; Moedjito *et al*, 2010). Studies from Mozambique, India and Kenya had higher proportions of IPD (2.2%, 1.2%, and 1%, respectively) (Roca *et al*, 2006; IBIS, 1999; Brent *et al*, 2006). Most patients with pneumonia are not bacteremic (Werno and Murdoch, 2008). This may explain why the proportion of IPD cases in this study was low since most subjects were diagnosed with pneumonia. Purulent CSF cultures give more positive results than blood cultures, as found in Bangladesh (0.3% positive for *S. pneumoniae* from blood cultures *vs* 11.2% positive CSF cultures) (Saha *et al*, 2009) and India (1.2% positive blood cultures *vs* 6.1% positive CSF cultures (IBIS, 1996).

The isolation of *S. pneumoniae* confirms the diagnosis of pneumococcal disease. However, bacteremia occurs in only a minority of cases of IPD (Werno and Murdoch, 2008). This occurs due to several factors, such as prior administration of antibiotics, the fastidious nature of this microorganism and the intermittent nature of bloodstream invasion by *S. pneumoniae*. Attempts to obtain positive results were made by excluding subjects with previous intravenous antibiotics, using Pediatric Bactec as transport media and processing the specimen for culture as soon as possible, since bacterial viability decreases over time. Prior oral antibiotic use may be a contributing factor to reducing positive results, since 21% subjects had previous oral antibiotic use. A study in India reported of subjects with prior antibiotic use, only 4% had positive blood

cultures compared to 5.7% of subjects who had no previous antibiotics (IBIS, 1996).

Contaminants can obscure the growth of pathogens, reducing sensitivity. While target rates for contamination have been set at 2-3%, actual rates seem to vary widely between institutes (Hall and Lyman, 2006). Our contamination rate was high (11.7%), similar to those in Kenya (11.9%) (Brent *et al*, 2006) and Mozambique (12%) (Roca *et al*, 2006), which might lower positivity of cultures. Incorrect aseptic technique with venipuncture is assumed to be the main source of contamination.

The pneumococcal serotype identified in this study was 7F, which was also reported as the most common cause of meningitis in Bangladesh (Saha *et al*, 2009). Serotype 7F is infrequently found in the nasopharynx and not included in the seven-valent pneumococcal conjugate vaccine. A study in Germany showed 7F causes higher case-fatality rates (OR 4.3; 95% CI 1.3-14.7) compared to other serotypes (Ruckinger *et al*, 2009).

The IPD case in this study was in a 3-month-old baby with a diagnosis of meningitis and lobar pneumonia. Arditi *et al* (1998) reported 10% of pneumococcal meningitis is associated with other comorbidities, such as pneumonia and otitis media. Clinical presentations of IPD vary, the most common diagnoses are pneumonia and bacteremia. Meningitis is occasionally found, but it accounts for the highest case fatality rates of IPDs. Our IPD patient died; this poor outcome is consistent with high mortality rates of pneumococcal meningitis as reported from Belgium (62.5%), Gambia (55%), and Mozambique (56%) (O'Dempsey *et al*, 1996; Roca *et al*, 2006; Vergison *et al*, 2006).

Cerebrospinal fluid analysis in this case was typical for bacterial meningitis,

except for only a slightly elevated protein level (65 mg/dl). Manifestations of meningitis in this case were classic, comprised of fever, convulsions, decrease in consciousness and a bulging fontanelle. The chest X-ray demonstrated bilateral consolidation. There are no characteristic radiological features for pneumococcal pneumonia (Dagan *et al*, 2009). However, Neuman and Harper (2003) found among febrile children with lobar infiltrates on chest radiograph, 76% (95% CI 63-85) had pneumococcal antigen detected in their urine, which suggests lobar infiltrate on chest radiograph is associated with pneumococcal pneumonia.

The *S. pneumoniae* isolated in this study was susceptible to penicillin. In a study from Bandung, which found two isolates, and Surabaya, which found six isolates, 100% of isolates were susceptible to penicillin (Kartasmita *et al*, 2010; Moedjito *et al*, 2010). The isolate in this study was not susceptible to tetracycline, dibekacin, and chloramphenicol. Resistance to chloramphenicol is found in as many as 17% of cases in India and 6% in Bangladesh (IBIS, 1996; Saha *et al*, 2009). In contrast to chloramphenicol, high resistance to Cotrimoxazole has been reported in several developing countries, which is possibly a result of its widespread use by community health care workers (IBIS, 1996; Brooks *et al*, 2007; Shah *et al*, 2009; Kartasmita *et al*, 2010; Moedjito *et al*, 2010).

Risk factors for IPD in our case were young age (3 months old), failure to thrive, tobacco exposure and overcrowding. Young age is perhaps the most important risk factor for acquiring pneumococcal infection because natural immunity is highly age-dependent (Dagan *et al*, 2009). The predilection for young age has been reported in Bangladesh, where 55% of IPD case occurred in babies ages ≤ 6 months

(Saha *et al*, 2009). Study in West Africa showed that failure to thrive (OR 8.08; 95% CI 1.66-39.25) and passive smoking (OR 2.99; 95% CI 1.10-8.15) were risk factors for IPD (O'Dempsey *et al*, 1996).

Our study had certain weaknesses. The main limitations were the method of study and the small sample size. The best method to estimate burden of disease is national active surveillance. This was a hospital-based study conducted at academic referral hospitals which might not reflect the spectrum of pneumococcal disease in the community. The subjects were inpatients who did not represent mild pneumococcal disease treated as outpatients. These limitations could cause the magnitude of pneumococcal disease to be underestimated.

In conclusion, this study attempted to estimate the burden of IPD. The results suggest pneumococcal disease is present in Jakarta. A community based nationwide epidemiological study is needed to confirm the burden of IPD. Another important factor is the type of serotype. An IPD vaccine for Indonesia should include serotype appropriate for this country. A cost-benefit analysis should be made to determine if a pneumococcal vaccine is cost effective for Indonesia.

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